ROTHAMSTED RESEARCH

Report for 1980 - Part 1

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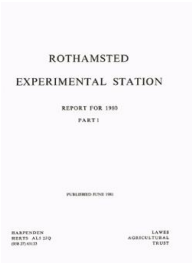

Soil Microbiology Department

J. E. Beringer

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SOIL MICROBIOLOGY DEPARTMENT

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Introduction

The Department has concentrated on studies of VA mycorrhizas and the *Rhizobium* legume symbiosis. Work on the microbiology of take-all disease and developing methods for handling soil micro-organisms has continued. Our inability to culture mycorrhizal fungi in the absence of growing plant roots continues to hamper research on these fungi. Effort is being devoted to finding suitable methods for producing large amounts of mycelium in axenic culture on host plant roots and to find methods for obtaining growth of the fungi in pure culture.

Much of the work on *Rhizobium* has been on studies of *Rhizobium phaseoli* and its symbiosis with the navy bean (*Phaseolus vulgaris*). This may become an important commercial dry bean crop in the UK within a few years and we hope to have suitable strains and techniques available for inoculating this crop. Encouraging responses to inoculation continue to be obtained. Methods for characterising rhizobia so that different strains can be identified in the field continue.

The Department has received grants from the ODA and NRDC to start in the winter of 1980/81 and to continue for 3 years. The ODA grant is to study the rhizobia which nodulate peanuts, chickpeas and pigeon peas in collaboration with Dr P. J. Dart at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) (India). The work will involve studies of strain recognition, competition and inoculation techniques. The NRDC grant is to produce improved strains of *Rhizobium* and VA mycorrhizas for inoculation purposes. It will involve the isolation and screening of micro-organisms on plants growing on soils which are low in indigenous endophytes, such as reclaimed gravel pits and acid upland pastures.

Vesicular–arbuscular Mycorrhiza

Field inoculation studies: Red clover, Sawyers I. The experiment set up in 1979 to compare the effects of inoculation with the three endophytes *Glomus caledonius* (a laminate spore

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type), *Glomus fasciculatus* 'E3', *Gigaspora margarita* and no inoculation at three levels of phosphate was continued. The growth response to added phosphate but not to inoculation, recorded in 1979, did not correlate with any increased P concentration in the shoot tissues which remained constant at around 0·2% in all treatments. Root samples taken in late 1979 showed a negative effect of phosphate on infection in both inoculated and uninoculated plants: *c.* 70% with no added P to *c.* 17% at the highest P level. Dry weights of plants harvested in midsummer 1980 were not affected by inoculation with any endophyte but were almost doubled by either level of added P. A second harvest in late autumn showed even bigger increases but no clear differences between any of the 12 treatments. Total dry matter production for 1980 ranged from 1200 to 1700 g m^{-2} (an average of 15 t ha^{-1}). This high yield, even where no superphosphate was added, is surprising for a soil containing only about 10 ppm Olsen P. Preliminary observations attribute these large yields to overall infection by *G. caledonius* because (1) resting spores of *G. caledonius* were found in abundance not only in the *G. caledonius*-inoculated plots, but also in the non-inoculated controls and in plots inoculated with *G. margarita* and 'E3', (2) mycorrhizal infection was consistently inversely correlated with the amount of phosphate added, suggesting one compensated for the other, and (3) lucerne responded to inoculation with *G. caledonius* in the presence of native endophytes at this site.

This experiment shows that in field comparisons of endophytes their ability to spread and infect in soil may be as important as their symbiotic efficiency and should be screened for when selecting inoculants. It also raises the useful possibility of replacing comprehensive inoculation of a perennial crop by establishment of infection centres from which rapid spread of introduced mycorrhizas can occur. (Hayman, Page and Clarke)

Spread of VA mycorrhiza inoculum in Sawyers I. Spread and residual growth effects of the 1978 field inoculation trial (Owusu-Bennoah & Mosse, *Rothamsted Report for 1978*, Part 1, 237–238), were monitored. Twenty-one months after inoculation a remarkable build up of spores of *G. caledonius* and sporocarps of *G. mosseae* had occurred throughout the plot. Again spore numbers were equally high on inoculated and uninoculated sites. They were much higher than those normally associated with agricultural soils and thus were comparable to those generally found in pot cultures in sterile soil. There was no longer a visible effect of inoculation on growth, and no significant differences between shoot dry weight of inoculated or uninoculated rows. (Mosse, Warner and Clarke)

