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ROTHAMSTED RESEARCH

Report for 1970 - Part1

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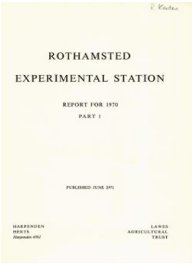

Soil Microbiology Department

P. S. Nutman

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SOIL MICROBIOLOGY DEPARTMENT

P. S. NUTMAN

The agricultural importance of soil microorganisms that are not pathogenic lies in their ability to break down and transform materials in soil, so providing nutrients for plants; they also affect conditions that influence the health of roots and react, sometimes unpredictably, to changing patterns of land use. Our three main lines of research are: (i) the transformation of nitrogenous compounds; (ii) the decomposition of natural and synthetic substances; (iii) interactions between roots and microbes.

Nearly half our programme is concerned with nitrogen fixation and nitrification; this work ranges from estimating the amount of nitrogen fixed in field crops to distinguishing the leghaemoglobins in the different groups of root nodulating plants, and from population studies of nitrifiers to measuring and accounting for the hydrogen produced by root nodules.

Collaborative work continues with the Plant Pathology Department on the growth of *Ophiobolus graminis* with reference to take-all decline, fungal nutrition and competition with the rhizosphere microflora. Work on plant-microbe interrelations includes studies on the factors affecting microbes in the rhizosphere and infection by mycorrhizal fungi which, under some conditions, can benefit crops by increasing phosphorus uptake. Among anaerobic processes studied are cellulose breakdown, nitrogen fixation and the production by anaerobes of phytotoxic substances. Work has begun on the aerobic slime-forming bacteria that break down cellulose and remove nitrate from solution.

The IBP (International Biological Programme) field and pot experiments on nitrogen fixation by lucerne were completed and work began on estimating nitrogen fixation by free-living soil microorganisms using the acetylene reduction technique. With Doncaster (Nematology Department) a film was made of the IBP experiments.

Studies on nodulation

***Rhizobium* culture collection.** Since 1969, 52 new accessions have been freeze dried, after testing on appropriate hosts. The collection now contains 345 cultures, 169 of *Rhizobium trifolii*, 41 of *R. leguminosarum*, 34 of *R. meliloti* and 102 of other groups.

TABLE 1

Rhizobium culture collection: numbers of ampoules issued in 1969 and 1970

	R. trifolii		*R. leguminosarum*		*R. meliloti*		Others	
	1969	1970	1969	1970	1969	1970	1969	1970
For research	65	12	19	24	20	28	73	64
For teaching	4	4	2	1	—	—	1	1
To farmers	—	1	—	1	—	2	—	—

Of 321 ampoules issued in 1969 and 1970, 120 were sent overseas. Table 1 shows the uses to which they were put. Details of our more important strains were sent to Dr. Hamatova in Prague for inclusion in the IBP World Catalogue of *Rhizobium* strains. (Gibbs)

Pectinolytic enzymes from the roots of red clover seedlings and their possible role in nodulation. Enzyme activity was measured in extracts prepared from the roots and on samples

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of the growth medium, using a viscosimetric assay with sodium polypectate and citrus pectin as substrates.

When seedlings were grown in distilled water and inoculated with infective rhizobial strains, slight but significant increases in the activity of root extracts occurred. However, not all infective strains caused increases, and neither nodules nor infection threads were observed over a period of 14 days after inoculation. Their roots developed poorly, which posed the possibility that pectolytic activity increased in response to conditions in the growth flasks rather than to infection.

