ROTHAMSTED RESEARCH

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Soil Microbiology Department

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Introduction

Research on vesicular-arbuscular (VA) mycorrhiza has been a dominant feature of departmental work this year and has proceeded along three lines, field experiments, inoculum production and basic studies on the endophyte. Field work to assess the importance of VA mycorrhiza in the phosphorus nutrition of crops is hindered by scarcity of inoculum because the endophyte cannot be grown in culture. To overcome this difficulty the nutrient film technique is being developed to provide abundant mycorrhizal roots for use as inoculum. The biochemistry of the endophyte is also being studied with the object of understanding its failure to grow in culture. Parts of the two latter programmes are being funded by the National Research Development Corporation and the Overseas Development Administration, respectively.

Departmental work on symbiotic nitrogen fixation in legumes has ranged from field studies on bean cultivation to fundamental work on characteristics of the *Rhizobium* cell. Collaboration with overseas research institutes on problems concerning tropical legume crops has recently been curtailed by financial stringency.

Other departmental interests include rhizosphere microbiology, especially in relation to the take-all disease of wheat, stability of soil aggregates and nitrification. The Rothamsted Collection of *Rhizobium* and work on the production of abnormal cells of *Rhizobium trifolii* will not be reported on this year.

Vesicular-Arbuscular Mycorrhiza

Field inoculation studies

Barley, Sawyers I. The growth response of barley cv. Ark Royal to inoculation with three different VA endophytes was tested in replicated 1 m² plots on a part of Sawyers I

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field that had been fallow for several years. The soil contained 10 ppm $NaHCO_3$-soluble P and half the plots were given 100 g of superphosphate, dug in lightly before sowing; this raised the $NaHCO_3$-soluble P in the top 10 cm to *c.* 40 ppm. Inoculum consisted of 20 g of infected soil and roots placed below the seed which was sown 10 cm apart. Inoculation with all three endophytes approximately doubled yield to 100 g grain m^{-2}, although uninoculated control plants were also well infected (45%) by indigenous VA fungi. Added superphosphate increased yield of control plants to 279 g m^{-2} but reduced infection by indigenous fungi from 45 to 11%. With inoculation the best endophyte further increased yield to 322 g m^{-2} but the other two reduced it to 214 and 204 g m^{-2}. The introduced endophytes were much less sensitive than the indigenous to added phosphate and infection remained above 40%. The different effects of the three endophytes on plant growth were not closely related to percentage infection either at the final harvest (13 weeks after sowing) or earlier. The best endophyte was *Glomus caledonius*, which had previously given good growth responses at this site, followed by a mixed inoculum previously shown to be less beneficial at this site, followed by E_3 (a form of *G. fasciculatus*). The experiment shows the importance of endophyte species. It also shows that even a relatively good phosphate forager like barley can benefit from inoculation in a soil well supplied with indigenous endophytes after addition of appreciable amounts of phosphate. (Mosse and Clarke)

Red clover, Sawyers I. The effects of inoculating red clover with different mycorrhizal endophytes and treating with superphosphate at 0, 30 and 60 kg P ha^{-1} were examined in the field. Sowing was delayed by the wet spring and plants failed to grow well or to flower. Plants responded positively to phosphate but differences between endophytes were too small to be evaluated. The plants were left *in situ* to measure symbiotic performance next year. (Hayman)

White clover in Welsh uplands. Field trials on the effects of mycorrhiza on white clover establishment in hill pastures were continued at Pwllpeiran EHF in cooperation with ADAS, Trawsgoed. Last year's endophyte-phosphate treatments at site 4 were repeated at site 1 which had proved to be more responsive in previous experiments (*Rothamsted Report for 1978*, Part 1, 238). Both inoculated and non-inoculated plants responded to phosphate fertiliser, superphosphate and basic slag being more effective than rock phosphate. Responses to mycorrhizal inoculation were variable and could not be evaluated properly because of poor seedling establishment in two of the three replicate blocks. Poor establishment here and in an adjacent seed inoculation trial was attributed to severe drought following wet sowing conditions. (Hayman)

Semi-leafless peas, John Innes. Treatment of soil with basamid and telone to kill virus-transmitting nematodes in a field at the John Innes Institute eliminated VA mycorrhizal fungi. Subsequent growth of semi-leafless peas was poorer than in adjacent untreated soil.