Use of NFT roots as inoculum in Sawyers I. The ability of VA mycorrhizal roots grown under nutrient film technique (NFT) to infect red clover in the field was tested. Fresh NFT grown roots of *Phaseolus vulgaris* cv. Jamapa, infected with E3, were applied at 6 and 42 g m^{-2} below the seed. Air dried infected roots, stored at room temperature for 5 months, were applied at 4·2 g m^{-2}. Controls were: (1) no inoculation, and (2) inoculation with standard pot grown material. Harvests taken 4 and 7 weeks after sowing showed that inoculation with fresh NFT-grown roots gave almost as much infection (25% for 42 g; 20% for 6 g at first harvest) as pot-grown inoculum (30%). The small difference in infection between the two quantities of NFT inoculum indicates that satisfactory infection might be obtained with smaller quantities of roots. Uninoculated controls had a low level of infection (8%) produced by an indigenous fine endophyte. Air-dried, stored roots gave little infection (10%). By 12 weeks after sowing growth differences were observed between the fresh inoculated and uninoculated roots. (Elmes, Hayman, Hepper and O'Shea)

White clover in Welsh uplands. Experiments to test the feasibility of inoculating white clover with selected mycorrhizal fungi to improve its establishment in hill grasslands

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continue at Pwllpeiran EHF in cooperation with ADAS, Trawsgoed. At the formerly responsive site 1, seedlings pre-infected with *Glomus fasciculatus* 'E3', *Glomus mosseae*, *Acaulospora laevis*, *Gigaspora margarita* and uninfected controls were compared in plots given 90 kg P ha^{-1} basic slag, rock phosphate, superphosphate or 1:1 rock:superphosphate, or given no phosphate. Plants grew poorly in all treatments because of the unusually cold, wet summer, but dry matter production was increased considerably by inoculation with 'E3' or by the addition of superphosphate. Plants given both superphosphate and inoculated with either 'E3' or *G. mosseae* grew best. In two other experiments in phosphate-amended plots widely distributed within site 1, seed inoculation with 'E3' and *G. mosseae* together had little or even a negative effect on seedling growth, especially in the wetter areas. (Hayman)

Glasshouse studies

Glasshouse trials on mycorrhizal inoculation of white clover. The same inoculation and phosphate treatments tested in the field were compared in pots of sterilised (γ-irradiation) or unsterile peat from site 1 (Welsh uplands) limed to just over pH7. Mycorrhizal infection established most rapidly with *Glomus mosseae*. Large growth responses to both *G. mosseae* and *G. fasciculatus* 'E3' were clearly visible by 5 weeks and were larger than the responses to added phosphate. When the plants were harvested at 10 weeks from the sterilised peat, *G. mosseae* had increased dry matter production over the controls by nearly 20-fold, 'E3' and *Acaulospora laevis* by around ten-fold, and *Gigaspora margarita* had no effect, whereas superphosphate (SP) increased growth four-fold, basic slag (BS) three-fold and rock phosphate (RP) very little. *G. mosseae* or 'E3', combined with either BS or SP, stimulated most growth. Plants grew up to twice as big in unsterile peat as in sterilised peat, but again controls grew poorly compared to plants inoculated with *G. mosseae*, 'E3' or *A. laevis*. *G. mosseae* plus BS increased dry matter production 40-fold over the uninoculated plants not given phosphate. Percentage P was highest in plants inoculated with 'E3' or *G. mosseae*. These results contrast with those obtained for unsterile site 4 (Welsh uplands) peat in both pot and field experiments (*Rothamsted Report for 1978*, Part 1, 238–239) where plants grew well irrespective of inoculation or phosphate treatment. Therefore an additional pot experiment was conceived to test whether differences in native endophyte populations between sites 1 and 4 were partly responsible. Inoculum from site 4 stimulated growth nearly 20-fold, compared to three-fold with SP, and also doubled shoot %P. Clearly the endophyte or endophytes indigenous to site 4 are far better symbionts than those indigenous to site 1. We are isolating them for use in future inoculation programmes. (Hayman and Page)

