ROTHAMSTED RESEARCH

Report for 1981 - Part 1

Full Table of Content

Soil Microbiology Department

J. E. Beringer

J. E. Beringer (1982) *Soil Microbiology Department ;* Report For 1981 - Part 1, pp 209 - 218 **- DOI: https://doi.org/10.23637/ERADOC-1-128**

SOIL MICROBIOLOGY DEPARTMENT

J. E. BERINGER

Staff

Head of Department J. E. Beringer, PH.D.

Principal Scientific Officers
Margaret E. Brown, PH.D.
J. M. Day, PH.D.
D. S. Hayman, PH.D.

Senior Scientific Officers
Christine M. Hepper, PH.D.
R. M. Macdonald, PH.D.
J. F. Witty, PH.D.

Higher Scientific Officers
E. S. P. Bromfield, PH.D.
Mrs Muriel Chandler
M. Dye, PH.D.
A. Fyson, PH.D.
Anne Warner, PH.D.

Scientific Officers
Sheila M. Smith, C.D.H.
J. R. Spokes, B.SC.
Mrs Rosemary Taylor, B.SC.

Assistant Scientific Officers
E. Davidson
Mrs Margot H. Ewens
P. Gee
Mrs Daphne V. Gibson
Mrs Carol A. Grace
J. O'Shea
R. Sen

Visiting Scientists
E. Bååth, PH.D.
Li Fu-Di
J. E. Ruiz Sainz, PH.D.
Maria Tavares, PH.D.

Students
H. F. Bitanyi, M.SC.
Michele Stein, B.SC.
S. Wang, M.SC.

Laboratory Attendants
Mrs Audrey Ballard
Mrs Joan Gostick

Personal Secretary
Mrs Pauline V. Mather

Introduction

Studies of symbiotic nitrogen fixation and vesicular-arbuscular mycorrhiza continue to be the primary interests of the Department. Work in both these areas benefits from outside support provided by the ODA and NRDC.

Work on VA mycorrhiza has benefited from the development of a method for producing large amounts of inoculum for field experiments by growing plants in peat blocks in nutrient film culture. This development (in conjunction with Mr L. Dingemans, a nurseryman) should overcome a major limitation to the size and number of field experiments which was imposed by previous methods for producing inoculum.

Studies of biological nitrogen fixation have concentrated on methods for measuring the efficiency of different host-*Rhizobium* combinations and on work related to the assessment of strains under field conditions. The selection and testing of strains that nodulate *Phaseolus* beans continues to yield promising results. Collaborative trials involving ADAS and the NVRS have yet again demonstrated the potential for inoculation and have shown that it should be commercially viable even for french beans. Two field trials were used by ADAS to demonstrate the effects of inoculation to farmers and processors.

Vesicular–arbuscular mycorrhiza

Peas at the John Innes Institute. Subsequent to a pilot experiment showing large responses of semi-leafless peas to mycorrhizal inoculation in sterilised soil (*Rothamsted Report for 1979*, Part 1, 186–187), the effects of mycorrhiza on leafless peas was studied

https://doi.org/10.23637/ERADOC-1-128

in more detail at a dense planting rate. *Glomus mosseae*, a species also found near the experimental site, was tested at five levels of added triple superphosphate (0–145 kg P ha^{-1}). By mid-summer plants showed definite growth responses to both inoculation and phosphate. Mycorrhizal infection in the inoculated and control plants ranged from about 20 to 60% and 1 to 18%, respectively, being least at the high P levels. As the soil had been sterilised nearly 2 years previously, it was evident that there was some re-invasion by mycorrhizal fungi. Dry weights at harvest were fairly similar between treatments. There was no obvious response to phosphate either in haulm weight or seed yield, but plants inoculated with *G. mosseae* were about 10% heavier at nearly all P levels.

Other plants sown less densely in the 1979 plots showed a strong effect from the residual inoculum of *G. mosseae* but little horizontal spread of the endophyte. Plants sown in the previously inoculated plots were much taller than the nearby controls. (Hayman and Grace, with Mr B. Snoad, John Innes Institute)

Maize at Grassland Research Institute. In 1980 maize after oilseed rape (RM) grew poorly with marked phosphate-deficiency symptoms, whereas maize after maize (MM) grew well. Root samples collected in June showed 12% mycorrhizal infection in the RM plots in contrast to 71% in the MM plots. Leaf P content of MM plants was double that of RM plants. The possibility of mycorrhiza affecting maize growth was considered because rape is not a host plant and therefore the indigenous mycorrhizal fungi would be less favoured in soil pre-cropped with rape than in soil supporting the strongly mycorrhizal host-plant maize. This was examined in 1981 with experimental field plots set up in blocks with different cropping histories, viz. MT (maize, then fallow for 6 months, then turnips (non-mycorrhizal species) for 6 months), MM and RM. Inoculation with *G. mosseae* was compared with no inoculation at two phosphate levels.

