ROTHAMSTED RESEARCH

Report for 1973 - Part1

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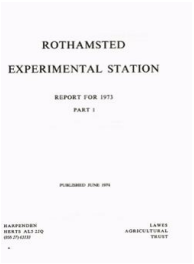

Soil Microbiology Department

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SOIL MICROBIOLOGY DEPARTMENT

P. S. NUTMAN

Soil microbes form a major proportion of the terrestrial biomass and a reserve of all the materials and activities needed to sustain life. Populations of soil organisms are well buffered against environmental change because of their great aggregate mass and their complexity and adaptability. Nevertheless, the rapid extension and intensification of agriculture and the pervasive effects of industrialisation impose strains that can lead to irreparable damage, so that developments in agriculture need to be judged against long-term effects on the soil and its inhabitants, about which all too little is still known.

This department studies a few aspects only of soil microbiology: the microbial cycling of nitrogen- and phosphorus-containing compounds in soil; the chemistry and microbiology of the breakdown of a small number of agricultural chemicals and related compounds; certain anaerobic processes and plant–microbe interactions. The latter includes the vesicular-arbuscular (VA) mycorrhiza, the effects of rhizosphere organisms on plant growth and health and associations of N-fixing bacteria and plants, especially those of nodulated legumes. The importance of biological nitrogen fixation is becoming increasingly recognised as the world-wide shortage of protein becomes more acute and the economics of using fossil energy for increasing agricultural production comes under scrutiny. Only a small percentage of a legume's photosynthate is consumed in nitrogen fixation; about the same as needed to reduce and assimilate an equivalent amount of nitrate nitrogen provided in fertiliser.

Work is continuing on the physiology of infection of clover by nodule bacteria, on the genetics of increasing symbiotic effectiveness, on soil anaerobes, on nitrification, and the effects of light on mycorrhizal infection, but these studies will not be reported upon this year.

Rhizosphere studies

Take-all decline. The take-all disease of wheat caused by *Gaeumannomyces graminis* usually declines after the third year of monoculture to a level that allows an economically acceptable yield of cereal. This interesting example of a natural change in the ecological relationship between a pathogen and host, if more fully understood, might be used for the better control of the disease.

Suggested explanations of decline are nutrient competition, specific microbial antagonism or loss of fungal virulence by a physiological change or virus infection. Results reported last year indicated a relationship between the decline of the disease and the nitrogen nutrition of the fungus in the soil, associated with the ratio of ammonium and nitrate ions in the wheat rhizosphere soil sampled in spring (but not in the bulk soil). This corresponded with changes in the numbers of ammonifying and nitrifying bacteria. A more detailed study with much more frequent sampling in 1973 showed, however, no correlation between disease incidence and these rhizosphere criteria which in all plots were related only to time of sampling.

The effect of sterile soil extracts taken from different cereal sequences on the development of the disease was examined in experiments with an added inoculum of *Gaeumannomyces graminis*. Seedlings were most infected when grown on extract from the third year sequence and less with extracts from subsequent years; infection thus corresponded to the decline pattern. Non-sterile extracts obtained by filtration through a 3·0 μm

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millipore membrane gave a similar pattern of incidence of disease but with much less infection. These experiments were then repeated with or without nitrogen added as potassium nitrate or ammonium sulphate which have been shown to affect the expression of the disease. Potassium nitrate alone did not alter the pattern of disease but sulphate of ammonia alone or with potassium nitrate significantly depressed infection of seedlings grown on sterile extracts made from the third and sixth year sequences or with non-sterile extracts from the third year sequence only.

Further collaborative work on the rapid induction of take-all disease in short-term greenhouse experiments and the possible effects of antagonistic microorganisms are described in the report of the Plant Pathology Department. (Brown)

***Azotobacter paspali:* its establishment in the rhizosphere, production of plant growth regulators and effects on plant growth.** *Azotobacter paspali* occurs abundantly in the rhizosphere of mature plants of *Paspalum notatum* and *Paspalum plicatum* where it fixes appreciable amounts of nitrogen (*Rothamsted Report for 1971*, Part 1, 95). *A. paspali* may also influence the plant by producing plant growth regulators of the types produced by *A. chroococcum*, viz. gibberellins, indolylacetic acid (IAA) and cytokinins.