In August the effects of inoculation with two mycorrhizal endophytes were compared with those of added superphosphate at 15, 75 and 150 kg P ha^{-1} and nitrogen fertiliser at 51 kg N ha^{-1}. Half of each plot was inoculated with rhizobia. After $9\frac{1}{2}$ weeks dry weights of mycorrhizal plants ranged from 2·2–2·6 g per plant, whereas control plants and those given nitrogen weighed *c.* 1 g each; plants given P weighed 1·0–1·8 g. Inoculation with rhizobia had no obvious effect.

Although this pilot trial lacked replication, the plants within each treatment were quite uniform. It was concluded that in this soil (containing *c.* 20 ppm Olsen P) semi-

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leafless peas depend strongly on mycorrhiza for satisfactory growth. (Mosse and Hayman, with Mr. B. Snoad, John Innes Institute)

Inoculation techniques. The establishment of pre-selected mycorrhizal endophytes in field-grown crops is difficult because of the large amount of inoculum required and competition from indigenous endophytes. Four methods of inoculation with mixed E_3 and LAM endophytes were tested on red clover in Sawyers I field at Rothamsted. In three methods crude inoculum from stock plants, consisting of soil with mycorrhizal root fragments, spores and hyphae was applied: (1) below seeds in furrows; (2) broadcast and raked in with the seeds; and (3) as multi-seeded pellets (*c.* 1 cm^3). In the fourth method the crude inoculum was concentrated by wet-sieving, suspended in 4% methylcellulose together with germinated seeds and applied as a slurry to the furrows (fluid drilling).

After 9 weeks, whole root systems of uninoculated control plants were infected to 5–10% with the indigenous endophytes and plants inoculated broadcast were infected to a similar extent. By contrast, plants inoculated by pellets were quite well infected (*c.* 25%) and those furrow-inoculated with crude or fluid-drilled inoculum were heavily infected (65–70%). Thus fluid drilling may prove to be a suitable technique for field inoculation because the quantities of inoculum needed are smaller than with other techniques, and other inocula such as legume rhizobia can be incorporated in the methylcellulose gel. (Hayman, Morris and Page)

Factors affecting the spread of VA endophytes. Interactions between root density (length per unit volume of soil), host species, linear spread of a VA endophyte and colonisation of the root system (total length of root infected) were evaluated in one soil contained in troughs 10 cm deep. Different root densities obtained by sowing seeds broadcast, or at 4 or 6 cm apart, persisted for 15 weeks. Root density greatly influenced colonisation and less markedly the rate of linear spread. In clover, linear spread and colonisation increased with root density but in fescue, maximum root density did not favour maximum spread. This was further investigated using five host species grown singly in pots (soil depth 10 cm). Effects of host species on spread overrode those of root density. Onions (root density 0·06 cm cm^{-3} soil) had 45% of root length infected (41 cm root), whereas fescue (root density 6·25 cm cm^{-3} soil) had only 2% infected root (156 cm). The two legumes, clover and bean, had the greatest length of infected root, 701 and 335 cm, respectively, representing 16% and 10% of total root length with root densities of 3·05 and 0·25 cm cm^{-3}, respectively. These figures show the possible difficulty in comparing percentage infection between different plant species because total root length can vary considerably. Soil fertility also had marked effects on total root length and may thus affect rates of colonisation and spread.

Inoculum of four endophytes was kept for 6 months in fabric containers which allowed hyphae to pass through but prevented the mechanical transfer of inoculum. In the absence of a host plant, the fungus grew from the inoculum into surrounding irradiated soil and became sufficiently well established to act as a source of infection for newly planted seedlings after the original inoculum was removed. However, the effective spread from the inoculum into the surrounding soil was less than 2 cm. The results indicate a limited saprophytic ability of the four endophytes used.