Growth of maize in June was better in blocks MM and RM than in block MT. However, this effect was reversed by late August when maize plants in MT were nearly twice as tall as those in the other two blocks. Shoot dry weights at harvest in October were about 20% higher in the MT block. There was no clearcut response to either phosphate or mycorrhizal inoculation. The native soil P level (about 50 ppm bicarbonate–soluble P) precluded any large response to P. The lack of growth response to either phosphate or inoculation was considered to be due mainly to widespread mycorrhizal infection throughout all the plots. More than half the root length was infected, even in the controls, implying an active and effective indigenous mycorrhizal population at this site. (Hayman, Grace, Spokes and O'Shea)

The spread of an indigenous VA mycorrhizal population in a field soil. A reclaimed gravel pit (Panshanger, Hertfordshire) was filled, levelled and drained by spring 1980, the topsoil having been stored for 12 years. A non-mycorrhizal host crop (oilseed rape) was sown in spring 1980. At this time, no indigenous mycorrhizal endophytes (either as spores or root infection) were observed. A host crop, barley, was sown in autumn 1980 and by summer 1981, mycorrhizal infection had developed throughout the crop (40–70% of the root length infected) and large numbers of mycorrhizal spores were found in the soil. We do not know how the infection developed. Such a rapid build-up from a residual population or from the spread of fungi from surrounding fields was quite unexpected. (Warner, Gee and Fyson)

Lucerne and maize at Panshanger. Two experiments were set up to determine: (1) whether mycorrhizal inoculation could improve plant establishment, (2) how crop rotations affected mycorrhizal endophytes in soil, and (3) whether the introduced inoculum could be built up *in situ* by judicious cropping.

https://doi.org/10.23637/ERADOC-1-128

Lucerne grew well in all plots irrespective of mycorrhizal inoculation. Maize also showed no response to inoculation. Soil and root samples showed that mycorrhiza became prevalent in all plots during the growing season, including the non-inoculated controls, thereby making it impossible to pursue the experimental objectives. The main finding was therefore the unexpectedly rapid spread of mycorrhizal infection through the plants which we believe came in from the surrounding fields because no infectivity was detected in the plots at the beginning of the experiments. (Hayman, Spokes and Grace)

Effect of nitrogen on mycorrhizal infection. Nitrogen was supplied to a legume (white clover) and a non-legume (onion) in two ways to determine whether the negative effect of nitrogen on infection by *Glomus fasciculatus* (E3) acted through the plant or through the soil. Accordingly ammonium nitrate (140 and 560 ppm) was applied either directly to pots of a nitrogen-deficient soil or to solutions into which roots from plants in pots had been made to grow. Mycorrhizal infection in onion was decreased from nearly 100% to about 65% with added nitrogen which raised the concentration of nitrogen in the roots from 1·7 to 5·7%. By contrast infection and nitrogen concentration in clover were lower than in onion and little affected by added nitrogen. It was concluded that the level of nitrogen in the plant rather than in the soil was the major factor affecting infection. (Wang and Hayman)

Interactions between VA mycorrhiza and *Azotobacter chroococcum* in reclaimed soil from Panshanger. Both VA mycorrhiza and *Azotobacter chroococcum* when inoculated separately on to roots of plants growing in field soils can improve growth and yields. Pot experiments were designed to examine the effect of these inoculants, added alone or together, on growth of lettuce, maize and peas in a soil from gravel pit workings. Mycorrhizal infection was established rapidly in the roots of all the plants but either had no effect on growth or was deleterious. The level of infection differed with the mycorrhizal strains used, and varied between 5 and 30% of total root length. Shoot weight of all plants was positively correlated with the actual root length infected rather than percentage infected. *Azotobacter* alone stimulated early shoot growth by 9–20% and harvest weight by 5–13%, but there were no correlations between numbers on the roots and growth. *Azotobacter* did not ameliorate the deleterious effect of the mycorrhiza and numbers of the bacteria were significantly decreased on mycorrhizal roots. No correlations existed between shoot weight and mycorrhizal infection when the two inoculants were added together. (Brown and Gibson)