The supernatant fluid of 14-day-old cultures of *Azotobacter paspali* were extracted for gibberellins and IAA. Extracts were separated by paper chromatography and eluates of each R_F value tested for growth regulators by standard bio-assays. At least three gibberellin-like substances were present in culture supernatants, one of which resembled GA3 and all of which behaved in bio-assays like those found in cultures of *Azotobacter chroococcum* and were produced in similar quantities. IAA was present in cultures, and bio-assays based on chlorophyll retention in oat leaves and on radish cotyledon expansion indicated that a cytokinin was also formed. (Brown and Barea)

Roots of the different seedlings were inoculated with known numbers of cells of *A. paspali* before transplanting into potting compost or soil from Brazil. Counts of *A. paspali* at 14-day intervals on N-deficient media showed that the inoculum declined rapidly even in the rhizosphere of *Paspalum notatum* and by eight weeks few cells were recovered from any sample. The *Azotobacter* declined less rapidly in compost than in the Brazilian soil, possibly because the richer compost supported better plant growth than the Brazilian soil. Failure to establish may also be related to the paucity of cysts in the inoculum which consisted mainly of filamentous vegetative cells. Vegetative cells of *Azotobacter chroococcum* do not establish well nor survive long in the rhizosphere.

To examine the effects on plant growth of inoculation with *Azotobacter paspali*, seedling roots of *Centrosema pubescens*, *Lolium perenne*, tomato, lettuce and wheat were dipped in cultures of *A. paspali* before translanting into a latosol from Brazil, or into standard John Innes compost number 3 and grown on for eight weeks. The development of all plant species grown in compost was affected by inoculation, but not that of plants grown in Brazilian soil. Tomatoes were most affected; stem length increased by 145% and leaf length (measured when plants had four true leaves) by 68%. Effects were still seen in plants with 12 leaves but were no longer significant. Inoculation shortened the development time of first and second trusses by seven and four days respectively. Dry weights of all plants were increased significantly by inoculation. (Brown)

Mycorrhizal studies

The vesicular-arbuscular mycorrhiza of legumes and tropical grasses. Inoculation with *Endogone* produced large increases (from 2–10 times) in the growth of the tropical legumes *Centrosema pubescens* and *Stylosanthes guyanensis* in phosphate-deficient soils from Brazil (cerrado soil) and from British Honduras. The tropical grasses *Digitaria*

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procumbens and *Brachearia* spp. also benefited from inoculation with selected strains of *Endogone*, even when the *Brachearia* was already heavily infected with indigenous mycorrhiza. In experiments with the above legumes and with the temperate *Trifolium repens* and *Lotus pedunculatus* mycorrhiza stimulated phosphate uptake and hence nodulation, nitrogen fixation and growth in P-deficient soils. Sometimes no nodules developed unless the plants were mycorrhizal. The tropical legumes were more dependent than the temperate ones on mycorrhizal-stimulated phosphate uptake, possibly because they possess fewer root hairs. When white clover and ryegrass were grown together mycorrhiza stimulated the growth of the legume more than the grass. (Mosse and Crush)