Experiments with seedlings grown on a mineral salts medium in which roots grew well and nodulated seemed to confirm this possibility. Comparisons were therefore made between uninoculated plants grown in this medium and those inoculated with *Rhizobium trifolii* TA1 (infective organism), or with a strain of *Rhizobium lupini* (non-infective organism). All gave increases in activity, but ability to affect pectin and sodium polypectate followed different time-course patterns, suggesting that the roots produce two enzymes, possibly with different functions as well as different substrates. The medium and root extracts had pectolytic activity more with uninoculated than with inoculated seedlings, probably because enzyme was destroyed by rhizobia in the inoculated plant cultures. Interpretation of results was difficult because of differences between replicates. However, root extracts assayed against sodium polypectate showed a consistent trend, the percentage drop in viscosity increasing from a base level of approximately 10% to a maximum of 70% in the 8 hours assay, representing an increase of at least 10-fold in the initial reaction rate. With seedlings inoculated with *R. trifolii* TA1, activity did not begin to increase until 5 days after inoculation, whereas with uninoculated seedlings it began at 3 days and with those inoculated with *Rhizobium lupini* at 2 days. Altering the mineral composition of the media changed the time when the increases began, but it was always earliest with *Rhizobium lupini*, and latest with *R. trifolii* TA1.

Increases in pectolytic activity may be caused by substances that accumulate in the medium and stimulate enzyme synthesis or activation, but this seems improbable because activity was not stimulated in all media, especially not in those where the pH remained at 5 or above. An effect of pH seems more probable, possibly modified by the composition of the medium, especially calcium concentration. These possibilities are difficult to test because control of pH is difficult.

Seedlings grown under conditions which permitted nodulation provided no evidence that pectolytic enzymes are involved in nodule initiation. Nodules are few in this system, in which many seedlings are grown together in a small volume of medium; the most obtained was 140 nodules per 100 seedlings at 10 days. More work needs doing in conditions better favouring nodulation, but it seems that if pectolytic enzymes are involved in nodulation, it can only be in very small amounts possibly acting at localised sites.

Pectin methyl esterase activity did not change significantly in root extracts or exudates. (Bonish)

Chemistry of the polysaccharides of *Rhizobium trifolii*. The amounts of extracellular polysaccharide produced by different strains of *R. trifolii* in defined liquid culture differ considerably; for a group of closely related strains from 0·6 to 5·5 mg/mg dry weight of cells. Adding yeast extract to the medium did not significantly increase these amounts. The purified polysaccharide contains approximately 50% hexose, composed usually of glucose and galactose in the ratio of 4 : 1; acetyl groups were readily hydrolysed by alkali and their removal increases the rate the polysaccharide is oxidised by periodic acid. (Hepper)

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Studies on the infection of clover by *Rhizobium trifolii*. Avirulent (non-nodulating) strains of *R. trifolii*, derived from virulent ones, failed to form infection threads in the root hairs of *Trifolium glomeratum* or *T. repens*, indicating that the infection process fails at a very early stage. The amount of deformation of the root hairs of *T. glomeratum* induced by these avirulent bacteria, was generally less than that induced by virulent or heterologous strains.

All strains of *R. trifolii* used, whether virulent or not, could convert tryptophane to indolyl-3-acetic acid. (Hepper)

The occurrence of *Rhizobium* bacteriophage in soil. The distribution and abundance of *Rhizobium trifolii* in soil are closely correlated with the occurrence of clover, and there is evidence that these bacteria are transferred between fields on farm implements and in other ways (Nutman and Ross, *Rothamsted Report for 1969*, Part 2, 148–167). The bacteriophages of clover nodule bacteria which may be important in determining both the numbers and qualities of the bacteria, seem to be widely distributed in soil; little is known of their origin and spread.

To see whether phage is airborne under normal glasshouse conditions, pots containing a mixture of sand, quartz chips and vermiculite, and watered with a solution of mineral salts, were set up in a glasshouse and periodically assayed for phage; those found were typed. Half the pots were seeded with clover and half left unseeded; one quarter of each group was inoculated with one of three nonlysogenic strains of *Rhizobium trifolii*, easily identified by their susceptibily to three groups of phages; the other quarter was not inoculated.