***Endophyte trial with* Glomus epigaeus.** *Glomus epigaeus* (*Canadian Journal of Botany* (1979) **57**, 539) supplied by Dr Barbara Daniels, has been introduced into the Rothamsted mycorrhizal culture collection. Because it forms large sporocarps on the surface of the soil it may be convenient for experimental use. It was tested in a pot trial in a temperate glasshouse with five other mycorrhizal fungi using onions as host plants in irradiated Rothamsted Barnfield soil (pH 6·8, 21 ppm Olsen P). Plants were harvested after 4 months. The mean shoot dry weights of *Glomus epigaeus* and YV (*G. mosseae*) inoculated plants were very similar and showed a 10-fold increase over the controls. E3 (*G. fasciculatus*) and *Glomus* sp. (isolated from Ashridge soil) inoculated plants both showed an 8-fold increase. The other two endophytes, a honey-colour spore type (*Acaulospora laevis*) and a large Brazilian species resembling *Glomus clarus* failed to infect. (Clarke)

Nutrient film technique. Pre-infection of plants grown in sand and grit, with P supplied as rock phosphate and other nutrients in dilute solution, gave good development of infection

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after transfer to the channels. In maize, Ca at 75 mg litre^{-1} gave better infection than 15 mg litre^{-1} or 150 mg litre^{-1}. The high level was probably approaching toxicity. The superior infection at 75 mg litre^{-1} may have been due to an interaction with rock phosphate. Solution analyses showed that P levels are lower with Ca at 75 mg litre^{-1} than 15 mg litre^{-1}.

Experiments with maize and clover emphasised the effects of P concentration on infection and the importance of pH on the solubilisation of rock phosphate. With N in the form of NH_4^+ the pH of the solution fell between daily adjustments and as a result the concentration of P in solution was raised and infection was poor. With 95% NO_3^-: 5% NH_4^+ the pH remained relatively constant and infection was satisfactory. In clover more infection was obtained with 95% NO_3: 5% NH_4^+ than when the plants obtained N by nitrogen fixation. With symbiotic N fixation the pH dropped causing a consequent solubilisation of rock phosphate. (Elmes, Mosse and O'Shea)

Axenic NFT for mycorrhizal plants. An axenic NFT system appeared to offer the possibility of producing relatively large quantities of VA mycorrhiza infected roots and external mycelium free of microbial contamination. Essentially a scaled down version of the non-axenic system was used with the plants growing in a PVC channel and the solution held in a 10 litre fermentor and circulated by a peristaltic pump. The channel was sterilised by sealing in an airtight container with formaldehyde then placed in a small laminar flow cabinet or in a modification of an isolator used for producing germ-free laboratory animals. Spread of infection in the channel and external mycelium production were unreliable although one plant became significantly infected and produced *c.* 6 mg fresh weight of external mycelium. It has been possible to maintain sterility for the duration of an experiment but so far without substantial spread of infection. The strong air stream affected the plants adversely and caused drying and salt accumulation on the upper roots. This, and possibly too high a concentration of P in solution, may have affected adversely the infection. To overcome the latter rock phosphate was sealed in a cloth bag with a stirrer bar so that it could be removed from the solution with a magnet. (Elmes, Hepper and Maskall)

Laboratory studies

Effect of calcium on VA mycorrhizal infection. Calcium is known to influence the infection of plants growing under NFT conditions (Elmes & Mosse, see above). The effect of calcium (5–75 mg litre^{-1}) was tested on the infection of *Trifolium repens* growing axenically on filter paper moistened with plant nutrient solution and inoculated with spores of *Glomus mosseae*. Increasing calcium levels stimulated root infection with a corresponding increase in the amount of external mycelium. Root hair length and shoot dry matter were not affected. (Hepper)

***Limited independent growth of* Glomus caledonius.** Although it has previously been found on agar that the hyphae of VA mycorrhizal endophytes cannot grow unless they are attached to their parent resting spore or to a living host root, it has now been possible to obtain growth, both by tip elongation and branching, from detached hyphae in agar media. This growth is stimulated by the presence of crushed resting spores and is dependent on the medium. The best mean figure obtained was a 4-fold increase. (Hepper)

Biochemical studies of the VA endophyte. A preliminary investigation of the metabolism of the endophyte *Glomus mosseae* (yellow vacuolate spore type) was performed using non-axenic external mycelium detached from roots of clover (*Trifolium repens*) grown by NFT.

