ROTHAMSTED RESEARCH

Report for 1978 - Part 1

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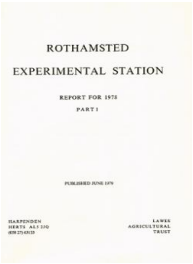

Soil Microbiology Department

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Introduction

Basic and applied research on vesicular-arbuscular mycorrhiza and on legume nodulation continue to be major interests of the Department. The importance of these symbioses lies respectively in their capacity to increase the uptake of phosphate from poorly available sources and to promote more efficient biological fixation of nitrogen, thereby saving energy and fertiliser costs. This part of our programme covers a range of agricultural crops including tropical species for which we undertake research on behalf of the Ministry of Overseas Development and with whom we are associated in training programmes and in field studies at tropical agricultural research centres abroad.

Work continues on rhizosphere microbiology in relation to crop health and disease, on microbial ecology, on the *in situ* assessment of microbial activity, on microbial effects on soil stability and porosity and on some aspects of the transformation of nitrogenous compounds in the soil.

New programmes were started on novel soil inoculation procedures, on physiology of the transport of fixed nitrogen compounds in legumes and on interactions between mycorrhiza and *Azotobacter* and between mycorrhiza and nematodes. Work on nitrification, anaerobic processes, *Rhizobium* ecology, nodule fine structure and some of the mycorrhizal work will not be reported this year.

General studies

Effects of repeated wetting and drying on the stability of soil aggregates. The natural and artificial aggregates of Rothamsted Parklands (grassland) soil were prepared and water stability assessed as described previously (Skinner, *Rothamsted Report for 1977*, Part 1,

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242). These were incubated for 7 days at 25°C and a water content of 20% with or without added carbohydrate and then subjected to repeated alternate wetting for 24 h and drying for 2–3 days. Drying was done in an incubator at 25°C above potassium carbonate. Measurements of stability and porosity were made after two, five and ten cycles of wetting and drying.

The proportion of water-stable natural aggregates > 1 mm was increased from about 50% when incubated in water to 64% when incubated with glucose, most of this increase being at the expense of the smallest particle fraction. The much less stable artificial aggregates behaved similarly; the corresponding figures being respectively 23 and 41%. Density of the soil particles was the same (2·34) for both types of aggregate.

Measurement of porosity characteristics, viz. porosity, coefficient of diffusion, efficiency of unit pore space for diffusion and complexity of the pore system indicated a slightly more open and rather simpler pore system in the artificial aggregates. These also remained constant irrespective of the incubation treatment or subsequent cyclical wetting and drying. Thus, the size, shape and gas diffusion characteristics of the pores, and the closeness of packing of particles in the aggregates, were unaffected by the treatment given. Increase in water-stability was not therefore accounted for by any re-arrangement of the soil particles but more probably by microbial action when carbohydrate was present. (Skinner, with Currie, Physics Department)

Enzyme cytochemistry of soil micro-organisms. Procedures for optimal staining and counterstaining of enzymically active soil bacteria (*Rothamsted Annual Report for 1977*, Part 1, 242) have continued. The activity of respiratory enzymes has been demonstrated in root-dwelling bacteria and fungi by incubating small intact root segments (< 1 cm long) in cytochemical reagents. After colour development the roots were cleared in chloral hydrate for microscopy. (Macdonald and Spokes)

Evaluation of a microbial 'fertiliser', made in USA. Bacterial fertilisers available in America are being considered for marketing in the UK. At the request of an importer one of these products is being examined here and at Letcombe to assess the manufacturers' claims to increase crop yield and accelerate the decomposition of straw.

This product consists of a mixture of micro-organisms, a substrate (whey) and an activator, containing organic cobalt. This is fermented before applying to soil with or without straw stubble. The treated soil is left several weeks before sowing the crop. The preparation is used at an approximate rate of 6 kg ha^{-1}, the proportions by weight of whey, activator and inoculant being 390:13:1.

The effect of this product and its components was examined in greenhouse experiments in two different soils, a Kettering loam of pH 6·8 containing 126 ppm N and soil from Horsepool field, Woburn Experimental Farm, containing 4 ppm N. The pH of the Woburn soil was adjusted to 7·2 with lime. Chopped straw was added to half of the experimental pots which were given potassium nitrate equivalent to 0, 20, 30, 60 kg N ha^{-1}.

Yields of lettuce and radish were increased significantly ($P = 0{\cdot}01$) in both soils by 30–60% with or without added straw, by the complete product and by whey and the activator alone, when nitrogen was added at 0, 20 and 30 kg N ha^{-1}.

A factorial field experiment was done on Hoosfield, which is low in soil nitrogen, using the complete product, the activator alone and the activator and whey applied in the Autumn. Half the plots received chopped straw in the Autumn, with or without 30 kg N ha^{-1}. Spring wheat was sown 3 months after inoculation and given nitrogen fertiliser at 30, 90 and 150 kg N ha^{-1}.

The microbial fertiliser treatments had no effect on germination or on weight of

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seedling plants, nor were the number of ears produced or the final yield affected although at tillering some significant increases in leaf weight were recorded in some treatments. Adding nitrogen fertiliser to plots in the autumn increased yield significantly. (Brown and Witty)

Take-all disease. The severity of take-all disease measured by visible root lesions is being correlated with the amount of living mycelium within the tissues, the amount of brown material produced in infected plant cells, and with microbial populations on the lesions.