Two days after setting up, all pots were free from phage, but after four months phage had appeared in 28% of the inoculated but in none of the uninoculated pots. Between 9 and 14 months most pots became infected with phage, whether with or without clover. Seeding the pots with clover stimulated multiplication of Rhizobium in the inoculated pots but at 14 months nearly all pots whether seeded or unseeded contained some rhizobia. Most pots contained more than one kind of phage and some contained phages that reacted with all four indicator strains. Clover bacteriophages and their host bacteria are equally common and both are probably airborne. (Kleczkowska)

Effect of temperature and combined nitrogen on nodule structure. Last year (p. 383) we reported that the temperature of the root affects nodule development and nitrogen fixation in *Trifolium subterraneum*. Light and electron microscopy were used to study the structure of nodules formed by three strains of *Rhizobium* on two varieties (one nodulating sparsely, one abundantly) when grown in sand or agar tubes at root temperatures ranging from 7°–19°C. At 19°C strain 0403 was effective in both varieties, but at 15°C it was more than twice as effective in the abundantly nodulating variety. These differences were associated with differences in nodule structure. At 15°C, the nodules of the 'sparse' variety formed infection threads but this was accompanied by disorganisation of the plant cells. Rhizobia were released in a few host cells, but they multiplied little and only a few developed into bacteroids.

At 7°C, only *Rhizobium* strain TA1 formed effective nodules, and only on the sparse host. Bacteroids and membrane envelopes developed as at warmer temperatures, but nodule degeneration was much slower. For effective combinations at other temperatures, bacteroids contained an electron-dense inclusion granule, as well as a crystalline inclusion, but rarely poly-β-hydroxybutyrate (PHBA) granules. Bacteroid morphology of nodules grown in sand or tube culture differed; nodules degenerated much more slowly in sand culture, which could account for their fixing much more nitrogen. Effec-

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tive nodules had peroxidase activity in the bacteroid membranes and in the plant cytoplasm outside the membrane envelope, but not in the space between, suggesting that, as in soyabean, this space does not contain leghaemoglobin. For host-strain combinations that did not fix nitrogen, membrane envelope formation was aberrant, very few rhizobia became bacteroids, PHBA granules were more prominent, and all host cells contained much starch. (Dart, Day and Roughley)

Giving ammonium sulphate or potassium nitrate to plants of *Trifolium subterraneum* with four-weeks old nodules caused the nodule and bacteroid structure to degenerate rapidly; changes occurred within 24 hours with the solution around the roots containing only 50 mg N/1. Degeneration began in the newly invaded host cells near the meristem and in the oldest tissue at the nodule base. Bacteroids changed from ellipsoids to spheres, and lost much of their staining density. Urea at the same N concentration caused slower degeneration, noticeable 4 days after addition.

Vigna sinensis (cowpea) nodules were also affected by compounds containing nitrogen, but plant structures degenerated first, especially the nucleus; little change was seen in bacteroid structure with the light microscope. (Day and Dart)

Root formation by legume nodules. The morphology of nodules on plants of *Trifolium subterraneum*, *T. pratense* and *Medicago sativa* at a root temperature of 20°C changed greatly when transferred to a temperature of 35°C. After 10 days, nodule meristems became much wider in the *Trifolium* sp. and much longer in *Medicago*. A few of the meristems divided to produce nodules with up to 40 lobes. Bacteroids soon degenerated and lost their ability to reduce acetylene and very few infection threads formed in the zone of newly formed tissue. After 10 days at 35°C, meristematic activity was prominent near the end of the nodule vascular bundles, and these points later developed into roots anatomically similar to normal secondary roots. (Day and Dart)

Factors affecting nitrogenase activity of legume root nodules

Incubation temperature. At temperatures up to 15°C, acetylene was reduced by nodulated roots at a constant rate for several hours and nodules maintained activity for at least 2 days. At warmer temperatures, the rate of reduction progressively slowed with time; above 35°C, enzyme denaturation is involved in this decline. From 20°–30°C, the most important factor is probably a progressive decrease in the pO_2 of the incubation atmosphere caused by the rapid respiration of the nodules and roots. Soyabean nodules at 30°C consumed more than 1 ml O_2/hour/g fresh weight nodules. Very little acetylene was reduced when O_2 concentration was less than 10%. When the pO_2 of the incubation vessel was maintained at 20%, acetylene was reduced at a constant rate until endogenous substrates became limiting. *Vicia atropurpurea* (purple vetch) nodules grown under a light intensity of 7500 lx showed effects of substrate limitation after 8 hours, so that during short days diurnal fluctuations in nitrogen fixation are to be expected. (Dart and Day)