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The distribution of the total radioactivity in the soluble compounds extracted from external mycelium incubated with ^{14}C-glucose between neutral sugars, amino acids, organic acids and nucelotides was similar for 2 and 4 h incubations. The proportion of the total radioactivity taken up which was present in the insoluble fraction (proteins and polysaccharides) was greater for the 4 h incubations. Measurements of this type may reveal changes in the metabolism of detached mycelium as it ages, and thus give clues to the nature of the dependence of the endophyte on the host plant. (Maskall, with Burrell, Insecticides and Fungicides Department)

'Active' mycorrhizal infection of lettuce. Differences in 'active' and total ('active' plus 'non-active') infection levels were investigated in lettuce that had been inoculated with *Glomus mosseae* (YV) alone or jointly with YV and either a 14-day-old *Azotobacter chroococcum* culture, or a cell-free culture filtrate. Infection levels were determined as a percentage of root length. Responses to inoculation were assessed after 36 days growth in steamed Delharding soil. 'Active' infection of the roots was measured following staining with nitro-blue tetrazolium for succinate dehydrogenase activity, and total infection after restaining with trypan blue. Presence of either *Azotobacter* or culture filtrate decreased the percentage root length showing 'active' infection. Proportions of active to total hyphae remained unchanged. Analysis showed root lengths of plants inoculated with *Azotobacter* plus YV did not differ significantly from those inoculated with YV alone, indicating decreases in percentage infection to be real. However, plants inoculated with the culture filtrate plus YV had significantly longer roots but no real decrease in actual length of root infected, thus decreases in percentage infection were artificial. The results suggest that the *Azotobacter* was either limiting infection by (a) physical/chemical exclusion from the root surface, (b) reducing the amounts and types of plant produced nutrients available to the endophyte, or both.

Rectilinear regression analysis of YV inoculated plants showed shoot dry weight to be a function of percentage 'active' infection ($r=0{\cdot}70$) but not of total infection ($r=0{\cdot}35$). Similar regressions of shoot dry weight on percentage 'active' infection were obtained from plants inoculated with YV and either *Azotobacter* or culture filtrate. Extrapolation suggests that 20–25% 'active' infection was necessary to produce significant increases in shoot dry weight over non-mycorrhizal plants. (Carr)

Vesicular-arbuscular mycorrhizas. An autoclavable hydroponic culture system has been developed and is being used for production of axenic VA mycorrhizas in *Trifolium parviflorum* from single spore inocula of *Glomus caledonius*. (Macdonald)

Electron microscope studies with VA mycorrhiza. Electron microscopy of ultra-thin sections of the mycorrhiza *Glomus caledonius*/*Trifolium parviflorum* and spores of *G. caledonius* has revealed the presence of bacterium-like organelles within the fungal spores, intercellular hyphae and in the thicker arbuscule branches. Colonisation and partial penetration of the spore wall of *G. caledonius* by bacteria has also been demonstrated. (Macdonald and Chandler)

A hitherto undescribed hyphal repair mechanism has been observed in germ tubes and hyphae arising from reproductive spores of *G. caledonius*. After excision of the hyphal apex, a septum is layed down across the hypha; the cytoplasm between the septum and apex is sacrificed and a new intrahyphal hypha grows from the septum, eventually emerging at the site of the excised apex. This repair is often accompanied by the production of regions of multiple branching similar to those which occur early after normal spore germination. (Macdonald)

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Legume inoculation studies

Laboratory studies on *Rhizobium*

***Long-term storage of* Rhizobium.** Investigation of factors affecting the 'shelf-life' of freeze-dried *Rhizobium* (*Rothamsted Report for 1979*, Part 1, 190) has continued. Loss of viability during the drying process, with strains 2001 and 3824, was independent of the growth phase and cell density of the inoculum (over a 1000-fold range). Three out of five strains tested survived initial drying in sucrose peptone medium (SPM) significantly better than in dextran sucrose glutamate (DSG), a commonly used suspending medium.

The half-lives of four freeze-dried strains stored at 4°C were estimated to range from 20 to 380 years. No significant difference was found between those dried in SPM and DSG.

No changes were detected in the symbiotic characteristics of these cultures due to the high temperatures used in the storage tests. (Dye)

***Rothamsted* Rhizobium *Collection*.** In the 2 years from January 1979, 576 cultures have been dispatched in response to 168 requests, 67 from overseas. In addition, 15 bags of peat inoculant have been provided for use in field trials outside Rothamsted.