The spread of the endophyte *G. caledonius* was monitored in a field experiment (*Rothamsted Report for 1978*, Part 1, 237–238). Spread could not be related to host species or plant size. A maximum spread of 1·7 cm per week was recorded.

Introduced endophytes can compete with indigenous strains and in one experiment suppressed their sporulation. In a Brazilian soil many single-spored sporocarps occurred together with a much coarser external mycelium. Maize grown in this soil contained few

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vesicles in the roots. Inoculation with another endophyte (E_3) completely suppressed sporocarp production and greatly reduced the coarse external mycelium. Many vesicles characteristic of E_3 occurred in the roots of maize grown in the inoculated soil. (Warner and Mosse)

Spread and residual effect of endophytes in last year's field inoculation trial (Owusu-Bennoah & Mosse, 1979) was monitored. *Glomus caledonius* had spread throughout the plot; up to 25 spores per 50 ml soil were recovered from positions 2·25 m from the nearest inoculation point. Lucerne, used as indicator plant, grew well throughout the plot. Inoculation had a positive residual effect of 17% on shoot dry weight. (Mosse and Clarke)

Nutrient film technique. This investigation has two objectives: (1) mass production of mycorrhizal roots for small scale field inoculation, (2) production of sufficient axenic material (infected roots and external mycelium) for biochemical studies of endophyte metabolism.

Bean (*Phaseolus vulgaris*) and maize (*Zea mays*) were used for mass inoculum production. Techniques of inoculation *in situ* i.e. in the flow culture trays, and rates of spread within the root mat were investigated. Spores or mycorrhizal roots placed in small pieces of capillary matting and inserted between the roots in trays produced much cleaner infected roots than pre-inoculation in soil but spread of the endophyte within the root mat was rather slow. Placing the phosphate into the tank containing the nutrient solution also improved cleanliness of the root mat. Placing the relatively insoluble phosphate source in a cartridge inserted in the flow line or in a muslin bag below the outflow pipe produced insufficient soluble P. Maize required 5·6 mg litre^{-1} iron, as NaFe EDTA for satisfactory growth and mycorrhiza development. Calcium levels in the nutrient medium affected the relative proportions of infected roots and external mycelium. Infection was inhibited by 11 mg litre^{-1} of N (95% as NO_3^-; 5% as NH_4^+) in the culture solution but developed well when the nitrogen level was maintained at 1 mg litre^{-1}. The pH of the culture medium rose with maize but fell with beans and required readjustment every two days. Good infections were also obtained in clover, lettuce, lucerne and capsicum in nutrient flow culture. NFT-grown mycorrhizal bean and maize roots used as inoculum produced normal VA infections in lettuce, onion, bean, clover and maize test seedlings. NFT-grown inocula of three endophytes, yellow vacuolate, E_3 and an unnamed *Glomus* sp. from Brazil, produced infections characteristic of each endophyte in bean and lettuce seedlings. Each inoculum was as good as the corresponding pot-grown inoculum of similar weight. Air-drying decreased, but did not eliminate, the infectivity of NFT inoculum. (Elmes and Mosse)

A prototype assembly for axenic NFT culture has been devised and tested. Essentially it is a scaled down version of the non-sterile system with the plant container inside a laminar-flow cabinet. In the second trial, without plants, the system remained sterile for 6 weeks and then became contaminated by insects entering the cabinet. (Elmes and Macdonald)

Laboratory studies on VA mycorrhiza

Estimating infection by VA endophytes. In a study of techniques used to assess mycorrhizal infection quantitatively a grid-line intersect method was compared with a visual estimate, and with a method based on detailed examination of 1 cm root pieces on microscope slides. The standard errors of the different methods were calculated and graphs were constructed giving the number of observations required for a given standard error for each method. (Giovannetti and Mosse)