The production of inoculum in nutrient film culture. A system of producing large quantities of vesicular–arbuscular mycorrhizal fungi in peat with lettuce as a host plant has been developed in association with Mr L. Dingemans of Lavinia Nursery, Middlesex. Inoculum produced in this way is one-tenth the weight of the equivalent volume of soil-grown inoculum. (Warner, Gee and Fyson)

Effect of amino acids on the growth of VA mycorrhizal fungi. Peptone has a stimulatory effect on the growth of hyphae from resting spores of *Glomus caledonius* (Hepper, *Soil Biology and Biochemistry* (1979), **11**, 269–277). This has been shown to be due to lysine, glycine and cystine; lysine being the most effective. The optimum concentrations found were 825, 556 and 4·6 mg litre^{-1} respectively. In combination cys+gly+lys enhanced growth more than cys+lys or gly+lys, the last two mixtures being more stimulatory than lysine alone. A mixture of cys+gly was no more effective than either added alone; both of which were equally effective. (Hepper and Jakobsen)

https://doi.org/10.23637/ERADOC-1-128

Chemical characterisation of VA mycorrhizal fungi. Extracts of soluble proteins were prepared from external mycelium collected from the roots of mycorrhizal *Trifolium repens* produced in non-axenic nutrient film culture. Samples were subjected to electrophoresis on polyacrylamide gels at pH 8·9 and the gels stained for enzyme activity. Extracts prepared from the mycelium of *Glomus mosseae* and *Glomus caledonius* each showed one band of acid phosphatase activity with Rm values of 0·35 and 0·54 respectively. Mycelium collected from plants with a dual infection of *G. mosseae* and *G. caledonius* gave two acid phosphatase bands with Rm values corresponding to those from the individual endophytes. (Maskall, Elmes and Hepper)

Bacterium-like organelles (BLOs). A range of species and genera of VA mycorrhizal fungi is being examined by electron microscopy for BLOs. One type of BLO, a small coccal structure which appears to divide by simple fission, was found in all fungal isolates. BLOs of other morphologies were restricted to single fungal species. Techniques are being developed for optimal preservation of BLOs for electron microscopy. (Macdonald and Chandler)

Biological nitrogen fixation studies

Field inoculation of *Phaseolus vulgaris*. Most of the *P. vulgaris* seed sown in the UK is either imported with captan seed dressing or is dressed locally prior to planting. Previous work at the NVRS, Wellesbourne, has shown captan seed dressings to be very toxic to *Rhizobium phaseoli* applied to the seed as a peat slurry (Taylor, Day & Dudley unpublished). In subsequent experiments it was shown that the toxicity associated with seed dressings could be overcome either by combine drilling a granular inoculant or by spraying peat inoculum suspended in water into the seed furrow at planting. This year's experiment was to determine the amount of granules and method of placement necessary to give nodulation on plants grown from captan-treated seed equivalent to that obtained with peat inoculant applied to non-dressed seed.

Granular inoculant was prepared by applying peat inoculum containing *R. phaseoli* strain RCR 3622 to 8–16 mesh pumice using methyl cellulose as an adhesive. The granules were hardened by absorbing the excess adhesive with dentist's plaster (a neutral $CaSO_4$). Granules were applied at 6, 12 and 24 kg ha^{-1} either in the furrow at sowing or broadcast and raked in to the surface soil immediately post-sowing. All plots receiving granular inoculants were sown with captan-treated seed. The above treatments were compared with no inoculation, and standard peat slurry applied to captan-dressed and non-dressed seed.