Approximately 150 copies of the new edition of the *Catalogue of Strains* have been distributed since publication at the beginning of 1979. Revision and reprinting of the *Catalogue* will be carried out early in 1981. (Dye and Taylor)

Field studies

***Inoculation methods for* Phaseolus vulgaris.** Although inoculation consistently increases yields of beans at both Woburn and the NVRS Wellesbourne the standard method of inoculation by application of peat-based inoculants to the seed is not satisfactory in commercial practice. *Phaseolus vulgaris* seed is often treated with fungicides which are toxic to *Rhizobium phaseoli*. Alternative methods of inoculation compatible with treated seed are necessary before inoculation will be acceptable to farmers. In joint experiments performed at the NVRS both liquid inoculant sprayed into the planting furrows and granular inoculants combined drilled with the seeds appeared to be satisfactory alternatives to the standard inoculation method. (Day and Ewens, with Dr J. D. Taylor and Mr C. L. Dudley, National Vegetable Research Station)

Multisite inoculation trials. Yield increases following inoculation have been obtained over several years at sites with no or low populations of *R. phaseoli*. To assess the extent to which inoculation is necessary throughout the commercial bean growing areas, a series of trials involving inoculation and differenct levels of N fertiliser were set up jointly with the NVRS and ADAS. Growth conditions were poor and yields low; responses to inoculation and N fertiliser were small. Inoculation gave significant yield increases in only two experiments, but at all sites yields of inoculated plants not receiving N fertiliser were equal to or greater than plants receiving 120 kg of fertiliser N. (Day, with Dr J. D. Taylor and Mr C. L. Dudley, National Vegetable Research Station, Mr M. Tuckwell and Mr P. Richard, ADAS Cambridge, and Mr I. Clarke, ADAS Ipswich)

Rhizobium phaseoli *strain selection*. The selection of improved strains of *R. phaseoli* for inoculation of *Phaseolus vulgaris* continued. As part of this programme *Rhizobium* strains for the International Bean Inoculation Trial distributed by the Centro Internacional de Agricultura Tropical, Colombia (CIAT), were tested at Woburn using a dry bean cultivar Longbow. One of the ten CIAT strains performed significantly better and three others were equal to or slightly better than our best local strains. Although the season was poor, plants inoculated with the best strain, CIAT 904, yielded approximately

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2000 kg ha^{-1} without any nitrogenous fertiliser and outyielded control plants receiving 120 kg N as fertiliser. (Day and Ewens)

Estimation of nitrogen fixation in the field. ^{15}N-labelled fertiliser has been used in field studies to evaluate N_2-fixation. The size of the soil-N pool can be calculated by isotope dilution using a non-fixing control. N_2-fixation can be calculated if the proportion of this soil-N taken up by a legume crop is assumed to be the same as the proportion of labelled fertiliser taken up (*Rothamsted Report for 1979*, Part 1, 191). It is implicit in the calculation that the control and fixing plant take up N from the soil at an isotope ratio which is proportional to the amount of ^{15}N added. Field experiments show that the isotopic enrichment of nitrogen taken up by control plants declines rapidly with time after the addition of a readily available ^{15}N salt and the correct proportionality only occurs when the N-uptake profiles of the control and N_2-fixing crops are the same.

It is seldom possible to exactly fit control to legume, but the effect of mis-matched N-uptake profiles is reduced when the $^{15}N/^{14}N$ ratio in the soil is stable.

The use of slow release ^{15}N-fertiliser formulations (oxamide, *Gypsum* pelleted ammonium sulphate, bacterial protein, ground plant material and glucose/ammonium sulphate) was investigated in pot experiments. Results showed that estimates of fixation relative to different controls are more consistent when slow release formulations are used. Field experiments using these compounds will continue. (Witty and Ritz)

Foliar feed experiments. Foliar application of N, P, K and S to *Vicia faba* significantly reduced yield in 1979 (*Rothamsted Report for 1979*, Part 1, 191). This effect was attributed to an interaction between leaf burning and extremely low soil moisture. The experiment was repeated in 1980 with a provision for irrigation. There was considerable rainfall over the spraying period so that irrigation was not required. Despite adequate soil moisture the foliar application of N, P, K and S had no significant effect on yield. (Witty, Day and Ewens)