Diurnal fluctuations. Possible fluctuations in acetylene reduction by nodules of subterranean clover, purple vetch, and soyabeans were studied with plants grown in controlled environments, where light–dark transitions are immediate. Nodules on plants grown in strong light (20 000 lx) usually reduced more acetylene during the light than during the dark period, but initially the activity/unit of nodule tissue decreased sharply at the beginning of the photoperiod. After a period of 2 hours, activity increased rapidly and then stabilised at a constant rate for the remainder of the photoperiod. This instability at the sudden transition from night to day in cabinets makes it advisable not to

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assay plants for acetylene reduction during the early part of a photoperiod. (Day and Dart)

Flowering. Acetylene reduction was followed throughout the life cycle of a typical short-day plant, soyabean (*Glycine max* var. Biloxi) and a long-day plant, subterranian clover (*Trifolium subterraneum* var. Yarloop). The plants were grown either with or without an inductive photoperiod to initiate flowering.

Three-week-old soyabeans were transferred from a 16 hour photoperiod to an 8 hour photoperiod (induced series), or were given an 8 hour photoperiod with dim light (430 lx) night break of 1 hour (non-induced series). Subterranean clover was grown in a 12 hour photoperiod, extended to 16 hours at 430 lx for the induced plants.

Soyabean nodules were visible 9 days after sowing, and developed nitrogenase activity by day 11, but Kjeldahl analyses showed that nitrogen did not move out of the nodules until day 21. Four days after transfer to inducing and non-inducing photoperiods, the total C_2H_2 reduction/plant was 50%, and activity per unit of nodule tissue, was 35% of that before transfer. After this initial decrease, activity in both series increased steadily until flowers appeared, when activity again declined, especially in the primary root nodules. The nodules of the induced series again reduced more acetylene as young pods appeared and pods and seeds grew.

Primary root nodules reduced acetylene until late in plant senescence at 14 weeks old, without the nodule shedding usually associated with flowering. Nodules were shed from the secondary roots during flower development and some further nodules formed on the secondary roots during pod set. Nitrogen accumulation followed the acetylene reduction pattern; half of the total nitrogen was fixed after flowering.

Nodules of induced and non-induced plants of subterranean clover reduced similar amounts of acetylene and fixed similar amounts of nitrogen during the early growth stages, in spite of large differences in growth habit. The pattern of nitrogen accumulation was similar in most respects to that for soyabeans, but with a much greater relative increase in nodule activity after flowering. The total activity of the nodules in the vegetative series was correlated with plant size and increased steadily with plant age.

Hydrogen production by root nodules. All legume nodules so far tested that reduced acetylene also produced some hydrogen; ineffective nodules produced only trace amounts. Hydrogen production was estimated by gas chromatography using a thermal conductivity detector, able to detect 0·1 μl H_2/ml of gas. Hydrogen was produced by effective pea and soyabean nodules, at temperatures from 10°–40°C, and by cowpea nodules from 10°–45°C; the optima for H_2 production and C_2H_2 reduction were coincident, i.e. at 40°C for cowpea, 30°C for soyabean and 35°C for peas.

The time-courses of H_2 production and C_2H_2 reduction were also similar and similarly affected by low O_2 tension. Over long periods of time the hydrogen levels encountered did not exceed 2% of the total atmosphere so that it is impossible that hydrogen production significantly inhibited either C_2H_2 reduction or nitrogen fixation.

The ratio of H_2 production to C_2H_2 reduced differed according to host plant and *Rhizobium* strain. Twenty host/strain combinations of peas (*Pisum sativum*) tested gave a range of 5·2 μM C_2H_4/μM H_2/g nodule to 63·4 μM C_2H_4/μM H_2/g nodule.