The pseudomonad flora associated with take-all disease was examined in wheat plants grown in pots of soil naturally infested with *Gaeumannomyces graminis* and taken from fields which had carried different numbers of consecutive cereal crops. Roots were sampled at 10 and 20 weeks and colonies counted on two selective media. Infected root material from field grown crops at similar stages of plant development was also examined for *Pseudomonas*.

At the tillering stage of growth (of plants in pots or from the field) *Pseudomonas* colonies, especially those producing fluorescent pigments, were more numerous on lesions from severely diseased roots than on those from less diseased roots. Healthy roots from plants grown in soil after a 3-year break also carried more *Pseudomonas* than those after several consecutive cereal crops.

At the stage of grain formation the number of *Pseudomonas* on the lesions was similar, irrespective of crop sequence. Healthy roots carried fewer *Pseudomonas* but again numbers were similar irrespective of the soil in which the plant was grown. At tillering, lesions on the most severely diseased roots carried the greatest proportion of *Pseudomonas* able to inhibit growth of *G. graminis* on agar (65%), but at earing, this proportion was decreased to 10%. On healthy pieces of root at tillering 40–50% of the inhibitory population was *Pseudomonas*, independently of the general level of root infection, but at earing, *Pseudomonas* was found only when infection was severe. These results show no firm correlation between the number of *Pseudomonas*, whether inhibitory to *G. graminis* or not, and the severity of infection arising from the natural supply of infective material in the soil. (Brown)

VA Mycorrhiza

Requirements for germination and growth of VA mycorrhizal spores. Of the seven spore sources of *Acaulospora laevis* (honey-coloured endophyte) tested, none gave > 6% germination on water agar. Germination of all samples was induced by placing the spores in soil between layers of 'Millipore' membrane; the maximum germination obtained was 80%; generally with extensive hyphal development. This stimulatory effect on germination varied with the soil pH, being most marked in soils of pH 4·5–6·5 and declining above this limit; spores buried in sand did not germinate.

Attempts were made to induce the spores to germinate under axenic conditions. Cold water extracts of soils mixed with water agar failed to stimulate germination possibly because they did not lower the pH sufficiently. Hot water soil extracts gave some germination but always less than that observed with buried spores and germ tube growth was often stunted. The use of buffers to stabilise the pH of the agar was partially successful. Up to 57% germination occurred on agars of between pH 3·7 and 5·5 buffered with 5 or 50 mM-sodium succinate but again germ tubes were very stunted. Agars buffered with citrate or β^1-dimethyl glutaric acid-KOH were unsuitable. One of the soils produced a volatile compound (probably not carbon dioxide) which induced germination of spores on water agar.

Surface-sterilised spores of *Glomus caledonius* (a laminate type) showed > 90%

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germination after 7–14 days on water agar. Storage of the spores in water at 6°C for a year had no effects on germination characteristics. When buried in soil between layers of 'Millipore' membrane in the absence of plant roots, the germination of the spores was the same as on agar.

The effect of a range of growth factors on hyphal development and growth from pregerminated spores was assessed by grading according to length and branching, or by estimating hyphal length by line intersection or by computer image analysis. The most stimulatory factors were yeast extract (400 mg litre^{-1}), bacteriological peptone (5 g litre^{-1}) and thiamine (5 mg litre^{-1}), which gave respective increases in hyphal growth of 2·4, 3·1 and 2·1 times over the control. By incorporating inorganic ions and other compounds possibly required for growth into a basic medium containing optimum concentrations of above nutrients, a 5·9-fold increase in hyphal growth was obtained compared with that on water agar.

Following Barrett's (*Recent Advances in Botany* (1961) **2,** 1725) report that VA mycorrhizal endophytes colonise and regrow from hemp seed bait, pieces of boiled seed of this plant or lima bean, dwarf bean, groundnut, soyabean, cucumber, maize or wheat were tested for their effect on hyphal growth. All seeds clearly stimulated growth, except wheat and maize which only did so marginally. Pieces of lima bean seed (cv. ACL 2594) gave a 2-fold increase in hyphal length on water agar and a 1·4-fold increase on the basic medium resulting in a mean hyphal length of 535 mm per spore, representing an 8-fold increase over the controls. The effect of the boiled seed was to increase hyphal length and branching apparently without colonisation. The stimulation probably came from a seed diffusate. After a period of about 4 weeks, growth appeared to cease without the utilisation of the spore lipid reserves. The addition of sugars (hexoses, pentoses and sugar alcohols) to the basic medium had no obvious effects on growth.

Attempts to subculture the fungus on media supplemented with pieces of seed failed; as soon as hyphae were detached from the parent spore they ceased to grow. Even when hyphal growth was considerable, the number of vegetative spores remained rather small – 4·9 per spore on medium with seed compared with 1·0 per spore on water agar. Such spores were generally confined to a small area close to the parent spore.