The effects of mycorrhiza on nutrient uptake. Our study of VA mycorrhiza at the IBP site at Meathop Wood was completed. An average of two out of three fine roots of herbaceous species were infected, usually for about one-third of their length. At least half of the roots of the woody hosts had ectotrophic mycorrhizal sheaths. In glasshouse pot experiments with soil from the wood very large increases in growth and phosphate uptake of indigenous plants (*Brachypodium*, *Viola*, *Fragaria*, *Fraxinus*, *Rubus* and *Betula*) were produced by inoculation with mycorrhizal fungi or the addition of mono-calcium phosphate. In ^{32}P-labelled soil, P-uptake of non-mycorrhizal plants was variable but generally less than one-tenth of that of mycorrhizal plants. *Brachypodium* was the most efficient absorber of soil phosphate in the absence of mycorrhiza. Several experiments using ^{32}P-labelled soil showed that all plants, whether or not mycorrhizal and of whatever species, used similar sources of soil phosphorus. The greater P-uptake by mycorrhizal plants can be attributed partly to the larger root systems of the better grown mycorrhizal plants and partly to the extensive mycelial penetration of the soil. Serial harvests suggested that seedlings are more dependent than older plants on mycorrhiza for phosphate uptake.

It is hoped to elucidate further the influence of mycorrhiza in natural conditions by using fungitoxicants or systemic fungicides to suppress P-uptake through the fungus. In preliminary experiments benomyl, benzimidazole and thiophanate methyl reduced infection in onions. (Mosse, Hayman and Arnold)

Factors affecting the occurrence and type of *Endogone* in agricultural soils continue to be studied. New evidence was obtained that swede, which is non-mycorrhizal, reduces mycorrhiza formation in nearby onion seedlings. (Hayman)

The amount of vesicular-arbuscular infection in clover and onion roots has been estimated by converting the fungal chitin to glucosamine which is then determined by a colour reaction as used by Ryde and Drysdale (*Physiological Plant Pathology* (1972), **2**, 7). (Hepper)

Mycorrhiza in root organ culture. Vesicular-arbuscular infections were for the first time successfully established in root organ cultures of *Trifolium pratense*. Pieces of root taken from liquid stock cultures were grown on a modified White's agar medium and inoculated with surface sterilised *Endogone* spores. Infection occurred within 7–14 days and when established produced extensive external mycelium-bearing vegetative spores and arbuscule-like branches. Often the first infections formed some distance from the inoculum, usually on young secondary roots. Entry points, arbuscules and vesicles within the root were anatomically similar to those in the roots of entire plants. The pH of the medium, which initially rises and then drops as the culture ages, appears to be an important factor influencing infection. (Mosse and Hepper)

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Lyophilised mycorrhiza as seed inoculant. The *mycorrhiza* in soyabean roots retained some viability after freezing at −30°C and then vacuum drying for 18 hours, with or without storage for up to two months. The dried mycorrhizal roots were mixed with lime to form a pellet around the seed. Only a small proportion of seedlings later developed mycorrhiza, whereas nearly all plants grown from seed pelleted with fresh spores or fresh roots became mycorrhizal; vacuum-drying alone without prior freezing at −30°C was unsuccessful. Grinding the freeze-dried mycorrhiza into a powder almost destroyed their capacity to produce infection on test plants. Although lyophilisation is possible with this material, recovery is as yet insufficient for this method to be used for routine preservation. (Crush and Pattison)

Studies on legume nodulation

***Rhizobium* in culture.** A selective medium for rhizobia (yeast mannitol agar containing brilliant green, sodium azide and pentachloronitrobenzene, *Rothamsted Report for 1972*, Part 1, 83) was further examined. The plate counts on this selective medium and on yeast mannitol agar (YMA) without inhibitors were the same for 18 out of 21 strains of rhizobia. The three remaining strains, one each of *R. phaseoli*, *R. lupini* and *R. meliloti* were inhibited by the selective medium, the latter strongly so. Micro-organisms in four Rothamsted soils were counted using the selective medium or YMA. Counts on selective media were about 10% of those on YMA for Broadbalk soils and from 57–75% for Park Grass samples.