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Growth from spores. VA mycorrhizal spores, which produce only limited mycelium unless a host root is infected by it, can be stimulated to regrow by detaching them from the hyphae and placing them on fresh medium. This 'second germination' growth can be as extensive as the first and some spores will regrow in this way for a third time. This suggests that the complete utilisation of essential stored compounds has not taken place and that growth stops for another reason, such as accumulation of toxic products around the hyphae. This possibility was investigated by incorporating activated charcoal or bentonite in the medium in the hope that toxic compounds would be adsorbed, but growth of hyphae from *Glomus caledonius* spores was not improved. (Hepper)

Effect of inorganic ions. The direct effect of soluble phosphorus on the germination and growth of VA mycorrhizal fungi has been investigated. There was no effect on the germination of *Glomus mosseae* or *G. caledonius* spores at up to 900 mg P litre^{-1}; above this level the total number of spores which germinated diminished. Growth from pre-germinated *G. mosseae* spores was adversely affected at 300 mg P litre^{-1} and considerably depressed above this level. *Glomus caledonius* spores were less sensitive, growth being inhibited at 600 mg P litre^{-1} and greatly reduced at 900 mg P litre^{-1}. At these relatively high phosphorus levels, the effects were probably due to non-specific ion inhibition since potassium sulphate (equivalent to 2500 K litre^{-1}, the same potassium level as that present when phosphorus was tested at 1240 mg litre^{-1}) completely stopped growth of *G. mosseae* hyphae and depressed the growth of *G. caledonius*. This concentration of potassium sulphate had no effect on spore germination. Soluble nitrogen (added as potassium nitrate) had no effect on the germination of *G. caledonius* at 500 mg N litre^{-1} (equivalent to 1390 mg K litre^{-1}) but depressed growth slightly at this level. (Hepper)

Effects on plant growth of mixed inocula of VA endophytes and root micro-organisms. Lettuce seedlings infected with a mixture of E_3 and YV mycorrhizal spores were planted in partially sterilised or untreated Rothamsted Delharding soil, after dipping the roots in a 14-day old culture of *Azotobacter chroococcum*. At final harvest top weights of lettuce were greater with the *Azotobacter*/mycorrhiza inoculum than with either inoculum alone. The effect was greatest in the untreated soil. Mycorrhizal infection in the roots was increased by the presence of *Azotobacter*. These results were confirmed with plants grown in a sterilised sand/grit mixture and which, in addition to the *Azotobacter*/mycorrhiza inoculum, were inoculated with soil suspensions (natural and partially sterilised) that had been passed through 3·0 μm Millipore filters to remove indigenous mycorrhiza. *Azotobacter*, the total bacterial population and spore-forming bacteria on the lettuce roots were counted midway through the experiment and at final harvest. The presence of *Azotobacter* and mycorrhiza depressed the total count from both soil treatments at the first harvest, but only in the partially sterilised soil treatment at the final harvest, and increased the spore-forming population in the partially sterilised soil treatment at both harvests. (Brown and Carr)

Effects of aldicarb. The nematicide aldicarb was added to cultures of *Glomus caledonius* in roots of *Trifolium repens* at 10 mg a.i. litre^{-1} in an attempt to control nematode infestation. Nematodes and *G. caledonius* spores were counted at monthly intervals for 9 months. Aldicarb reduced the number of nematodes but also reduced sporulation by the fungus. This inhibitory effect contrasts with neutral or stimulatory effects noted with other species of mycorrhizal fungi (*Rothamsted Report for 1977*, Part 1, 238). (Spokes and Macdonald)

***Association between VA endophyte and* Azotobacter.** Forced associations between

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Azotobacter spp. and *Glomus caledonius* have been obtained by establishment of the bacteria within the fungal cytoplasm. Limited cytopathic effects have been observed and conservation and multiplication of the bacteria within the fungus and infectivity of the parasitised fungus for plants are being investigated. (Macdonald)