The uninoculated plants had very few nodules and nodulation was severely reduced with peat slurry inoculation on seed dressed with captan. The two higher rates, i.e. 12 and 24 kg granules ha^{-1}, placed in the furrow gave nodulation equal to or slightly better than peat inoculation to undressed seed and even the lower rate (6 kg ha^{-1}) gave only slightly impaired nodulation, caused by sporadic very poorly nodulated or unnodulated plants rather than a general lowering of nodule numbers. Broadcasting of granules was far less effective, 24 kg granules ha^{-1} being necessary to give adequate nodulation. The difference was probably due to a pronounced dry spell following sowing. (Day, Ewens and Witty)

***Rhizobium* in gravel pit soils.** Topsoil samples from seven recently infilled sand or gravel pits were examined for the presence of rhizobia. Bacteria forming effective nodules on *Trifolium pratense*, *Lotus corniculatus* and *Vicia faba* were present on all the sites. *Phaseolus vulgaris*, *Melilotus officinalis* and *Medicago sativa* formed effective nodules in

https://doi.org/10.23637/ERADOC-1-128

only three of the soils. No nodules were formed on *Lupinus angustifolius*. The performance of nine legume species with or without the addition of *Rhizobium* inoculum was compared on one site. The soil was calcareous (pH 7·5–8·0) and had a very low organic matter. The highest dry matter yields were obtained for *Trifolium fragiferum*, *T. pratense* and *Melilotus officinalis* which consistently outyielded *Lotus corniculatus* and *Medicago sativa*. Other species (*Lupinus angustifolius*, *Phaseolus coccineus*, *Onobrychis viciifolia* and *Vicia faba*) grew very poorly though a significant yield response to *Rhizobium* was obtained for *Lupinus angustifolius*. (Fyson, Gee and Warner)

Populations of *Rhizobium* in the soil. In the *Rothamsted Report for 1979*, Part 2, 77–90, Nutman and Hearne presented a detailed survey of populations of *Rhizobium* in the soil. We have attempted to provide extra information on the effect of the appropriate host plant on populations. For convenience we counted *R. leguminosarum* and *R. trifolii* from all samples and used the same sampling technique as was used by Nutman and Hearne. We improved the dilution procedure which had the effect of reducing estimates of the populations of *Rhizobium*.

The effect of previous legume crops on populations was tested by comparing counts (Table 1). The counts are in numbers of rhizobia per gram dry weight of soil and are approximate. Samples were taken in July and August 1980.

TABLE 1

The effect of previous legume crops on Rhizobium *populations*

Crop	*R. leguminosarum*	*R. trifolii*
Fallow (no legume for 20 years)	10^2–10^4	10^2–10^3
First year grass/white clover (no clover for 4 years)	10^3–50^5	10^{-4}
Spring barley (grass/white clover 5 years ago)	10^3–10^5	10^3–10^5
First year *Vicia faba* (no peas or *V. faba* for 6 years)	10^4–10^5	10^2–10^3
Potatoes (*V. faba* 5 years ago)	10^5–10^6	10^3–10^5

It is clear from these data that populations are relatively small, are variable and that the hosts (*V. faba* for *R. leguminosarum* and clover for *R. trifolii* do not have a consistently beneficial effect on the population of the appropriate *Rhizobium* species.

Similar populations were observed for soil samples taken from two other sites where *V. faba* was being cultivated for the first time for at least 20 years. Our counts indicate that *R. leguminosarum* and *R. trifolii* are well adapted to life as soil bacteria and the results suggest that periodical cultivation of the appropriate host plant is not needed to maintain adequate populations. (Taylor and Beringer)

Competition among strains of *Rhizobium leguminosarum* for nodule formation. Six strains of *R. leguminosarum*, five from the Rothamsted Collection of *Rhizobium* and one from Tanzania were tested for their abilities to compete with each other in forming nodules on two varieties of peas (Sobat and Onward) and field beans (var. Minden). Two methods were used to identify the different strains. In one a limited range of antibiotics was used which was found to give a clear discrimination on the basis of intrinsic resistance. The reliability of this method depended upon establishing the right concentration of each antibiotic for the strains used. The other method, used to supplement the first, was phage typing which was very reliable.

Two characteristics of *Rhizobium* strains thought to influence competition were also examined. These were the production and sensitivity to bacteriocins and the ability to respond chemotactically to low molecular weight organic molecules. Two strains (RCR1013 and RCR1045) produced bacteriocins; strain RCR1001 was sensitive to both

https://doi.org/10.23637/ERADOC-1-128

and RCR1056 was sensitive only to that produced by RCR1013. All five strains were positively chemotactic to mannitol and yeast extract.