General studies

Microbiology of wheat roots infected with take-all disease. The study on the microbiology of roots infected with take-all taken from winter wheat grown in phased sequences in Little Knott field, Rothamsted, was completed. The microbiological findings were interpreted in terms of the number of consecutive wheat crops grown, the amount of disease on the roots and infectivity of soil samples. Results depended entirely on the source of the experimental material and interpretation of data differed with age of plant and the sequence in the monoculture from which plants or soil came. Relationships were considered between the aerobic bacteria, *Pseudomonas* species and bacteria inhibitory to growth of *Gaeumannomyces graminis* var. *tritici* on agar found on lesioned tissue and (a) the severity of disease on roots supplying the lesions and (b) the infection produced on axenic seedlings inoculated with the lesioned tissue. Aerobic bacteria on lesions from tillering and mature plants were positively correlated with disease on the donor roots. Inhibitory bacteria from these same lesions were positively correlated with disease on axenic seedlings. *Pseudomonas* species showed no correlations.

During the years of this study take-all in Little Knott did not develop in the expected manner so that the involvement of specific groups of bacteria in development of take-all decline could not be clearly demonstrated. Therefore the microbiology of infected roots was examined at another site, Highfield, Rothamsted, where large differences in the amounts of disease on roots from first year and fourth year consecutive cereal crops

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were recorded. Wheat plants were sampled in April, June and August 1980 and roots examined for the different bacterial groups. Over the season changes in numbers of aerobic bacteria and the percentage of *Pseudomonas* in the bacterial population generally followed the same patterns as in Little Knott. At no sampling did the percentage of inhibitory bacteria in the population differ between crops though they did in Little Knott.

Axenic wheat seedlings were inoculated with lesioned tissues from roots collected in August and the amount of disease produced was negatively correlated with the percentages of both *Pseudomonas* and inhibitory bacteria. In Little Knott inhibitors were positively correlated. Aerobic bacteria showed no correlations.

New wheat seedlings grown for 3 weeks in soil collected in August, and representing the second and fifth crops, were found to be more diseased than seedlings similarly grown in soil samples collected at the beginning of the season. Axenic seedlings inoculated with lesions from these second and fifth crop plants became infected but no correlations were found between the microflora of the donor lesions and the amount of disease on the axenic seedlings. Thus, like results from Little Knott, those from Highfield still do not demonstrate clear associations between the microflora on the roots and take-all disease. Correlations were found at both sites but they were different for each site. (Brown and Page)

Anatomical studies of take-all lesioned tissue. Staining techniques were used to show active respiratory enzymes in invading hyphae in take-all lesions. Inocula of *Gaeumannomyces graminis* var. *tritici* were placed 3 or 8 cm from the seeds of axenic wheat seedlings grown on mineral salts agar. Whole pieces of root were removed at regular intervals after reaching the inocula and incubated in the enzyme reaction mixture. Root infection was very similar for both placements of inocula, but hyphae from the 3 cm inoculum spread less rapidly through the cortex to the endodermis than those from 8 cm. At first all cortical cells contained active hyphae irrespective of inoculum placement, but later this applied only to the 3 cm position. At 8 cm only cells adjacent to the endodermis contained active hyphae. At 3 cm all invaded cortical cells contained blue formazan deposits, but at 8 cm only cortical cells adjacent to the endodermis were stained. Ultimately more of the root tissue below the inoculum placed at 3 cm was discoloured brown than above the inoculum, but at 8 cm placement the root was more discoloured above the inoculum. Within this discoloured tissue enzymically active hyphae were concentrated in groups in the endodermal region and surrounded by many lignitubers. These groups were linked by a few almost inactive hyphae. There were few lignitubers between the groups.

Thus the progress of infection and host reaction differs slightly with the position of the inoculum in relation to the distance from the seed. (Brown and Page)

Physical studies on soil micro-organisms. The techniques of differential sedimentation and density gradient centrifugation have been applied to soil suspensions for the physical isolation of purified concentrated samples of the non-filamentous component of the soil microflora. Application of the same techniques to crushed nodules from roots of *Lupinus angustifolius* resulted in the isolation and physical fractionation of bacteria and bacteroids. (Macdonald and Martin, West of Scotland Agricultural College) Non-filamentous soil micro-organisms have been isolated from samples of Park Grass soil for studies on plant nutrient (particular P) immobilisation. (Macdonald, with Brookes, Soils and Plant Nutrition Department)

A combination of wet sieving and centrifugation in stepped density gradients has allowed the quantitative recovery of azygospores of the entomogenous fungus *Conidiobolus obscurus* from soil. The method is being used to assess the concentration, distribution