Alnus glutinosa, *Hippophae rhamnoides* and *Casuarina cunninghamiana* nodules, all non-legumes, did not produce detectable hydrogen at any temperature.

Acetylene reduction by *Casuarina* root nodules. Temperature greatly affects acetylene reduction by detached nodules from *C. cunninghamiana*. Only little is reduced at 2°C, but

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transfer to 26° after 24 hours at 2°, immediately restores the rate to that typical of 26°C. The optimum temperature for reduction is 30°–35°; some acetylene is reduced at 45° but none at 46°C. Up to 26°, ethylene was produced at a linear rate for 24 hours after detaching the nodules, but above 26° its production slowed with time. Some acetylene was reduced in an argon atmosphere, but at less than a tenth of the rate with 20% O_2 in the atmosphere. As with legume nodules, a water film on the surface of the nodules restricted acetylene reduction. Small water tension around the roots likewise limited acetylene reduction. Dim light and short days slowed reduction per weight of nodule tissue, and it slowed with time much sooner when plants were given less than 10 000 lx than when given 18 000 lx. Hydrogen and carbon dioxide concentrations up to 10% did not inhibit acetylene reduction. (Dart and Day)

Estimates of nitrogen fixed by nodulated lucerne (IBP). Experiments at five sites (two on clay loam, two on sandy soil and one on chalky loam) were concluded. Their object was to test the feasibility and usefulness of an experimental design using fertiliser (N, P, K and lime) and inoculation treatments to estimate nitrogen fixed by lucerne in the field (*Rothamsted Reports for 1967–69*). The strip feature in the design for separating the inoculation treatments to minimise contamination, and the grouping together of some fertiliser treatments to make the experiments smaller were satisfactory and enabled statistically valid comparisons to be made; pooled standard errors per plot averaged 17·9% of the mean nitrogen content of the herbage.

The use of massive inocula of an ineffective (non-nitrogen fixing) strain of *Rhizobium meliloti* to suppress effective nodulation by indigenous bacteria gave a useful second estimate of the amount of available nitrogen in the soil during the first year at some of the sites. At all sites the introduced ineffective strain was overgrown by effective bacteria during the second year. The non-legume (ryegrass) was therefore used to assess amounts of available soil nitrogen and these values were subtracted from the nitrogen contents of the lucerne herbage to give estimates of fixation listed in Table 2.

TABLE 2

Minimum estimates of nitrogen fixation kg N ha^{-1} in first and second years, by indigenous bacteria (O), or introduced effective strain (E) with (M) or without soil amendment (U)*

			Rothamsted		Woburn		Stopsley Chiltern hills
			Stackyard 3 cuts	Pastures 3 cuts	Stackyard 3 cuts	Bare fallow 3 cuts	3 cuts
First year	O	U	70	0	92	32	40
		M	97	43	108	32	33
	E	U	132	91	91	86	59
		M	230	153	162	116	47
			4 cuts	2 cuts†	2 cuts	2 cuts†	3 cuts
Second year	O	U	220	18	190	24	90
		M	306	58	213	0	90
	E	U	235	34	157	57	97
		M	342	70	239	40	116

* The M treatment added lime (to bring soil pH to 7·0), P and K
† Overgrown by weeds

The lack of enough good bacteria in the soil limited fixation, especially during the first year. This limitation differed between sites and was greater where lime and PK were not given; it was remedied by inoculation with the effective strain. A smaller benefit from

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inoculation continued into the second year and this was usually increased by improving the host's nutrition.