The growth of *G. caledonius* was also examined on a medium used to culture a mucoraceous mycoparasite (also an haustorial biotroph) which had limited permeability to glucose (Binder & Pierce. *Canadian Journal of Botany* (1976), **54,** 1403) but this was found to be inhibitory. (Hepper)

The germination of *Gigaspora margarita* originating from Florida, was tested at 2, 10, 15, 20, 26 and 31°C with spores placed between 'Millipore' filters buried in the soil. Germination after 3 weeks was respectively 0, 0, 2·5, 50, 75, 90%. Most germ tube growth occurred at 31°C. Even after 9 weeks spores did not germinate below 15°C. This spore type which has not been reported in surveys of soils in Britain or the South Island of New Zealand is probably restricted to tropical conditions. (Clarke)

A range of culture media was prepared to simulate published analyses of plant roots and phloem exudates in an attempt to provide a suitable chemical environment for the culture of endomycorrhizal fungi. The media contained amino-acids, amides, sugars, nucleic acid bases, nucleotides and inorganic salts at concentrations similar to those at which they occurred in roots. The growth of the fungi on these media was compared with that on 0·1% peptone in agar supplemented with biotin and thiamin (basal medium). In no case did growth exceed that on the basal medium although hyphal morphology and branching frequency were modified by high concentrations of many of the materials tested. (Macdonald and Spokes)

Enzymic lysis of VA mycorrhizal fungi. Giles (*Cytobios* (1975) **14**, 49–61) has shown that

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Azotobacter spheroplasts can be incorporated into protoplasts of the ectomycorrhizal fungus *Rhizopogon*. Pine seedlings infected by the modified fungus reduced acetylene. Preliminary experiments on protoplast formation in VA mycorrhizal fungi have been carried out. A mixed lytic enzyme preparation extracted from the culture supernatant of *Trichoderma harzianum* released protoplasts from *Mortierella rammaniana* when the fungus was suspended in an osmotic buffer. No protoplasts were released from the taxonomically related VA mycorrhizal fungi *Glomus mosseae* and *Glomus caledonius* although cytoplasmic leakage occurred. Digestive juices from the gut of the snail *Helix pomatia* also failed to stimulate protoplast production although they did alter the structure of hyphal walls in *G. mosseae* and *G. caledonius*. Combinations of the lytic enzymes listed above with commercial lipase and cellulase sometimes lysed *G. mosseae* and *G. caledonius* but protoplasts were not formed. (Macdonald)

Development of VA mycorrhiza (E_3 and YV) in plants fed with nutrient solution in sand and nutrient film culture. Sodium nitrate, ammonium nitrate and ammonium sulphate were added twice weekly in complete nutrient solutions containing 5, 10, 20 and 40 ppm N to lettuce plants grown in sand and inoculated with E_3. Plants given up to 20 ppm N as ammonium sulphate had about 50% of their roots infected but this fell to 7% with 40 ppm N. Plants fed with sodium or ammonium nitrate never exceeded 20% which dropped to 10% with 10 ppm N and to nil with 40 ppm N. The pH of the nutrient solution in the pots fell to about 5·4 for the ammonium sulphate-fed plants and to 6·0 and 6·3 respectively for those given ammonium or sodium nitrate; all within an acceptable pH range for E_3 endophyte. The results confirm those obtained last year with soil-grown lettuce where infection was more sensitive to added calcium nitrate than to ammonium sulphate. They support the view that levels of VA infection in the field may be controlled as much by nitrogen fertiliser as by phosphate levels in the soil. (Owusu-Bennoah and Mosse)

Maize and wheat inoculated with E_3 or YV were grown in sand culture with Hewitt's solution at full, 1/4 and 1/10 strength in which the proportion of nitrate to ammonium nitrogen varied from 100 to 95, 50 and 0%. Very little infection developed in the wheat plants fed with full strength nutrient solution. The 1/4 strength solution produced most infection in both wheat and maize; 1/10 strength seemed to favour vesicle development. In all treatments YV-inoculated maize weighed more than E_3-inoculated plants but wheat grew better when inoculated with E_3. (Thompson)

The possibility of obtaining large amounts of relatively clean mycorrhizal roots from plants grown in nutrient film cultures was investigated. In a preliminary survey twelve graminaceous and twelve leguminous plants grown in sand culture were screened for suitability for large scale inoculum production. *Phaseolus vulgaris* cv. Jamapa became strongly infected (80%) by two different endophytes whereas the variety Canadian Wonder was only very slightly infected. The other species averaged 40% infection. Buckwheat appeared to be immune.

Nutrient film cultures set up with *Phaseolus vulgaris* cv. Jamapa showed heavy infection with normal development of arbuscules, vesicles and mycelium; the infectivity of this material is being tested. Different levels and forms of phosphate in the nutrient solutions markedly affected the rate of infection; in all treatments infection was more extensive after 10 than after 5 weeks. The plants nodulated well in the N-free medium, symbiotic N_2 fixation providing all the N needed for normal growth. Many spores of the endophyte (E_3) developed within some of the older nodules. Young uninfected seedlings placed in the flow cultures with older infected plants became infected after 2–3 weeks. (Mosse, Thompson and Smith)

Factors affecting spread of endophytes. The spread of the endophyte (E_3) was compared

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in soil sown with *Trifolium repens*, *Vicia faba*, *Lactuca sativa*, *Festuca rubra* or *Allium cepa*. The soil was cut into sections and presence or absence of infection was recorded in roots washed from each section. After 40 days most spread had occurred with clover followed by bean, lettuce, *Festuca* and onion. *Festuca* had the largest aggregate root length followed by clover, bean, lettuce and onion. With the exception of *Festuca*, root density and rate of spread were correlated.

Effects of aeration on spread of the endophyte E_3 were compared in soil mixed with fine sand, fine or coarse grit, either continuously supplied with water through wicks, or watered manually at intervals. Clover in fine sand and soil with continuous watering induced the greatest spread rate of 0·8 cm per week and coarse grit watered manually the least, 0·48 cm per week. With *Festuca*, the watering regime made little difference; in coarse grit infection spread 0·4 cm per week and in fine sand only 0·25 cm per week. These rates are low compared to some observed with another endophyte in the field. A maximum spread of 2 cm per week was recorded with lucerne.