The value of the selective medium for isolating *Rhizobium* from heavily contaminated sources was also examined by estimating the recovery of *R. trifolii* when mixed with suspensions of soil. From spread plates of the two media, *Rhizobium*-like colonies were inoculated on to sterilised seedlings of red clover. About 30% of the total colony counts on the selective medium nodulated clover compared with only 3% of isolates from YMA. The selective medium would be of little use in isolating rhizobia direct from soil but is clearly of value in allowing rhizobia to grow preferentially in the presence of contaminating organisms and is now being used routinely for work with the Rothamsted collection of *Rhizobium*. The absence of surface spread of bacteria on plates of the selective medium is of great advantage. (Skinner and Pattison)

Reduction of the dye Nile Blue to the colourless state by *Agrobacterium* but not by *Rhizobium* species was used by Hamdi (*Folia Microbiologica* (1969), **14**, 92–93) to distinguish these genera. Agrobacteria typically develop a greenish-yellow pellicle and a greenish tinge to the liquid medium in one to two days, whereas cultures of rhizobia remain blue in colour. Using eight strains of rhizobia and five of agrobacteria we were unable to distinguish between these genera at the recommended concentration of dye in the medium. However, when Nile Blue was used at half the recommended concentration (namely at 25 ppm) the rhizobia cultures remained blue and formed a deposit of dark-blue particles, but agrobacteria cultures became colourless or greenish with a narrow zone of oxidised dye at the surface and with a greenish yellow deposit. At still lower concentrations some of the dye was also decolourised by rhizobia. Using 113 strains of rhizobia and 25 of agrobacteria the Nile Blue test (at 25 ppm) was compared with other tests used for distinguishing agrobacteria from rhizobia, viz. the ability of agrobacteria but not rhizobia to form 3-ketolactose from lactose, to form hydrogen sulphide in bismuth sulphite medium and to grow well on glucose peptone agar. Only one of the 36 strains of *R. trifolii* reduced Nile Blue and none reduced 3-ketolactose. Similarly good agreement between responses to the tests were found for 28 strains of *R. leguminosarum* except that 10 non-nodulating strains gave typical *Agrobacterium* reactions and also grew well on nutrient agar and on glucose peptone agar, often producing an alkaline reaction. These results throw serious doubt on the presumptive non-nodulating strains

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and they have now been removed from our collection. Except for a few strains of *R. meliloti* the remaining rhizobia were negative in both tests. All strains of *Agrobacterium radiobacter* var. *tumifaciens* reduced Nile Blue but four failed to form 3-ketolactose. The ability of these strains to reduce Nile Blue was correlated with H_2S production and formation of alkali on glucose peptone agar. (Skinner)

Studies on the effect of yeast extract and casein hydrolysate (at from 0·1–1·0%) on cells of rhizobia were extended to include five commercially important strains for clover, lucerne, lupin, soyabean and cowpea (*Rothamsted Catalogue* numbers: 221, 2011, 3211, 3407 and 3824 respectively). With 0·1 or 0·35% yeast extract viable counts of the order of 10^9 cells/ml of culture were achieved, satisfactorily for the production of inoculants. Good yields were also obtained with some strains (3407, 2011 and 3211) at the higher concentrations of yeast extract but the viability of strains 3824 and 221 was depressed. Casein hydrolysate also supported large viable populations of four of the strains but depressed the viability of strain 3407 at 1% and of strain 221 (TA1) at all except the lowest concentration. All of 18 amino acids so far tested caused cell distortion of strain 221, and some groups of acids such as a mixture of valine, isoleucine, methionine, arginine and proline induced the formation of many large spherical granular bodies *c.* 5–7 μm in diameter. (Skinner and Roughley)

IBP World Catalogue of *Rhizobium*. A preliminary version of the 'World Catalogue of *Rhizobium* Collections' was published in August by the International Biological Programme. This lists about 3000 strains of rhizobia able to form root nodules on some 430 species of host plants. These strains are held in 59 collections housed in 29 countries. At the IBP/PP meeting 'Nitrogen Fixation and the Biosphere' held at Edinburgh in September, arrangements were made for us to undertake periodic revision of this catalogue. (Skinner)

The fine structure of hereditarily ineffective red clover nodules. The simply inherited major genes *ie*, i_1, *n* and *d* separately determine ineffectiveness in *Trifolium pratense* in symbiosis with *R. trifolii* strain 0403. The features of *ie* and i_1 nodules as seen by light microscopy were described by Bergersen and Nutman (*Heredity* (1957), **11**, 175). Each kind of ineffective nodule has a central zone containing infected cells enclosed in an endodermis and an outer cortex containing vascular traces; starch is abundant and leghaemoglobin is not seen.