The competitive abilities of the different strains were compared by inoculating plants with equal numbers of the strains in pairs and also as a mixture of all six strains. Inoculations were done in triplicate and 60 nodules were checked per plant for paired combinations and 150 per plant for the mixture. Strain MR4 from Tanzania was the most competitive strain in all tests. Strain RCR1056 was the next most competitive strain, but this was affected by host genotype, as were the competitive abilities of the other strains. For example strain RCR1056 outcompeted strain 1012 on the pea variety Sobat, but when Onward was the host plant strain 1012 was far more competitive. Similar results were obtained when peas and field beans were compared with this and the other strains. Studies of the role of genotype interactions will be continued and an attempt will be made to identify the characteristics involved by genetical means. (Bitanyi and Beringer)

Bacteriocin production by *Rhizobium*. The occurrence, activity and significance of bacteriocin production by *Rhizobium* were investigated. Approximately 25 strains of each species from the Rothamsted Collection of *Rhizobium* (RCR) were tested. Bacteriocins were produced by 87% of *R. trifolii*, 71% of *R. japonicum*, 56% of *R. leguminosarum*, 39% of *R. phaseoli* and 23% of *R. meliloti* strains. Twenty-eight fresh *R. phaseoli* isolates from plants grown in a Cambridge soil all produced bacteriocins. These isolates and the RCR strains were equally sensitive to each others' bacteriocins.

Bacteriocins produced by six strains of *R. leguminosarum* gave inhibition zones in 40% of all possible pairings when tested against 22 strains of other fast-growing *Rhizobium*. Similarly *R. phaseoli* produced zones in 29% of cases, *R. trifolii* 9% and *R. meliloti* 1%.

The comparative advantage due to bacteriocin production was studied using strains of *R. leguminosarum* obtained from the John Innes Institute, Norwich. Strain J.I. 491 (M^+) is isogenic with J.I. 490 (M^-) but in the latter, the genes for bacteriocin production had been inactivated by transposon mutation. M^+ and M^- strains were mixed singly with an indicator strain (J.I. 487), which was inhibited by the bacteriocin, and used to inoculate pea plants. Whether the indicator was mixed with M^+ or M^- strains, rhizosphere counts of it were the same. This showed that either the bacteriocin was not formed or it was inactive under the experimental conditions. Over 90% of the nodules formed on peas contained the M^- strain when paired with the indicator. Therefore any extra inhibition due to bacteriocin production by the M^+ strain would not have been detectable. A more suitable indicator strain is being sought. (Dye and Sen)

Identification of *Rhizobium*. A method based on intrinsic antibiotic resistance (IAR) for identifying large numbers of *Rhizobium* strains (*Journal of General Microbiology* (1980), **118**, 437) was found to be unsatisfactory for *R. phaseoli* and isolates from *Cicer arietinum* (*Rhizobium* spp.). The number of different IAR patterns we determined always exceeded the number of strains tested. With 90 nodule isolates from plants inoculated with a mixture of three strains of *R. phaseoli*, the technique gave 18 different IAR patterns. When 24 strains of *Rhizobium* spp., each replicated three times, were examined 68 different patterns of resistance were obtained. Single colony isolates from one strain of *R. phaseoli* also gave several different IAR patterns. All strains tested with fluorescent antibody were readily identified. Attempts to correctly identify strains with IAR by simplifying the scoring system or allowing up to two differences in the resistance patterns were unsuccessful. It was not possible to define the source of variation although incubation time and inoculum concentration were shown to affect the IAR patterns. (Stein, Bromfield, Dye and Day)

The IAR technique described above was modified and gradient plates were used to

https://doi.org/10.23637/ERADOC-1-128

characterise several cultures of *R. phaseoli* and *Rhizobium* spp. (isolated from *Cicer arietinum*). Differentiation between cultures was facilitated by use of cluster analyses. The validity of the method for strain identification was demonstrated by flourescent antibody tests which gave corresponding identity for 50 nodule isolates from plants inoculated with a mixture of three strains of *R. phaseoli*. The technique permitted 15 of the 16 cultures of *R. phaseoli* that we tested to be distinguished on 14 antibiotics. Two cultures which exhibited similar IAR patterns were shown to be the same strain obtained from different collections. The method was less suitable for characterising cultures of the slow growing *Rhizobium* spp. because several antibiotics induced growth which lacked a clearly defined boundary between resistance and susceptibility. Although 15 of the 16 cultures of *Rhizobium* spp. could be differentiated, several isolates were only distinguishable by a difference on a single antibiotic. Similarity between stock cultures and derivative nodule isolates suggested that IAR on gradient plates was a stable property unaffected by plant passage. (Bromfield and Stein, with White, Statistics Department)

The carbon cost of nitrogen fixation. Although large and well-recognized differences exist in the symbiotic performance of *Rhizobium* strains within the root nodule little fundamental information is available on the reasons for these differences. The efficiency with which available carbohydrate is used by the symbiosis in nitrogen fixation is an important factor.