The effect of inoculum density on rate of infection was examined by diluting heavily infected P-deficient soil with irradiated (uninfected) soil of the same kind, in the proportion of 0, 1, 12·5, 75 and 100% infected soil. Clover seedlings became infected 15 days after germination in all but the 1% dilution, in which infection did not develop until 45 days. At 60 days roots in all dilutions were at least 60% infected and the 1% dilution averaged 74%. However, these plants were smaller with only half as much root, presumably due to their later infection in a P-deficient soil. (Warner)

Effects of pesticides on VA mycorrhiza. Effects were examined of a range of pesticides on the development of infection and on spore populations in field trials of barley, maize and potato at Woburn, and barley and maize at Rothamsted. These were given no pesticide or one or more of the following: benomyl, dazomet, aldicarb, carbofuran, thiabendazole, phorate, chlorfenvinphos and chlortoluron. Benomyl, dazomet and phorate applied singly decreased spore numbers and VA infections to about half of the controls. Other pesticides had little effect. Aldicarb, by contrast, although having little effect in two trials, markedly increased mycorrhizal infection in barley at Woburn when applied at 5·6 kg ha^{-1} in combination with 'Nitro-Chalk' at 38 kg N ha^{-1}. This resulted in 58% of root length infected compared to 8% in the controls, and increased spore numbers by 50%. However, with more 'Nitro-Chalk' (75 kg N ha^{-1}), infection was low (1–4%) in all treatments. (Ocampo and Hayman)

It is claimed that the systemic fungicide aluminium tris (ethyl phorphonate), known as 'LS 74783' or 'Aliette', is translocated downwards in plants and inhibits certain phycomycete pathogens. Its activity was tested against three endophyte species in pots and against indigenous endophytes in the field. It did not reduce infection in lettuce seedlings sprayed with 49 g a.i. $litre^{-1}$ before transplanting into infected (E_3) soil (90% in treated v. 80% in control plants) or in seedlings sprayed 10 days after emergence in a soil infested with the endophytes, E_3, VY and *G. microcarpus* which showed 80% infection compared to 70% in the controls. Drenching an infested soil did not reduce infection and immersing the inoculum in the fungicide for 24 h had no adverse effect on its infectivity. (Clarke)

A 0·2% aqueous suspension of 'LS 74783' was sprayed on to the leaves of *Trifolium dubium*, *Lolium perenne* and *Avena fatua*, which were 60, 10 and 0% mycorrhizal respectively. The roots of each of the plants were supplied with 50 μc of ^{32}P phosphate. Analysis of the leaves after 5 days failed to reveal any effect of 'LS 74783' on phosphate uptake, indicating that the fungicide is ineffective in preventing P uptake by established endomycorrhizal fungi. (Macdonald, Spokes and Ocampo)

Aqueous mixtures of three fungicides were applied at two concentrations (triadimefon at 5 and 25 ppm; etridiazole at 5 and 50 ppm and chloroneb at 10 and 40 ppm) for 16 h to

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inocula consisting of spores and hyphae of *Glomus mosseae*, *G. fasciculatus* and *G. microcarpus*. The inocula were washed free of fungicide and mixed with γ-irradiated soil/sand mixtures into which lettuce seeds (cv. Little Gem) were sown. The fungicides were not phytotoxic.

None of the fungicide-treated inocula of *G. fasciculatus* formed mycorrhizas before 4 weeks; untreated inocula had infected 10% of roots at this time. Infection in plants given treated inocula slowly increased, reaching that of the controls at 12 weeks (74% of roots infected). Plants infected by the *G. mosseae* inoculum treated with the lower concentration of chloroneb were as much infected at 4 weeks as the controls whereas other fungicide treatments reduced mycorrhizal development by about 50%. At the 8 and 12 week harvests all fungicide treatments had reduced infection to about two-thirds of that recorded on the controls.

Plants given *G. microcarpus* inocula treated with chloroneb were consistently more infected than the controls – up to 2·75 times more after 12 weeks.

The triadimefon-treated inocula infected about half as many roots as the controls but no differences could be seen between the etridiazole treatments and controls. (Spokes and Macdonald)

Mycorrhizal development in host and 'non-host' plants when grown together or in succession. Mycorrhizal infections were examined in ten crop species grown separately and in pairs in sterilised and unsterile soil and inoculated with different endophytes. No infection was observed in cabbage, kale, oilseed rape or swede (supposedly non-mycorrhizal Cruciferae) and only traces in sugar beet (supposedly non-mycorrhizal Chenopodiaceae) when these plants were grown alone. However, slight ($< 5\%$) infection (cortical mycelium and vesicles but no arbuscules) developed in some of these crops when a mycorrhizal host plant was present and there were many clumps of endophyte mycelium on their root surfaces, usually attached to aborted entry points. *Glomus fasciculatus* E_3 was more infective than *Gigaspora margarita*. Infection in the host plants barley, lettuce, maize, potato and onion was occasionally depressed by the presence of another plant, irrespective of whether the second plant was a host or 'non-host'. It appears that the barriers to mycorrhizal infection in some plants are intrinsic, probably dependent on characteristics of the root cortex or epidermis rather than on the release of infection-inhibiting factors from the roots. (Ocampo, Martin and Hayman)