Ineffective nodules of plants homozygous for the gene *ie* at first develop normally; an apical meristem is differentiated and the young nodule cells become penetrated by infection threads. After about three days large vesicles form on the infection threads. Host cell wall formation is stimulated, especially near the hypertrophied infection threads and vesicles, but meristematic development continues so that nodules become as large as, but no more numerous than effective nodules formed on heterozygotes. Some vesicles contain much zooglaeal-like material and detach from the threads becoming foci for further deposition of fibrous wall-like material which stains for lignin, both externally and within the vesicle. This is accompanied by much membrane formation in the host cytoplasm. Some rhizobia escape from the vesicles and may retain some zooglaeal material and also become enclosed in fibrous material. In some cells the bacteria multiply and enlarge into a bacteroid-like form within membrane envelopes which also enclose many small vesicles. These imperfect bacteroids are sparse and lie among untransformed rods. After about seven days the rhizobia and host cytoplasm rapidly degenerate leaving much wall and zooglaeal material and membrane fragments within the cells and in the intercellular spaces. Plastids in infected cells are large and ovoid and contain starch and much phytoferritin in crystalline arrays.

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Ineffective nodules of i_1 homozygotes are much smaller and more numerous than nodules on effective plants. Initial development is normal but as in the *ie*-type nodules many infection thread vesicles are formed. Rhizobia are released into the cytoplasm in groups but then remain enclosed by a common membrane envelope so that large parts of the cytoplasm become filled by a bacterial zooglaea. The bacteria divide little and most rapidly lyse; in some nodules a very small proportion enlarge to form bacteroids. The host cytoplasm is rich in membranes, either as vesicles or myelin-like whorls. Degeneration of the nodule is usually complete within 12 days.

The *n* homozygotes also form small ineffective nodules. Infection threads are infrequent and invaded cells few, but these may contain large amounts of zooglaeal matrix. There is a little sporadic bacteroid formation. The breakdown of the zooglaeal matrix varies from cell to cell. The degeneration of rods and bacteroids occurs rapidly and is already well advanced in eight-day-old nodules. Plant cytoplasm and organelles appear otherwise little affected by the invasion. Starch accumulates in cells where bacteroids had formed.

Ineffective nodules of *d* homozygotes are also fewer and smaller than those of effective plants but rhizobia and residual infection threads are very rarely found. The whole central zone of the nodule is formed of large, roughly isodiametric thin-walled cells with very little cytoplasm. Development of vascular traces is restricted. Any bacteria released had completely degenerated by four days. (Chandler, Dart and Nutman)

Nitrogen fixation in Broadbalk, in the Broadbalk and Geescroft Wildernesses and in Manor Wood. An *in situ* assay of nitrogenase activity was impracticable at Rothamsted because of the slow rate of diffusion of acetylene through the clay soil. An alternative method was devised in which 17-cm long cores, 11 cm in diameter enclosed in steel tubes were taken from the field and incubated in large confectionary jars closed with Suba Seals. Acetylene (500 ml) was added and the initial gas sample taken after 16 hours. Acetylene reduction was highly correlated with soil moisture at all sites. As previously reported (*Rothamsted Report for 1972*, Part 1, 87) high rates of N_2-ase activity on the field plots were sometimes associated with crusts of blue-green algae. The activity of the 17-cm deep cores with surface soil removed was small (less than 50 g N/ha/day). Only on plot 5 (added minerals and no nitrogen) were cores with plants more active than cores without plants. All cores from the arable field were much drier than those from the Wilderness site.