Ectotrophic mycorrhiza: phosphorus source of Sitka spruce seedlings. Sitka spruce seedlings inoculated with the ectomycorrhizal fungus *Thelephora terrestris* were grown in Woburn soil and in a forest nursery soil labelled with ^{32}P. Mycorrhizal seedlings made similar growth in the two soils but non-mycorrhizal seedlings weighed 12% less in the Woburn and 60% less in the forest soil. In both soils the specific activity $\left(\frac{^{32}P}{^{31}P + ^{32}P}\right)$ of phosphorus taken up by the mycorrhizal seedlings was higher (2·3 *v.* 1·9 in Woburn soil and 9·3 *v.* 7·1 in the forest soil) than that in the non-mycorrhizal, indicating that the mycorrhiza enabled the plant to take up relatively more of the labile P and implying that the fungus did not mobilise any soil phosphate unavailable to the Sitka spruce roots. (Mosse and Mr. J. Thomas, University of Surrey)

Legume inoculation studies

Laboratory studies on *Rhizobium*

***Long-term storage of* Rhizobium.** Optimal conditions for freeze-drying *Rhizobium* have not been defined and the 'shelf-life' of dried cultures not adequately estimated (Dye (1979) In: *Recent advances in biological nitrogen fixation*. London: Edward Arnold, pp. 437–474). Good survival during the drying process, a low death rate during storage and freedom from genetic alterations are all necessary for an effective preservation technique. The difficulty in making long-term storage studies has been overcome by using the 'accelerated storage test' of Damjanovic and Radulovic (*Cryobiology* (1968) **5,** 101–104). Dried cultures are heated at elevated temperatures and the death rate at each temperature calculated. This allows an estimate of survival under normal storage conditions.

Using the standard Rothamsted drying method (Dye, 1979) and subsequent storage at 4°C, a ten-fold reduction in viable count is calculated to take 100 to 1000 years, depending on the strain. The 'accelerated storage test' is being used to examine variables such as culture age, cell density and type of suspending fluid. (Dye)

Testing pre-inoculated seed. Evidence from Australia, where suppliers inoculate legume seed with rhizobia just before delivery to the farmer, shows that survival on the seed is poor, few viable rhizobia remaining after 2 weeks at ambient, or 4 weeks at refrigeration temperatures. As some firms in N. America and Europe store such pre-inoculated seed for up to several months before dispatch it is clearly desirable to test imports to the UK before their distribution to customers.

To estimate the number of rhizobia on pre-inoculated seed it is necessary to release the bacteria into aqueous suspension before counts can be made, often difficult when the adhesive holding rhizobia to the seed is unknown. A sample of commercial pre-inoculated legume seed, prepared with a strong adhesive, was used to investigate how efficiently three different methods would remove rhizobia into suspension.

Seeds in water were treated: (a) in a Whirlimixer for 2 min; (b) in a high speed blender for 20 min; (c) in a wrist-action shaker for 2 h. Dilutions of the resulting suspensions were inoculated on lucerne, the nodulated plants counted after 4 weeks and estimates of rhizobia made from MPN counts (Dye, 1979). The first treatment gave a count of 78 rhizobia per seed and the other more vigorous methods about ten times as many.

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These results indicate that counts from pre-inoculated seed must be interpreted with caution if the suspending method is not specified. (Dye)

Fine structure of ineffective nodules. *Rhizobium leguminosarum* can form ineffective nodules on *Trifolium subterraneum* (Hepper & Lee, *Plant and Soil* (1979) **51,** 441–445). A comparative study of such nodules, formed by strains 1013 and 1020, and the ineffective nodules formed by *R. trifolium* strain 6 showed differences in development and structure.

Bacterial development was slow with strain 6 and very few pleomorphic forms were found, even in 5-day old nodules. By 7 days degeneration was well advanced with clumping of the bacteria in many cells. At no stage did typical bacteroids form.

Cells of 1-day old nodules formed by strain 1013 were invaded with many pleomorphic bacteria. In 3-day old nodules these resembled normal bacteroids, and the amount of starch in the cells was greatly decreased. However, no nitrogenase (C_2H_2 reduction) activity was recorded and the nodules remained white. At 5 days most cells were still normal and degeneration was only well advanced by the 9th day with secondary infections in the collapsed cells.