There is evidence from field studies that mycorrhizal development is affected by previous cropping, and speculation that negative effects may occur if the preceding crop is a 'non-host'. This was examined in glasshouse pot experiments, using sterilised soil amended with an inoculum of *Glomus fasciculatus* E_3 and unsterile soil, with or without preplanting with 'non-host' plants. In all combinations VA infection was not depressed in soil previously cropped with a 'non-host', whether or not its roots were retained in the soil. Indeed the early establishment of infection was stimulated by 'non-hosts', particularly in sterilised soil where, for example, 3 weeks after inoculation with E_3 the proportion of root length infected by mycorrhiza in barley was 70% in soil containing roots of the preceding oilseed rape plants, 29% in fallow soil containing inoculum and 4% in fresh soil given the same inoculum stored at 2°C. Lettuce after cabbage and maize after sugar beet showed similar effects. Some VA hyphae were observed growing in moribund 'non-host' roots. These results indicate that, far from having a detrimental effect, the roots of plants generally regarded as 'non-hosts' can improve the survival, infective vigour and perhaps quantity of active mycelium of VA mycorrhizal fungi in soil. (Ocampo and Hayman)

Field inoculation trial (Sawyers I, Rothamsted). Onion, lucerne and barley were inocu-

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lated with either LAM (a form of *G. caledonius*) from open pot cultures or a mixed inoculum containing YV (*G. mosseae*) produced in sand culture. The trial consisted of three blocks, half of each treated with formalin to kill the indigenous endophytes. At the beginning of June seed was planted in holes 3 cm above 10 g of the inoculum. Inoculation very significantly increased shoot weight of lucerne cut at 13 weeks by 60 and 360%, of onion by 75 and 510% and of barley by 42 and 32% respectively for the two endophytes. Differences between endophytes were significant for onion and lucerne, LAM inoculum being the better, but not for barley. Although inoculated plants were more heavily infected (about 80%), the control plants also became mycorrhizal, even in the formalin treated plots, showing approximately 30% of infected root tissue. Many new spores arose from the LAM inoculum but few from the mixed inoculum; spore numbers in the uninoculated plots were lower. Percentage infection was independent of spore numbers and the stimulation of growth was not related to level of infection. There was a marked phosphate gradient in the experimental area. Soil analysis showed a gradient of P content along the plot from 0·5 to 1 ppm $CaCl_2$ soluble P and from 8 to 13 ppm $NaHCO_3$ soluble P; the greatest growth improvement from inoculation occurred at the more fertile end of the plot. All lucerne plants were effectively nodulated. (Owusu-Bennoah and Mosse)

Field inoculation trial (white clover in Welsh upland soil). Field trials were continued at hill grassland sites at Pwllpeiran Experimental Husbandry Farm in conjunction with ADAS, Trawsgoed, (*Rothamsted Report for 1977*, Part 1, 241). White clover S184 seedlings were raised in the glasshouse, inoculated with *Rhizobium* and given either phosphate (controls) or an inoculum of specific mycorrhizal endophytes. They were transplanted to the field in June and harvested in September. All plants grew better than last year at the same unimproved site, mycorrhizal inoculation increasing plant growth most where no phosphate was added and having least effect at the standard rate of basic slag (1883 kg ha^{-1}). Of the plants at this site not harvested last year, the mycorrhizal ones continued to grow best. In scattered microplots at another 1977 site, and at a new site, the same mycorrhizal inoculum did not improve clover yield. In other tests at the new site, using different endophytes, there were neither large differences between inoculation treatments nor clearcut responses to phosphate, whether added as superphosphate, rock phosphate or basic slag. (Hayman and Morris)

These differing field responses were investigated in glasshouse pot experiments. In γ-irradiated peaty soil from the site responding to inoculation mycorrhizal plants were twice as large as the non-mycorrhizal controls in all phosphate treatments (0, 19 and 75 mg basic slag per pot – the latter being equivalent to the standard field rate). Non-mycorrhizal plants given 75 mg basic slag were also about double the size of control plants not given P. In all treatments replicate mycorrhizal plants were much more uniform than controls. There was no response to mycorrhiza in irradiated soil from the two other 1977 sites. In unsterile soil from all three sites there was a small response to P but no apparent response to mycorrhizal inoculation. Plants grew slightly better in irradiated than in unsterile soil except for controls without P which were more than 50% bigger in the unsterile soil, an effect attributed to the indigenous endophytes.

Soil from the new site, which proved to be unresponsive, was used to compare the efficiency of four endophytes (*Glomus fasciculatus* (E_3), *G. mosseae* (YV), *Acaulospora laevis* (HON) and *Gigaspora margarita* (MARG) and a non-mycorrhizal control in the presence of different phosphate fertilisers. In irradiated soil, growth differences were striking; YV-inoculated plants given superphosphate were eight times as large as controls with no P. Superphosphate alone increased growth of controls five-fold. Without phosphate, YV inoculation increased growth by 4·5 times and E_3, HON and MARG increased

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it three-fold. With basic slag, controls, HON- and MARG-inoculated plants attained only about half the size of those inoculated with E_3 or YV. With superphosphate differences between five inoculation treatments were small. With rock phosphate, controls showed no growth response and E_3 was the best inoculum. In all endophyte-phosphate combinations, plants grew better in unsterile soil than in the corresponding treatment in irradiated soil, especially the controls. The differences between treatments in unsterile soil were much smaller. Controls grew as well as inoculated plants and, where no phosphate had been added, yields were six times larger than for controls in irradiated soil. This may also be attributed to indigenous endophytes.