Activity at the Manor Wood site to a depth of 17 cm in the soil was very variable and strongly influenced by soil moisture. Using longer cores appreciable activity was found to a depth of 50 cm and at this level soil moisture varied little.

Acetylene reduction was measured on the part of the Broadbalk Wilderness site left uncultivated since 1882 and now a mixed woodland and on the ungrazed 'stubbed' sections where trees are removed annually and which is now a mixture of coarse grasses and herbaceous dicotyledons. These two sites have accumulated nearly 2 t N/ha since 1882, representing some 49 and 39 kg N/ha/annum respectively from biological nitrogen fixation. Estimates of N-fixation in the 'stubbed' section was high in the surface top 17 cm of soil but decreased rapidly with depth. The rate of acetylene reduction in the wooded section of Broadbalk Wilderness was low during 1973 (which was a dry year). The pH of this soil is about 7 and of the Geescroft Wilderness about 4, but rates of fixation were of the same order at both sites. (Day, Dart and Van Berkum)

Effects of inoculation, root temperature and nitrogenous fertiliser on grain legume symbiosis. The effects of inoculation of *Phaseolus vulgaris* with strain 3610 and with nitrate of 0, 30 and 90 kg N/ha applied at sowing was examined in 1973 on the varieties Seafarer (baked bean type) and Processor (dry bean type) grown at Woburn in soil already

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containing *R. phaseoli*. Seafarer nodulated more rapidly than Processor. Up to the early pod fill stage inoculated plants grew best; added nitrogen decreased nodulation and did not increase growth; yields were low. Except with 90 kg N/ha treatment uninoculated plants podded earliest, they also yielded more grain than uninoculated plants; yields increased with added nitrogen. Seafarer and Higuerello (dry bean type) inoculated with strain 3610 were grown in a glasshouse with ^{15}N-labelled nitrate added at sowing at rates equivalent to 0, 30 and 60 kg N/ha. The added nitrogen inhibited nodule formation and N_2-ase activity but did not affect yield. (Eaglesham, Day and Dart)

Nitrogenase of *Cicer arietinum* nodules formed at 23°C was active when assayed at incubation temperatures from 6–40°C. Activity was maximal between 20–33° and declined rapidly to be almost non-existent at 40°C. *Cicer* plants inoculated and grown continuously at 23°C were transferred either to continuous or to intermittent root temperatures of 33° and 36°C for periods up to seven days. Nitrogenase activity declined rapidly under continuous exposure to high temperatures becoming zero after 48 hours at 36°C, and only 17% of controls after 72 hours at 33°C. When the higher temperature treatment was given for 5–7 hours during the day N_2-ase activity also declined rapidly. However, plants nodulated with strain Ca-2 but not with strain Ca-1 recovered slightly from the third day of daily cycles at 33°C but not when subjected to cycles at 36°C. Plants transferred back to 23°C after 5–10 cycles at higher temperatures regained from 60–100% of their normal activity. This was associated with the formation of new red bacteroid tissue in the nodules. Plants grown with periods at higher temperatures produced less dry matter and fixed less nitrogen than those kept at 23°C. (Islam and Dart)

Nodulated plants of cowpea (*Vigna unguiculata*) grown in a glasshouse were similarly transferred at four weeks to waterbaths in which the temperature was raised to 36° and 40°C for periods of 5 h each day. The 36°C treatment did not affect N_2-ase activity but three daily cycles at 40°C decreased activity by 20% and five cycles by 90%. After a further eight days at normal temperatures activity was fully restored. A few plants were less affected by the 40°C stress, losing only 25% activity after five cycles, suggesting the possibility of breeding for resistance to temperature stress.