Development of nodules formed by strain 1020 was similar to those formed by strain 1013, but the host cell walls were very thin and appeared to lack rigidity, and the envelope surrounding the bacteria was usually incomplete. Degeneration occurred much earlier. In 3-day old nodules the cytoplasm in many host cells was so disorganised that the starch was no longer peripheral but randomly distributed among the degenerating bacteria. (Misra, Chandler, Hepper and Nutman)

Field studies

***Nodulation and nitrogen fixation by the field bean* (Vicia faba).** In an experiment on the effects of irrigation and nitrogen fertiliser on *Vicia faba* the fertiliser was labelled with ^{15}N and applied at the same levels to barley. The uptake of ^{15}N-labelled nitrogen by the barley was used as a measure of the apparent soil N pool. Knowing the percentage of N derived from fertiliser by the beans and their total N content, it was possible to calculate the amount of N fixed using the *A* concept (Fried & Broeschart, *Plant and Soil* (1975), **43,** 707–717). The total nitrogen in the crops increased from 260 kg ha^{-1} without fertiliser nitrogen to 305 kg ha^{-1} with 150 kg ha^{-1} of fertiliser nitrogen and thereafter was unaffected by fertiliser N up to 450 kg ha^{-1}. Nitrogen fixed was 235 kg ha^{-1} without added N and declined linearly to 50 kg ha^{-1} with 450 kg ha^{-1} of added N. In this experiment *c.* 600 kg fertiliser N ha^{-1} would have been required to suppress N-fixation completely. See *Rothamsted Report for 1978*, Part 1, 241 for a note on this experiment.

Foliar application of N, P, K and S increased the yield of *V. faba* (*Rothamsted Report for 1977*, Part 1, 236). In a further experiment in 1979 a significant reduction in yield was obtained despite the fact that a large proportion of applied fertiliser was taken up by the plants. This effect is attributed to an interaction between leaf burning and extremely low soil moisture during the spraying period. (Day, Roughley and Witty)

The growth and nitrogen fixing ability of a determinate mutant of *V. faba* was compared with that of the indeterminate variety Maris Bead, with and without irrigation, in conjunction with staff at the Plant Breeding Institute, Cambridge. Initially, growth, dry weight of nodules and nitrogenase activity were greater with the determinate mutant, but from July onward there were no significant differences between the varieties. Irrigation, however, increased the dry weight of nodules, total and specific nitrogenase activity and total nitrogen in the plant with both varieties. A major component of this difference was probably attributable to less nodule damage by *Sitona* on irrigated plots. Irrigation

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also delayed the onset of translocation of plant nitrogen into the seed but by the end of August both irrigated and non-irrigated plants had accumulated similar amounts of N in the seed. Yields without irrigation were 3470 and 3240 kg ha^{-1} for Maris Bead and the determinate mutant, respectively. Corresponding yields with irrigation were 3700 and 3140 kg ha^{-1}. (Day and Roughley, with Mr. R. B. Austin, Dr. D. A. Bond, Miss M. A. Ford and Mr. C. L. Morgan of the Plant Breeding Institute, Cambridge)

Nodulation of soybean cv. Malayan by rhizobia of the cowpea miscellany. Studies of the competition between strains of *Rhizobium* spp. (cowpea miscellany) isolated from *Glycine max* cv. Malayan grown in Nigeria and *R. japonicum* (*Rothamsted Report for 1978*, Part 1, 240) were continued using antibiotic-resistant mutants. All 20 strains isolated from cv. Malayan, a promiscuously nodulating cultivar, when tested singly in competition with *R. japonicum* CB1809 *str*^{r} at 30°C, formed fewer than 50% of the nodules, and 14 other strains formed fewer than 25%. Competition was influenced by root temperature. Three strains of *Rhizobium* spp. were poor competitors with CB1809 *str*^{r} between 24° and 33°C but at 36°C they formed more nodules (74–88%). Another strain of *Rhizobium* spp. formed the majority of the nodules between 27° and 36°C whereas CB1809 *str*^{r} formed the most at 24°C. (Roughley, Bromfield and Day)