Caution should therefore be exercised in predicting field responses to mycorrhiza from results of pot experiments. Whereas responses in irradiated soil from one site showed a close similarity to the field results, those in unsterile soil did not. Plants in irradiated soil from the unresponsive site also displayed larger endophyte-phosphate interactions than those grown in unsterile soil in pots or in the field. The experiments also reveal differences in the activity of native endophytes between sites. (Hayman and Hampson)

Legume nodulation

Rothamsted *Rhizobium* Collection. In the 2 years from January 1977, 547 cultures have been dispatched in response to 151 requests, 64 from overseas. In addition, 21 bags (140 g size) of peat inoculant have been sent out for use in field trials. Plant testing of strains for symbiotic activity is continuing. A new edition of the '*Catalogue of Strains*' has been prepared and will be available from January 1979. (Dye)

Until 1965 Allen and Hanburys manufactured most inoculants used in the UK and their quality was monitored at Rothamsted. Since then inoculants have been imported by seed merchants and Rothamsted lost control over the quality of the products used by growers. Arrangements have now been made for the testing of stocks held by the UK distributors of inoculants obtained from reputable companies in Australia and America under a scheme offered by ADAS. The department has undertaken the work until ADAS sets up the necessary procedures. Twenty-one packets of inoculant have been tested; all those within their expiry date being of adequate quality (i.e. containing more than 10^8 effective rhizobia g^{-1}). The methods and standards used are based on those employed by the Australian Inoculant Research and Control Service. (Dye and Roughley)

Large bodies of *Rhizobium*. Amino acids affect *Rhizobium* cells differentially according to their concentration and type and may cause inhibition of growth, cell-wall distortion or the formation of spherical large bodies with volumes many times greater than that of normal cells. Large bodies of this kind occur in other bacterial genera, either spontaneously or in response to chemical stimuli and if they are deficient enough in cell wall may represent the first stage of L-phase growth, characterised by pleomorphy, very small colonies and viable filter-passing units. The possible importance of the latter in some infective processes has been recognised.

A mixture of valine (0·7 g), isoleucine (0·7 g), arginine (0·4 g), methionine (0·3 g), and proline (1·1 g) produced large bodies reliably in *Rhizobium trifolii* TA1 when used as a 2% solution in an assay cylinder on a surface-inoculated plate of yeast extract-mannitol agar (YMA). Large bodies towards the edge of the inhibition zone were most abundant where the total amino acid concentrations were 0·05–0·8%. Individual amino acids, especially valine, also induce large bodies though these differ morphologically from those produced by the mixture.

Proliferation of large bodies and distorted cells to form microscopic colonies of

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similar cells has taken place in soft agar medium (YMA with 0·5–0·8% of agar) containing 10% of horse serum, when incubated in air enriched with 10% CO_2. The colonies contain many pleomorphic elements characteristic of true L-phase growth but these are not lysed in hypotonic solution indicating that too much cell wall remains for true L-phase growth to occur. Lysozyme, 2 mg ml^{-1} in 4% EDTA, pH 6·5, lysed the large bodies produced by the amino acid mixture without apparently affecting the cell walls of normal cells of TA1. (Skinner and Roughley)

Infection and nodulation of *Stylosanthes capitata* and *S. hamata*. *Stylosanthes capitata* is a potentially important forage legume in very acid soils of the dry tropics and *S. hamata* in similar regions but with soils of higher pH. The isolation of rhizobia from nodules of *S. capitata* and the induction of nodulation are difficult and merit investigation. Seedlings of two introductions of *S. hamata* and one of *S. capitata* were grown with their roots enclosed in test tubes on N-free agar at pH 4·5, 6·0 and 7·2; root temperature was maintained at 30°C. *S. hamata* was inoculated with two *Rhizobium* strains from a moderately acid soil (pH 5·5–6·0) and an alkaline soil (pH 8·0–8·5) and *S. capitata* with one strain from a very acid soil (pH 4·2–4·8).

Nodulation of *S. hamata* began at all pHs 8 days after sowing inoculated seedlings irrespective of the origin of the *Rhizobium* strain. *S. capitata* nodulated poorly and late at all pHs. Nodules were first initiated after 3 weeks at pH 4·5 and after 4 weeks at pH 6·0; at pH 7·2 no nodules had formed by 6 weeks.

Inoculated roots of *S. capitata*, but not uninoculated roots, characteristically formed fluffy, structureless outgrowths in the axils of the secondary roots, most abundantly in the middle zone. Nodules appeared to arise only from within these outgrowths but not all outgrowths were sites for nodule formation. No similar outgrowths formed on *S. hamata*. These structures and the details of the infection process are being further investigated. (Date, Chandler and Roughley)

The nodulation of soyabean cv. Malayan by cowpea rhizobia. It has been long known that cowpea rhizobia will nodulate some cultivars of soyabean. Recently, Dr. Pulver, International Institute of Tropical Agriculture, Nigeria observed that the cultivar Malayan nodulated with indigenous cowpea strains which failed to nodulate the more productive American cultivars. We are examining the compatibility of the Malayan cultivar with cowpea strains, in competition with *Rhizobium japonicum*.

Of the nine cowpea type strains from Nigeria and elsewhere three did not form nodules and a further two did not nodulate all plants of the Malayan cultivar. No generalisations were possible as to the likelihood of a strain from a particular host being infective, e.g. of two strains from *Centrosema* one nodulated and the other failed to nodulate Malayan. Malayan soyabeans nodulated with all eight strains of *R. japonicum* of widely differing origins. All strains which formed nodules also fixed nitrogen.