To measure the efficiency of nitrogen fixation, techniques have been developed to measure respiratory CO_2 production, hydrogen evolution and $^{15}N_2$ incorporation by detached or attached nodules over short periods (10 mins to 1 h). The same nodules are subsequently exposed to acetylene and from the decay in respiration and nitrogenase activity both the background respiration and the carbon cost for the assimilation of fixed N can be determined. These values in conjunction with those from the $^{15}N_2$ exposure allow the carbon cost of N_2-fixation to be calculated.

Initial experiments with strains of *Rhizobium leguminosarum* which can or cannot utilise hydrogen evolved during nitrogen fixation give total carbon costs which range from 6·4 g C per g N fixed (2% of energy to nitrogenase used for H_2 production) to 10·5 g C per g N (38% of energy to H_2 production). When allowance is made for root and nodule respiration not coupled to nitrogenase these values are equivalent to 4·2–6·9 g C per g N fixed.

Work is continuing with genetically modified strains of rhizobia and it is hoped the technique will form a rational basis for the selection of improved inoculants for the field. (Witty, with Dr F. R. Minchin, Grassland Research Institute, and Dr N. J. Brewin, John Innes Institute)

***Rhizobium* genetics.** We need to know what functions of *Rhizobium* strains are important for nodulation and nitrogen fixation to decide upon rational procedures for producing improved strains. One of the best methods for determining whether a function is important or not is to inactivate the gene(s) involved by a technique which induces a single mutation per bacterium. The mutant strains can then be compared with the parent and any differences in symbiotic abilities are probably due to the loss of the function in the mutant strains. Changes of this type are most easily induced by transposon mutation. Transposons are discrete sequences of DNA that are able to integrate within the chromosome or other replicons in a bacterium. One insertion occurs per bacterium and when this is within a gene the function of that gene is inactivated.

Techniques are available to introduce transposons into *Rhizobium* strains. However the most common procedure has the disadvantage that it can make the rhizobia symbiotically defective for reasons other than the mutation caused by introduction of the

https://doi.org/10.23637/ERADOC-1-128 pp 8

transposon. To avoid this problem we have introduced the most useful transposon for inducing mutations in *Rhizobium* (Tn5) into plasmids which can be transferred from *Escherichia coli* to *Rhizobium*, but are not replicated or maintained in the *Rhizobium* recipients. Therefore selection for the inheritance of the transposon results in transposon mutation. Plasmids RA1, R64 and R136 belonging to the incompatibility groups C, Iα and FII respectively are available for use as transposon donors. (Li and Beringer)

General studies

Physical studies on soil microorganisms. The effect of mechanical and chemical dispersion on the release of microorganisms from sorptive and mechanical interactions with soil have been investigated. Desorbed microorganisms were extracted from soil differentially on the basis of sedimentation velocity in soil elutriators. The extracted cells were partially purified by sieving, filtration and isopycnic flotation. Samples of specific fractions of the soil microflora were analysed for phosphorus and nitrogen to investigate microbial immobilisation of these plant nutrients. The relative frequency of indicator microorganisms in soil and in extracted populations is being compared in order to estimate the representativeness of physically extracted samples of the microflora. (Macdonald and Spokes, with Brooks, Soils and Plant Nutrition Department, and Dr N. J. Martin, West of Scotland Agricultural College)

Comparative anatomical studies of wheat roots invaded by *Gaeumannomyces graminis* var. *tritici* or *Phialophora radicicola* var. *graminicola*. Transverse sections of roots treated to show active respiratory enzymes in invading hyphae have been examined. The rate of invasion of both fungi was faster the nearer the inoculum was placed to the seed. The pattern of invasion and host reaction differed with the two fungi. Hyphae of *Gaeumannomyces* containing active enzyme were confined to small segments of the cortex and pericycle and associated with brown host deposits and lignitubers. Infected, but not uninfected, cells contained blue formazan deposits. Hyphae of *Phialophora* containing active enzyme were found throughout the cortex and were concentrated in the layer surrounding the endodermis. Vesicles with formazan deposits were also in this layer. Hyphae did not penetrate the stele and infected host cells did not contain formazan deposits. (Brown and Gibson)

Staff and visiting workers

Joan Gostick joined the Department in January as a part-time laboratory attendant.