Strains of *Rhizobium* spp. and *R. japonicum* were characterised by their symbiotic performance on two hosts, *Vigna unguiculata* cv. Ife Brown and *Glycine max* cv. Bossier. Isolates from *G. max* cv. Malayan grown in Nigeria form a group intermediate between *R. japonicum* and *Rhizobium* spp. but are more closely related to the latter. We suggest on the evidence of symbiotic performance that *R. japonicum* is a specialised group within *Rhizobium* spp. (Bromfield and Roughley)

Breeding for high symbiotic effectiveness in red clover. Highly effective families (H × H) were compared with the original cultivar (cv. S123) in Saxcil cabinets in a 12 h day at high (25 000 lx) and low (12 500 lx) light intensities, with or without supplementary CO_2 during the light phase to give a final concentration of 1000 ppm. The day/night temperatures and humidities were 22/17°C and 17/88%, respectively. At both light intensities and CO_2 contents of the atmosphere the H × H families gave the highest yields. Nodule efficiency in promoting yield (per mg nodule) appeared to be unaffected by light intensity or CO_2 content of the air. Acetylene reducing activity per nodulated root was related to yield but, as found previously (*Rothamsted Report for 1974*, Part 1, 247), specific activity was unaffected by plant type, or by light intensity or supplementary CO_2. These results underline the importance of the host factors that regulate under diverse environmental conditions the amount of nitrogen fixed to attainable growth. (Nutman and Hearne)

The relationship between flowering behaviour and symbiotic effectiveness in red clover was examined in a series of diallel crosses between highly effective plants that differed in times of flowering. The five complete 6 × 6 diallels tested were of the following non-overlapping flowering categories: first early, second early, mid-season, late and very late; 96 days elapsed between the first and last plants to flower. With few exceptions the 150 families in the diallels out-yielded the original cv. S123 by large margins; the mean dry matter yields of the crosses were 101·5 and of S123, 84·2 mg per plant.

The 'first early' crosses in general yielded more than the late crosses with the mid-season families lying in-between, but there were many exceptions. Two of the earliest flowering parents when crossed in all combinations with the other four early parents yielded significantly less than the remaining families of the same diallel, and some late-flowering plants gave progeny that were symbiotically more effective than some of their earlier flowering counterparts. From the latter it should be possible to breed highly effective late flowering lines. (Nutman, Hearne and Capel)

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General studies

Stability of soil aggregates. The experiments on aggregates of Rothamsted Parklands soil (*Rothamsted Report for 1978*, Part 1, 231) were repeated on a less stable Barnfield soil (unmanured, plot 80). Measurements on water stability, gaseous diffusion and porosity characteristics of the aggregates were made immediately after aerobic or anaerobic (N_2 atmosphere) incubation for 1 week, and subsequently after five cycles of alternate wetting (to 25%) and drying over a 3-week period. The initial small proportions of water-stable aggregates >1 mm diam for natural (11%) and artificial (2%) material changed little when incubated with water but increased to *c.* 50%, aerobically or anaerobically, with glucose amendment. Wetting and drying had an effect only after initial anaerobic incubation with glucose, when stability increased to 78%. Artificial aggregates behaved similarly although with stability always less than the corresponding samples of natural aggregates. With Barnfield soil anaerobic incubation gave larger increases in stability than did aerobic incubation, the reverse of that found with Parklands soil (Skinner, *Journal of Soil Science* (1979) **30,** 473–481).

As with Parklands soil, measurements of crumb porosity and gaseous diffusion were both greater in the artificial than in the natural crumbs but porosity decreased significantly after incubation with glucose, and diffusion decreased following anaerobic incubation. Thus, conditions favouring microbial growth modified the pore characteristics, possibly by occluding them with microbial cells or end-products (see Currie, Physics Department Report, p. 157).