Plants of cowpea were fertilised with nitrogen at mid pod-fill either by spraying leaves with urea or by adding urea, ammonium sulphite or potassium nitrate directly to the roots. Nitrogenase activity assayed five days later was little affected by the 0·5% urea spray but 1–3% urea decreased activity by more than 90% and the leaves were badly scorched. Adding 25 kg N/ha to the root medium similarly decreased activity.

The symbiotic efficiency of three soyabean varieties, Chippewa, Grant and Kent, inoculated with strains CC705, CB1809 and Sm1b was examined in day/night temperature regimes of 33/30° and 27/24°C. At 27/24°C there were no effects of strain on cultivar but at 33/30°C yields of each cultivar with CC705 were less than half of those with the other strains.

In our work with tropical legumes in controlled environment cabinets the type of illumination was found to affect yields. After six week's growth, dry weights of peanut, pigeon pea and soyabean var. Kent were more than 10% larger under Warm White fluorescent tubes than under Daylight tubes, while pigeon pea and *Phaseolus vulgaris*, cowpea var. K2809 and soyabean var. Grant yielded 10% more under Daylight tubes. *Phaseolus vulgaris* var. Higuerello and *Vigna mungo* and *V. radiata* grew equally well under both kinds of tube, though soyabean and *Phaseolus vulgaris* grew taller under Warm White tubes. (Eaglesham, Day and Dart)

The effect of the composition of the root medium on the growth of *Vigna* spp. As reported earlier (*Rothamsted Report for 1972*, Part 1, 86) plants of *Vigna radiata* and *V. mungo* failed to grow or were dwarfed and abnormal in a quartz–sand and grit mixture to

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which minerals, peat, etc., had been added. Plants grew well in Rothamsted silty loam, Kettering clay loam, a neutral fen peat soil and a fen silty-loam soil. Plants that grew poorly in some Woburn sandy soils grew abnormally in those with more organic matter. The plants in Kettering loam were not nodulated presumably because much inorganic combined nitrogen was present.

Growth and nodulation was good in a sand and grit mixture amended with 10 or 30% by volume of Kettering loam; 10% addition produced better plants than those grown in Kettering loam alone, probably because the 10% mixture allowed better nodulation. Both species of *Vigna* failed to grow in 10% Kettering loam soil that was first ignited at 450°C for 4 hours to remove combined nitrogen and organic matter.

The 10% Kettering loam mixture supplied the equivalent of 30–35 kg N/ha. The effect of further addition of combined nitrogen was examined using ammonium nitrate at rates up to 100 kg N/ha, or continuously supplied at 20 ppm in culture solution to inoculated and uninoculated *Vigna radiata* and *V. mungo*. The inoculated plants without addition of nitrate grew better than the uninoculated ones given nitrogen. In glasshouse conditions additions of 40–60 kg N/ha delayed nodulation. The two species responded differently to nitrogen application. *V. radiata* yielded most and had most nodule mass when given 20 ppm nitrogen in the culture solution but most nitrogen was accumulated when 40 kg N/ha was applied. For *V. mungo* the contrary results were obtained. However, in the better growing conditions in growth cabinets nodulation was only slightly delayed, even when 100 kg N was applied, but number and weight of primary root nodules was decreased by added combined nitrogen; there was virtually no fixation above 80 kg/ha over a six-week growing period. Nodule efficiency (N_2-ase activity/g nodule) was at a maximum early in nodule development and then declined. Nitrogen fixation was stimulated in *V. radiata* by additions of up to 30 kg N and for *V. mungo* by adding 10 kg N/ha. (Islam, Dart and Day)

Leghaemoglobin. An improved method for purifying leghaemoglobin (Lb) was developed, involving gel filtration and two chromatographic separations on DEAE cellulose columns; contaminating peroxidase enzymes were reduced to a very low level. EPR of purified ferric cowpea Lb and its derivatives measured at pH 7 at liquid helium temperatures gave 'clean' spectra with the following g values:

$Lb(Fe^{III}).H_2O$	5·99	2·00
$Lb(Fe^{III}).F$	6·05	2·00 (43 gauss splitting)
$Lb(Fe^{III})_1N_3$	2·79, 2·20, 1·72	