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and persistence of these spores in soil. (Macdonald and Spokes, with Wilding, Entomology Department)

Nitrification. A selective and diagnostic growth medium has been developed to facilitate isolation of pure cultures of ammonia oxidising bacteria. Selectivity depends on the inclusion of antibiotics and the absence of organic materials. Addition of the dye neutral red makes the medium diagnostic for acidogenic colonies which become red; non-acidogenic heterotrophs produce pale orange colonies. (Macdonald and Spokes)

Staff and visiting workers

J. E. Beringer took up his appointment as head of Department in April. Barbara Mosse, F. A. Skinner and N. Walker retired during 1980, each after many years service.

Anne Warner was awarded the degree of Ph.D(London).

During the year J. E. Beringer attended conferences and presented papers in Helsinki (Finland), Lake Tahoe (USA) and Canberra (Australia); Muriel Chandler visited the Max Planck Institute, Cologne (W. Germany); J. M. Day visited IAEA, Vienna (Austria), and ICRISAT, Hyderabad (India); Christine M. Hepper gave a course on VA mycorrhizas at the University of Helsinki (Finland) and F. A. Skinner attended a meeting at FAO, Rome (Italy). Several members of staff attended meetings and presented papers in the UK.

Visitors Mr S. M. Palacios and Mr F. Le Tacon returned home and we welcomed Professor C. A. Parker and Dr I. Jakobsen for short visits. We are grateful for help received from our sandwich students R. Page and K. Ritz. We should also like to acknowledge support from the ODA and NRDC for research on the culture of VA mycorrhizas.

Publications

Thesis

1 Warner, A. (1980) Spread of vesicular-arbuscular mycorrhizal fungi in soil. Ph.D. Thesis, University of London.

General Papers

2 Beringer, J. E. (1981) Finding nitrogen fixation genes. *Nature, London* **289,** 16.

3 Hayman, D. S. (1980) Mycorrhiza and crop production. *Nature, London* **287,** 487–488.

4 Hepper, C. M. & Mosse, B. (1980) Vesicular–arbuscular mycorrhiza in root organ cultures. In: *Tissue culture methods for plant pathologists*. Ed. D. S. Ingram & J. P. Helgeson. Oxford: Blackwell Scientific Publications, pp. 167–171.

5 Munns, D. N. & Mosse, B. (1980) Mineral nutrition of legumes crops. In: *Advances in legume science*. Ed. R. J. Summerfield & A. H. Bunting. London: HMSO, pp. 115–125.

Research Papers

6 McEwen, J., Day, J. M., Roughley, R. J., Witty, J. F. *et al.* (1981) The effects of irrigation, nitrogen fertilizer and the control of pests and pathogens on spring-sown field beans (*Vicia faba* L.) and residual effects on two winter wheat crops. *Journal of Agricultural Science, Cambridge* **96,** 129–150.

https://doi.org/10.23637/ERADOC-1-137

7 MACDONALD, R. M. & SPOKES, J. R. (1980) A selective and diagnostic medium for ammonia oxidising bacteria. *FEMS Microbiology Letters* **8,** 143–145.

8 MACDONALD, R. M. (1980) Cytochemical demonstration of catabolism in soil micro-organisms. *Soil Biology and Biochemistry* **12,** 419–423.

9 OCAMPO, J. A. & HAYMAN, D. S. (1980) Effects of pesticides on mycorrhiza in field-grown barley, maize and potatoes. *Transactions of the British Mycological Society* **74,** 413–416.

10 OCAMPO, J. A. & HAYMAN, D. S. (1981) Influence of plant interactions on vesicular–arbuscular mycorrhizal infections. II. Crop rotations and residual effects of non-host plants. *New Phytologist* **87,** 333–343.

11 WARNER, A. & MOSSE, B. (1980) Independent spread of vesicular–arbuscular mycorrhizal fungi in soil. *Transactions of the British Mycological Society* **74,** 407–410.

12 WITTY, J. F. (1979) Acetylene reduction can overestimate nitrogen fixation in soil. *Soil Biology and Biochemistry* **11,** 209–210.

13 WITTY, J. F., ROUGHLEY, R. J. & DAY, J. M. (1980) Reduction of yield of *Vicia faba* by foliar fertilisation during the seed-filling period. *Journal of Agricultural Science, Cambridge* **94,** 741–743.

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