The nodulation of Malayan by *R. japonicum* CB1809 and cowpea strain CB1024 was studied at temperatures between 24° and 39°C. The soyabean strain CB1809, nodulated all Malayan plants between 24° and 36° whereas nodulation by CB1024 was sporadic; only 50% of the plants nodulated within the range 24°–33°C. When inoculated with equal numbers of these two strains, CB1809 formed all the nodules. Preferential nodulation by the effective soyabean strain was also shown in a field trial at Woburn where the soil was heavily inoculated with a mixture of 11 cowpea-type isolates from Nigeria and the seed inoculated with CB1809. Eighty-five percent of the nodules were formed by CB1809. (Roughley, Chandler, Day and Bromfield)

Nodulation and N-fixation by the field bean (*Vicia faba*). *Vicia faba* was sown with a

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Stanhay drill to give populations of 3, 4, 5 and 6 $\times$ 10^5 plants ha^{-1}. Establishment was however, extremely variable (see Field Experiment Section, McEwan, p. 117) and this invalidated comparisons between populations. Plant establishment with and without pathogen control was similar. There was no significant effect of pathogen control on dry matter accumulation or yield of beans, but as last year, there was a significant increase in post-flowering nitrogenase activity. The apparent anomaly between the lack of response in total dry matter accumulation and increases in nitrogenase activity will be investigated in 1979 using ^{15}N. (Roughley, Day and Gill)

In previous experiments with *V. faba* the seasonal pattern of nitrogenase activity showed at least two peaks. An experiment was done to determine whether this was an effect of soil moisture or due to the host's ontogenetic development. Irrigated and non-irrigated precision-sown (600 000 plants ha^{-1}) populations of *V. faba* receiving 0, 50 and 150 kg N ha^{-1} were sampled at weekly intervals throughout the growing season, for dry matter accumulation and nitrogenase activity.

Soil moisture was generally high and 25 mm of irrigation was necessary only on 19th July at early podfill; this had no significant effect on dry matter accumulation, nitrogenase activity or final yield. Irrespective of nitrogen fertiliser nitrogenase showed three peaks of activity at 9–10, 13–15 and 19–20 weeks from sowing which were not related to soil moisture.

Heavy rain followed the application of the nitrogenous fertiliser and nodule development and nitrogenase activity at the first sampling (8 June, 9 weeks from sowing) was not significantly affected by the fertiliser. Nitrogenase activity was however significantly reduced by 150 kg N ha^{-1} from 10 to 15 weeks after sowing but not thereafter; 50 kg N ha^{-1} affected nitrogenase activity only on the sample taken on 16 June. Top dry weight was significantly increased by applied N up to 17 weeks after sowing but not thereafter.

Nitrogen increased yield from 6·8 t ha^{-1} to 8·2 t ha^{-1} but this effect was in part due to absence of severe lodging in plants given nitrogenous fertiliser; those without had thinner stems. (Day, Roughley and White)

Ureides (allantoin and allantoic acid) in xylem exudate as a measure of nitrogen fixation in legumes. Pate *et al.*, *Planta* (1974), **120,** 229–243, reported that allantoin is a major compound for the transport of nitrogen in some sub-tropical grain legumes; its occurrence is restricted to nodulated plants (Matsumuto *et al.*, *Plant and Cell Physiology* (1977) **18,** 353–357). This suggested that its analysis may provide a quantitive assay for nitrogen fixation. The nitrogenous components of xylem exudate, which carries the products of N-fixation, include NO_3^- from soil and fertiliser, ureides, amides and amino acids, arising from nitrate reduction or fixation. In those species with negligible nitrate reductase in their roots the NO_3^- represents nitrogen from soil and fertiliser, the other components being products of fixation. In other species with appreciable nitrate reductase in their roots the amide and amino compounds arising from nitrate reductase or fixation can be apportioned using ^{15}N fertiliser. (Day and Roughley)

Nodulated roots of *Vigna unguiculata* and *Phaseolus vulgaris* were exposed to $^{15}N_2$ in a gas-tight incubation chamber with a circulating gas phase. The aerial part of the plants were excluded from the incubation vessel with a two part lid sealed around the stem. The apparatus was flushed with CO_2 and 20 % O_2 and $^{15}N_2$ introduced, without evacuation, by absorbing the CO_2 on sodalime. The $^{15}N_2$ was recovered quantitively and re-used (for full details see Witty and Day, *International Atomic Energy Agency, Technical Report Series*, 1979).

After overnight exposure to $^{15}N_2$ the plant top was removed and xylem exudate collected. The exudate was fractionated into allantoin and allantoic acid, total amide

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and amino-N and NO_3^--N. Determinations of isotopic ratio showed the highest level of labelling in the allantoin and allantoic acid fraction. (Day and Witty)

Glasshouse grown *Vigna unguiculata* and *Phaseolus vulgaris* inoculated with effective strains of *Rhizobium* were harvested at 3-hourly intervals over a complete diurnal cycle. At each harvest nitrogenase activity was determined by acetylene reduction, and the xylem exudate was analysed for allantoin and allantoic acid (ureide) content. Nitrogenase activity was considerably higher at night than during the day. This diurnal nitrogenase profile, the inverse of those previously published, was confirmed at controlled root temperatures and shown to be a function of root temperatures above those optimum for nitrogenase activity.