Except where fixation was negligible because of flooding and weed infestation in the second year at two sites, these estimates fell within the ranges reported in the literature. Parallel experiments using undisturbed cores of soil mounted in pots gave results very similar to those of the first year in the field but the binding of roots within the small core volume prevented its use beyond this period. The results of the two kinds of experiment were so well correlated that a core experiment would give nearly as much information as the full-scale field trial on the principal factors limiting fixation, and on amounts of nitrogen fixed. (Bell and Nutman)

Mycorrhiza and rhizosphere studies

Staining mycorrhizal roots. Two improved procedures were developed for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for assessing infection rapidly. Non-pigmented roots, fresh or fixed in formalin–acetic–alcohol, were heated at 90°C for 1 hour in 10% KOH to remove host cytoplasm and nuclei. After rinsing in water and acidifying with dilute HCl, they were stained by simmering for 5 minutes in 0·05% trypan blue in lactophenol. This gave excellent differentiation of fungal structures in whole onion roots. Larger or older roots usually needed more than 1 hour in 10% KOH. Pigmented roots were heated in 10% KOH for at least 2 hours, washed with fresh KOH, and immersed in an alkaline solution of H_2O_2 (approx. 10 vol) at 20°C until bleached (10 minutes–1 hour); they were then rinsed thoroughly in water, acidified in dilute HCl, and stained as already described. Even heavily pigmented roots, such as those of Sitka Spruce, were successfully cleared and bleached to reveal infections by fungi such as *Olpidium* sp. (Phillips and Hayman)

The establishment of *Endogone* mycorrhiza in agar media. Mycorrhizal infections have until now been established in mineral media in aseptic conditions, only when plants were nitrogen deficient and fungal penetration was assisted by some means, most reliably by inoculation with a *Pseudomonas* sp. (*Rothamsted Report for 1961*, 80). However, clover seedlings (*T. parviflorum*) readily became infected without the aid of bacteria in a medium containing, in g/l, 0·5 KNO_3, 0·2 Mg SO_4, 0·2 $CaCl_2$ (hydrated), 10·0 activated charcoal, 15 agar, 0·007 Fe (as Fe-EDTA) and 0·6 $CaHPO_4.2H_2O$ or calcium phytate; pH adjusted to 6·5 before autoclaving. The effects of calcium phytate, inositol, glucose-6-phosphate and calcium phosphate are also being tested. (Phillips and Mosse)

Turner (*Ann. Bot.* (1955), **19**, 73) reported that charcoal contains toxic substances (removable by alkali-acid washing) that stunt clover seedlings grown in Jensen's medium, but failed to produce the stunting with various charcoal extracts. Adding charcoal to a mineral medium containing $FeCl_3$, which we used to establish mycorrhizal infection in clover seedlings, stunted the clover and made it chlorotic. However, when the stunted plants were given Fe-EDTA they recovered, and when the original medium contained it the plants grew normally; indeed given Fe-EDTA plants with added charcoal, whether alkali-acid washed or not, then grew better than those without. These effects were found with two sources of charcoal that differed in their deleterious effects without Fe-EDTA. (Phillips)

Specificity of *Endogone*. Single strain cultures of seven *Endogone* spore types on various host plants are now established in pots. New stock plants must be made each year because few spores and sporocarps are produced in pots kept for two years and none

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after three years, although root infection persists. Some soils specially favour spore formation by some *Endogone* species. Three new, so far undescribed, spore types were found in Brazilian soils and it is hoped to establish these also in pots. A spore type from Nigeria did not become established and may require tropical conditions.

Different spores types affected the growth of onions and ryegrass very differently in the same soil (up to four-fold increase in weight with onions) and the effect of the same spore type on the same host differed in different soils. In general the yellow vacuolate and laminate spore types have similar effects and these can differ greatly from the effects of reticulate spore-type mycorrhiza. Responses may also be affected by other micro-organisms carried on the spore inoculum because washings from different spore types also had different effects on the growth of ryegrass. Spore types that differ in the nature of their outer walls may carry different bacterial floras. (Mosse)