E. Bromfield, E. Davidson and Valerie Harju were appointed to work on an ODA-funded project to collaborate with ICRISAT (Hyderabad, India) in studies of the rhizobia that nodulate peanuts, chickpeas and pigeon peas. A. Fyson and P. Gee have joined Anne Warner on a project funded by the NRDC to isolate and test improved strains of *Rhizobium* and mycorrhizal fungi.

We were sorry to lose S. Maskall and R. Elmes who left in September when an ODA-funded grant to study methods for culturing VA mycorrhizal fungi terminated.

The Department has, as usual benefited from work done by visitors and sandwich course students. Visitors I. Baldani, D. Kishinevsky, M. Müller and K. Rao spent periods of 1–3 months working with us and Mr Li Fu-di returned to the People's Republic of China in January after spending a year in the Department studying various aspects of *Rhizobium* genetics. We are grateful for help received from our sandwich course students, P. Edwards, Julie Kirk and from Ruth Neiland who worked as a temporary assistant.

During the year several members of staff attended and presented papers at conferences

https://doi.org/10.23637/ERADOC-1-128

in the UK and abroad. The following staff presented papers and participated in meetings abroad. J. E. Beringer, Workshop on Biological Nitrogen Fixation Technology for Tropical Agriculture, Cali (Colombia), Eighth North American *Rhizobium* Conference, Winnipeg (Canada), Bat Sheva Symposium on Nitrogen Fixation, Rehovet (Israel). J. M. Day, IAEA coordination meeting on the use of isotopes in nitrogen fixation research, Vienna (Austria). D. S. Hayman, Seventy-third Annual Meeting of the American Phytopathological Society, New Orleans (USA), Fifth North American Conference on Mycorrhizae, Quebec (Canada), Symposium on Biological and Chemical Interactions in the Rhizosphere, Stockholm (Sweden), IAEA consultants' meeting on 'The Use of Isotopes in Studies of Nutrient Availability to Food Crops by Endomycorrhizae', Vienna (Austria). R. M. Macdonald, Fifth North American Conference on Mycorrhizae, Quebec (Canada). J. E. Beringer also gave seminars and discussed research at the University of Mexico, Cuernavaca (Mexico), and at the Universities of Erlangen-Nurnberg and Regensburg (Germany), as did D. S. Hayman at the University of Florida, Gainesville, and at the USDA, Orlando (USA). J. M. Day is visiting ICRISAT (Hyderabad, India) for 6 months (starting November 1981) to coordinate the collaborative ODA-funded research projects. Most scientific staff attended, and in many cases presented papers, at meetings in the UK.

Publications

General Papers

1 Beringer, J. E. (1981) Genetics of symbiotic nitrogen fixing microorganisms. In: *Current perspectives in nitrogen fixation.* Ed. A. H. Gibson & W. E. Newton. Canberra: Australian Academy of Science, pp. 131–136.

2 Beringer, J. E. (1981) The identification, location and manipulation of genes in *Rhizobium.* In: *Genetic engineering of symbiotic nitrogen fixation and conservation of fixed nitrogen.* Ed. J. M. Lyons, R. C. Valentine, D. A. Phillips, D. W. Rains & R. C. Huffaker. New York and London: Plenum Press, pp. 55–63.

3 Beringer, J. E. (1981) Mycorrhizas and nitrogen-fixing symbioses. In: *Aspects of crop growth.* ADAS Agronomy Conference January, 1981. *MAFF Reference Book* No. 341. London: HMSO, pp. 62–69.

4 Beringer, J. E. & Day, J. M. (1981) The role of biological nitrogen fixation in U.K. agriculture. *Proceedings of the Fertiliser Society* No. 205, 1–16.

5 Beringer, J. E., (Brewin, N. J. & Johnston, A. W. B.) (1982) Genetics. In: *Ecology of nitrogen fixation II. Rhizobium.* Ed. W. J. Broughton. Oxford: Oxford University Press, pp. 167–181.