For all aggregates examined, large differences in water-stability were not reflected in the values for porosity and diffusion characteristics. So far, the evidence suggests that changes in aggregate stability brought about by microbial action are not closely related to changes in underlying physical structure. (Skinner)

Infectivity of take-all lesions. Samples of tissue from lesions on wheat roots caused by *Gaeumannomyces graminis* were taken from soil-grown plants and the amount of mycelium present in the darkened tissue examined in 5 μm transverse sections cut with a freezing microtome and stained with 0·05% trypan blue, and by measuring the activity of respiratory enzymes in pieces of lesion, a technique developed by Macdonald for demonstrating activity of fungi in roots. Succinate and glutamate dehydrogenases were present in hyphae concentrated in areas of the lesion and were surrounded by areas of non-reacting tissue. Mycelium in the lesion was shown by both methods to occupy only a proportion of the damaged tissue and the amount was positively correlated both with the disease assessment for the roots and with the amount of infection produced on test seedlings inoculated with lesion tissue. The observations and correlations were confirmed with axenically grown seedlings infected with *G. graminis* only. (Brown)

Ecology of *Entomophthora*. *Entomophthora* spp. cause epizootics in arthropods and may be useful in integrated pest control programmes. Resting spores of some species overwinter in the soil and may infect populations of insects in the following spring. Since germination of resting spores *in vitro* is erratic and culture of the fungus is difficult, little is known of the inoculum potential of soil containing resting spores. A physical extraction method has been developed based on wet sieving and differential sedimentation through interfaces between water, sucrose and potassium silicate solutions. The spores are stained and concentrated on membrane filters for microscopic counting. When spores obtained from homogenised infested aphid cadavers are added to soil, *c.* 70% may be recovered by this method. (Macdonald)

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Nitrification. Four new isolates of *Nitrosospira* have been obtained from arctic soils and are being compared with congeneric isolates from tropical soils. Isolation of pure cultures from different environments is continuing. To improve the efficiency of this process, a selective and diagnostic medium for ammonium-oxidising bacteria has been devised and is being tested against representative strains of all genera currently in cultivation. (Spokes and Macdonald)

Staff and Visiting Workers

Dr. P. S. Nutman retired at the end of October after spending 40 years in this department, the last 21 years as its Head. R. J. Roughley, Joan A. Crawley and S. P. White resigned, and E. S. P. Bromfield and J. D. Gill left the department at the termination of financial support by the Overseas Development Administration (ODA) for work on tropical legume crops.

Christine Hepper and C. S. Maskall were each awarded the degree of Ph.D. (London).

R. J. Roughley made two visits to the International Institute for Tropical Agriculture, Nigeria, under different auspices, to plan work on soybeans (ODA) and advise on funding of the nitrogen fixation programme (UNDP), and one to ICARDA, Syria, to establish a programme on nitrogen fixation in chickpeas and lentils. J. M. Day spent 3 months at the University of the West Indies, Trinidad, to complete work on the ODA pigeon pea project. D. S. Hayman gave a seminar at Wädenswill, Switzerland and visited research centres in Brazil to advise on mycorrhizal programmes there (British Council and Brazilian Science Research Council.) Christine Hepper presented papers at the Fourth N. American Conference on Mycorrhiza, Fort Collins, Colorado and visited the University of California. Margaret E. Brown and F. A. Skinner attended a symposium on straw decay at Hatfield College of Technology and several members attended a meeting on nitrogen fixation at the University of Sussex.

Dr. Manuela Giovannetti returned to Italy, and Dr. Aruna K. Misra to India, after stays of 11 and 10 months, respectively. Dr. J. P. Thompson returned to Australia in February. We welcomed Dr. Rosemary S. Bradley, Brazil and Dr. C. Marques-Pinto, Portugal for short visits. Recently-arrived visitors are S. M. Palacios from Mexico and F. Le Tacon from France. We acknowledge the help received from our past sandwich course students Esther M. Fletcher and Eileen J. Morris and their recent replacements, R. Page and K. Ritz.

Publications

Books

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4 Maskall, C. S. (1979) Characterisation of leghaemoglobins from legume root nodules. Ph.D. Thesis, University of London.

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