Influence of daylength and light intensity on *Endogone mycorrhiza*. The establishment and development of mycorrhiza in onion roots and the influence of mycorrhizal infection on plant growth were studied in controlled environment cabinets at 18°C with three day-lengths (6, 12 and 18 hours) and two light intensities (12 000 lx and 24 000 lx). In irradiated soil containing little phosphate, infection from *Endogone* inoculum developed faster with more light. Arbuscules were larger at the higher light intensity and there were more vesicles at 24 000 lx and with longer daylengths than with 12 000 lx and in shorter days. *Endogone* stimulated plant growth within 2 weeks with 18 hours of 24 000 lx, but not until around 4 weeks with shorter days. The largest growth increase (ratio of dry weights of mycorrhizal plants to non-mycorrhizal plants after 10 weeks) was 14·2 (18 hours, 24 000 lx), and the smallest was 3·5 (6 hours, 12 000 lx). When given $Ca(H_2PO_4)_2$ (0·2 g/kg soil), the non-mycorrhizal plants were about 50% heavier than the mycorrhizal plants in 18 and 12 hours light. With 18 hours of 24 000 lx, mycorrhizal plants not given phosphate were 50% heavier than mycorrhizal plants given phosphate. (Hayman)

Effect of *Endogone* mycorrhiza on plant growth. Last year's report that mycorrhizal infection increased phosphorus uptake and improved the growth of onions in soils deficient in phosphate was confirmed with both irradiated and unsterilised soils. The sources of extra phosphate taken up by the fungus are being studied by comparing the uptake by mycorrhizal and non-mycorrhizal plants of relatively insoluble sources of phosphate that occur in soil; and by measuring the relative uptake of ^{32}P-labelled phosphate added to soil, after an equilibration period.

In sand given a nutrient solution lacking P, mycorrhizal and non-mycorrhizal onions grew equally well with $Ca(H_2PO_4)_2$, $CaHPO_4$, sodium phytate, calcium phytate, phytin, lecithin, ATP and DNA. With glucose-6-phosphate and RNA, the non-mycorrhizal plants were about 50% heavier; with gafsa rock phosphate the mycorrhizal plants were more than twice as heavy. Adding 10% of sterilised soil to the sand had little effect on the growth of the mycorrhizal plants but sometimes halved growth of the non-mycorrhizal ones; adding it to sand containing rock phosphate, more than doubled the weight of both mycorrhizal and non-mycorrhizal plants. (Hayman)

The specific activity of mycorrhizal plants grown in two ^{32}P-equilibrated soils was greater than that of non-mycorrhizal plants, indicating that the fungus probably used phosphate from the labile pool and not from the unlabelled organic soil phosphorus. (Mosse and Hayman)

The effects of additional phosphate ($Ca(H_2PO_4)_2$) on the host-fungus balance was studied in irradiated and unsterilised soils. In pots containing soil in which the freshly

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added phosphate was uniformly distributed and nitrogen and potash were supplied weekly, there was an optimum amount of phosphate for maximum plant growth. For non-mycorrhizal plants in several irradiated soils the optimum usually lay between 0·5 and 1·0 g ($Ca(H_2PO_4)_2$/kg soil, but for mycorrhizal plants it was much smaller (0·2–0·4 g), especially in the sterilised soils. With phosphate exceeding the optimum for mycorrhizal plants, infections seemed to become pathogenic (corresponding non-mycorrhizal plants were heavier) and more vesicles formed; with even more phosphate, neither vesicles nor arbuscules formed and the intercellular hyphae became much finer and ramified throughout the cortex. With phosphate additions of 1·5–3·0 g/kg soil, the infections died out, although the fungus remained viable in the soil and caused new infections when the phosphate was diluted by adding fresh soil. Possibly changes in soil pH produced by the $Ca(H_2PO_4)_2$ contributed to the fungal response. (Mosse)

Bacteriostasis versus rhizosphere stimulation. Several hundred bacterial isolates from the rhizosphere and rhizoplane of wheat plants and from root-free soil were assayed for their sensitivities to rhizosphere stimulation, using the buried slide technique, and to bacteriostasis, by incubating suspensions of them on agar discs in contact with soil; techniques were described in *Rothamsted Report for 1968*.

All isolates from the root region and 40% from plant-free soil were stimulated by roots and inhibited on agar discs over soil, whereas 60% of the plant-free soil isolates were neither stimulated by roots nor inhibited on agar discs over soil. The root region therefore provides a habitat where inhibitory factors are overcome and where the nutritional requirements of a physiologically active group are fully met. Away from the root, these bacteria do not multiply either because the soil contains an active inhibitor or because essential nutrients supplied only by living roots are missing.