Purified soyabean Lbs and their derivatives have similar EPR spectra. The purified acid-metLbs of both soyabean and cowpea have a very small signal at $g = 2$ at liquid nitrogen temperature, whereas the crude (i.e. ammonium sulphate fractionated) Lbs have a large signal. This signal was not associated with contaminating peroxidase enzymes, but was present in oxyleghaemoglobin ($Lb.O_2$) fractions purified by column chromatography. The signal was gradually lost during autoxidation of crude Lb, and this was associated with a corresponding increase in the $g = 6$ signal, suggesting that an Fe ligand is involved in the large $g = 2$ signal. The $g = 2$ signal was not generated by reduction of crude or purified ferric Lb by dithionite. Superoxide ion (O_2^-) may be involved in producing this $g = 2$ species and superoxide dismutase was found in the soluble (cytoplasmic) fraction of nodule homogenates. This enzyme converts O_2^- to oxygen and peroxide.

Partially purified $Lb.O_2$ from *Cicer arietinum* var. Kabuli had an absorption spectrum closely resembling that of soyabean $Lb.O_2$ (contrary to other reports). (Maskall and Dart, with Dr. J. F. Gibson—Imperial College)

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Microbial degradation of herbicides and related compounds

Investigations were directed to studying co-metabolism of mono-chlorophenols and mono-chlorobenzoates by various phenol-utilising organisms but especially by spore-forming *Bacillus* spp. Several such bacilli were isolated and tested for their co-oxidising capacity. One benzoate-grown *Bacillus* sp. was found to co-metabolise 3-chlorobenzoate by a novel pathway, in which 5-chlorosalicylic acid and 5-chloro-2,3-dihydroxybenzoic acid were intermediates. The metabolism of benzoic acid itself by this bacillus has been studied because it seemed to have a new metabolic pathway. Previously, only *Azotobacter vinelandii* was known to form salicylic acid as an intermediate between benzoic acid and catechol, but salicylic acid was detected in benzoate cultures of this *Bacillus* sp. In addition 2,3-dihydroxybenzoic acid but not catechol was found to be a metabolite of benzoate.

Work on the microbial degradation of 1-naphthol, a product of hydrolysis of the insecticide carbaryl, was continued. Traces of salicyclic acid were detected in pseudomonad cultures grown on 1-naphthol and the identity of other intermediates is being studied.

Work has also begun on the possible microbial decomposition of the herbicide Asulam and soil enrichment culture techniques based either on Asulam or the closely related sulphanilamide have been set up. (Spokes and Walker)

Staff and visiting workers

A. Eaglesham and R. MacDonald joined the department to work on grain legumes and pesticide breakdown respectively. Visiting workers included Dr. J. R. Crush of Grasslands Division, DSIR, Christchurch, New Zealand, and Miss Irene Miehlmann of the Institute for Microbiology, Göttingen University under the auspices of the International Association for Exchange of Students for Technical Experience.

J. Day visited Brazil and Centro Internacional de Agricultura Tropical Colombia and P. J. Dart the FAO/IAEA Division Vienna for consultations and discussion of international research programmes. D. S. Hayman worked for one month at the Estación Experimental del Zaidín in Granada.

Papers were read by Margaret Brown on phytohormone production by soil micro-organisms at Rome in March, by F. A. Skinner on microbial adaptation to extreme environments at Nijmegen, Holland, in June, by Barbara Mosse on VA mycorrhiza at the 'Global Impacts of Applied Microbiology' congress in São Paulo, Brazil, in July, by J. Day, P. J. Dart, P. S. Nutman and F. A. Skinner at the IBP meeting at Edinburgh in September on 'Nitrogen fixation and the biosphere', and by P. J. Dart at the 'Grain legume workshop', IITA, Ibadan, Nigeria, in November. P. S. Nutman lectured at universities in South Africa in October.

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