The volume of xylem exudate in both species increased rapidly after daybreak reaching a maximum soon after midday and then declined to zero for the 2 h period before dawn. The concentration of ureide was inversely related to the quantity of exudate collected, exceeding 1000 ppm N at night but falling to less than 400 ppm N when the volume of exudate was greatest. Total ureide increased throughout the morning and was greatest during the period of maximum exudate flux and declined progressively during the night as exudate flow diminished to zero. At this time nitrogenase activity attained a maximum indicating that ureides formed during the night are stored either within the nodule or root and then transported to the aerial parts of the plant when transpiration recommences. Thus there exists no correlation between nitrogenase activity and ureide content in the xylem exudate; acetylene reduction giving an indirect measure of current enzyme activity whereas ureide content of the xylem exudate measures the flux of fixed —N which may either be from current fixation or from stored nitrogen resulting from earlier fixation. (Day and Roughley)

The effect of fertiliser nitrogen on ureides and nitrate content in xylem exudate was examined in *Vigna unguiculata* and *Phaseolus vulgaris*, grown in a mixture of sand and grit in pots in a greenhouse at 25° to 32°C day and 18°C night. Plants were watered to excess at least twice daily with nutrient solutions (Summerfield, Huxley & Minchin, *Experimental Agriculture* (1977) **13,** 81–92) prepared with tap water (*c.* 7 ppm N) and supplemented with either 25, 50 and 75 ppm N.

Plants supplied with combined nitrogen made rapid growth up to flowering but senesced earlier and had low harvest indices. Plants using fixed nitrogen remained green and continued to fix until late pod-fill. They had higher harvest indices and yields were not significantly different from those given combined nitrogen.

Xylem exudate was collected and nitrogenase activity determined at weekly intervals. With increasing levels of N the concentration of ureides (allantoin and allantoic acid) decreased and that of NO_3^- increased in both hosts. In *Phaseolus* there was little fixation or ureide transported at 75 ppm N whereas in *Vigna* both were still considerable at 75 ppm; about equalling those with *Phaseolus* at 50 ppm. The exudate concentration of NO_3^- in both species was greater than that of the nutrient solution. The concentration of NO_3^- in the xylem exudate of *Phaseolus* was always greater than that of *Vigna*.

Although the relationship between C_2H_2 reduction and amount of ureide transported is not simple for reasons given above, the general profiles of total ureide transported and C_2H_2 reduction are similar. (Day and Roughley)

Breeding for high symbiotic effectiveness in red clover. Data from crossing programmes reported earlier (involving more than 8000 plants in more than 1000 families) have now been examined by modified diallel analysis using regression in Genstat. This has established highly significant effects of selection in two independent programmes using *Rhizobium trifolii* strain 0403 (moderately effective in fixing nitrogen) and strain 5 (highly effective), leading to average yield increases over the original cultivar in the

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second generations of 16 and 34% and in the third generations of 56 and 55% respectively. Smaller increases were found in the first generation crosses and these were related to the size of the population of plants from which selections were made and the intensity of the selection procedures; crosses between cultivars showed heterosis. Breeding from plants with yields equal to the mode of the cultivar's response also increased yield (by 8 and 30% in the third generations of the two programmes) whereas the progeny of plants of poor effectiveness yielded less than the controls. Within each cross type there remained highly significant parental effects in most of the variates measured – viz. primary nodulation time, nodule number, leaf area and yield; high yield was correlated with early nodulation. (Nutman, with Riley, Statistics Department)

Plants selected and bred for high effectiveness with *Rhizobium trifolii* strains 0403 or 5 were examined in Saxcil cabinets which provided either long days (16 h) or short days (10 h) and day/night temperatures of 16/11°C, 22/17°C or 27/22°C. Plants were either inoculated at sowing or after a delay of 18 days. Under all conditions the highly effective selections fixed more nitrogen and out-yielded the original cultivar but most strikingly in long days at the optimum temperature regime of 22/17°C. Smallest yields were obtained at 16/11°C and delayed inoculation depressed yield in all comparisons. Treatment effects were also found in leaf area, number of branches and petiole length. Highly effective lines are known to flower earlier than the original cultivar (*Rothamsted Report for 1976*, Part 1, 291) but dissection of mainstem primordia at 60 days showed no effect of treatment on progress towards flowering. These results show that the heritable factors for high symbiotic effectiveness are relatively insensitive to day length or to stress imposed by temperature or inoculation delay and that the highly effective phenotype is independent of conditions that allow flower formation. Crosses were made to investigate further the connection between flowering and the symbiotic response. (Nutman and Poonam Rao)

Staff and Visiting Workers

Courses on mycorrhiza were taught by Barbara Mosse at the School of Tropical Agriculture, Hawaii and by B. Mosse and D. S. Hayman at the Agronomic Institute Campinas, São Paulo, Brazil and at the Forest Products Research Institute, Kumasi, Ghana (under the auspices respectively of the British Council and the International Federation for Science). Margaret Brown participated in an International Symposium at the University of Oxford and presented a paper and also attended the 11th International Congress of Soil Science, Edmonton, Canada. F. A. Skinner went as visiting professor to the Soil and Water Sciences Department, University of Alexandria, Egypt. R. J. Roughley visited microbiological laboratories at Moscow and Leningrad, USSR, under the ASCAR agreement, and at Prague, Czechoslovakia; he also made two short visits to IITA to assess programmes and discuss collaborative work. J. F. Witty attended the Steenbock Kettering Symposium, Madison, Wisconsin and also IAEA in Vienna to discuss the use of isotopes in N fixation.

Visiting workers included Dr. P. de Sousa, Brazil; Dr. H. G. Diem, Senegal; Dr. R. Date, Australia and Mr. K. A. Raja, Pakistan.

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