6 Beringer, J. E., (Brewin, N. J. & Johnston, A. W. B.) (1982) Symbiotic nitrogen fixation in plants. In: *Bacteria and plants.* Ed. M. E. Rhodes-Roberts & F. A. Skinner. Society for Applied Bacteriology Symposium No. 10. London: Academic Press, pp. 43–50.

7 Brown, M. E. (1982) Nitrogen fixation by free-living bacteria associated with plants—fact or fiction. In: *Bacteria and plants.* Ed. M. E. Rhodes-Roberts & F. A. Skinner. Society for Applied Bacteriology Symposium No. 10. London: Academic Press, pp. 26–41.

8 Hayman, D. S. (1981) Mycorrhiza and its significance in horticulture. *The Plantsman* **2**, 214–224.

https://doi.org/10.23637/ERADOC-1-128

Research Papers

9 Bromfield, E. S. P. & (Jones, D. G.) (1980) Studies on acid tolerance of *Rhizobium trifolii* in culture and soil. *Journal of Applied Bacteriology* **48**, 253–264.

10 Bromfield, E. S. P. & (Jones, D. G.) (1980) Studies on double strain occupancy of nodules and the competitive ability of *Rhizobium trifolii* on red and white clover grown in soil and agar. *Annals of Applied Biology* **94**, 51–59.

11 Bromfield, E. S. P. & (Jones, D. G.) (1980) A strain marker in *Rhizobium trifolii* based on the absorption of congo-red. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, Abteilung II* **135**, 290–295.

12 Bromfield, E. S. P. & (Ayanaba, A.) (1980) The efficacy of soy-bean inoculation on acid soil in tropical Africa. *Plant and Soil* **54**, 95–106.

13 Bromfield, E. S. P. & Roughley, R. J. (1980) Characterisation of rhizobia isolated from nodules on locally adapted *Glycine max* grown in Nigeria. *Annals of Applied Biology* **95**, 185–190.

14 Brown, M. E. (1981) Microbiology of roots infected with the take-all fungus (*Gaeumannomyces graminis* var. *tritici*) in phased sequences of winter wheat. *Soil Biology and Biochemistry* **13**, 285–291.

15 Brown, M. E. (1981) Infectivity of lesioned wheat root tissue related to the presence of dehydrogenase enzymes in hyphae of *Gaeumannomyces graminis* var. *triciti* in the lesions. *Soil Biology and Biochemistry* **13**, 519–525.

16 Hayman, D. S., Morris, E. J. & Page, R. J. (1981) Methods for inoculating field crops with mycorrhizal fungi. *Annals of Applied Biology* **99**, 247–253.

17 Hepper, C. M. (1981) Techniques for studying the infection of plants by vesicular–arbuscular mycorrhizal fungi under axenic conditions. *New Phytologist* **89**, 641–647.

18 Macdonald, R. M. (1981) Routine production of axenic vesicular–arbuscular mycorrhizas. *New Phytologist* **89**, 87–93.

19 Macdonald, R. M. & Chandler, M. R. (1981) Bacterium-like organelles in the vesicular–arbuscular mycorrhizal fungus *Glomus caledonius*. *New Phytologist* **89**, 241–246.

20 Macdonald, R. M. & Spokes, J. R. (1981) *Conidiobolus obscurus* in arable soils: a method for extracting and counting azygospores. *Soil Biology and Biochemistry* **13**, 551–553.

21 Macdonald, R. M. & Spokes, J. R. (1981) Ecology of *Conidiobolus obscurus* (*Entomophthora obscura*) in soil. *Journal of the Science of Food and Agriculture* **32**, 632.

22 (Martin, N. J.) & Macdonald, R. M. (1980) A density gradient procedure for separating microbial cells from soil. *Journal of Applied Bacteriology* **49**, No. 3, vii.

23 (Martin, N. J.) & Macdonald, R.M. (1981) Separation of non-filamentous micro-organisms from soil by density gradient centrifugation in Percoll. *Journal of Applied Bacteriology* **51**, 243–251.

24 Roughley, R. J., Bromfield, E. S. P., (Pulver, E. L.) & Day, J. M. (1980) Competition between species of *Rhizobium* for nodulation of *Glycine max*. *Soil Biology and Biochemistry* **12**, 467–470.

25 Spokes, J. R., Macdonald, R. M. & Hayman, D. S. (1981) Effects of plant protection chemicals on vesicular–arbuscular mycorrhizas. *Pesticide Science* **12**, 346–350.

https://doi.org/10.23637/ERADOC-1-128