To test whether inhibition of root-region isolates by soil might be caused by lack of nutrients in the discs, the discs were prepared with added sugar, casamino acids and nutrient agar with 1·0% glucose, also with root exudates from wheat seedlings grown aseptically either in distilled water or in a complete mineral solution. Only the two kinds of root exudate and sterile mineral solution overcame the soil inhibition.

Air-drying and re-moistening the soil before testing the discs, diminished but did not remove the inhibition. Sterilising the soil by heat removed the factor and increased growth beyond that on discs without soil.

Hence, inhibition is probably of microbial origin. Tests for inhibition were also made with cold-water extracts of soil filtered through a range of millipore filters and then incorporated in discs. Bacterial growth was stimulated by filtrates passing the 0·22 and 0·65 μm pores, was unaffected by the 1·2 μm filtrates and inhibited by the 3·0 μm filtrates. This also points to the inhibitory affect being of microbial origin and resembling the inhibition of fungal growth by soil, which is also overcome by living roots. Thus, the stimulation of microbial growth in the rhizosphere may be explained partly as removal of inhibition and partly as a provision of nutrients. (Brown)

Plant growth-regulating substances produced by microorganisms from soil and rhizosphere. Microorganisms isolated from rhizosphere and rhizoplane of 6, 42 and 82 day old wheat plants, and from plant-free soil produced plant growth-regulating substances with the properties of gibberellins and IAA. Substances inhibiting extensions of pea internodes and lettuce hypocotyls were also produced, especially by bacteria from the root region of 6 day seedlings. Bacteria producing growth promoters were most abundant on roots of older plants. Plant development may be influenced by microorganisms producing growth regulators in the root region where they are most readily absorbed

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by plants. Seedlings grown aseptically with added GA3 and IAA, or grown with a soil inoculum, develop similarly but differ in their morphology from those grown aseptically without additives. (Brown)

Studies on nitrification, soil anaerobes and on cellulose and pesticide decomposition

Ecology of nitrifying bacteria. Selected plots of Broadbalk, Barnfield and Park Grass were surveyed for numbers and types of autotrophic nitrifying bacteria. The modified Winogradsky plate counting technique used has the advantage (over 'most probable number' methods) that the organisms can be counted, isolated and identified. Contrary to common assumptions, *Nitrosomas* species were not always the dominant nitrifiers in arable soils and were frequent mainly in soils that had received organic manures. Species of *Nitrosocystis* and *Nitrosospira*, hitherto regarded as rare, were commonly isolated. (Walker and Soriano)

Microbial degradation of chlorine-containing organic compounds. Further work on the co-oxidation of chlorobenzoic acids by benzoate-grown *Azotobacter* organisms, partly in collaboration with Briggs of the Chemistry Department, confirmed that these organisms formed 3-chlorocatechol from 3-chlorobenzoic acid.

Some bacteria from soil, able to use propane, were used to see whether they degrade the nematicides 'D-D' and 'Telone', the active ingredient of which is a dichloropropene. Results in Warburg manometers, however, were indecisive, because of the difficulty of making respirometer measurements with volatile substrates. (Walker and Fletcher)

Nitrogen-fixing clostridia. Total counts of clostridial spores (DRCM method) are often smaller than counts of nitrogen-fixing types. Eight strains of *Cl. butyricum*, some strains of which can fix nitrogen, and four strains of *Cl. pasteurianum* from different culture collections were tested for growth in DRCM at 35°C and 25°C and for ability to blacken the medium as is characteristic of clostridia.

Though *Cl. butyricum* does not always reduce sulphite to sulphide efficiently, all strains blackened the medium within 3 days at 35°C, and six strains did so within 7 days at 25°C. One *Cl. pasteurianum* strain blackened DRCM within 2 days at 35°C, the others took from 7–14 days. At 25°C no blackening occurred before 15 days; two strains had not caused blackening by 20 days. Failure to blacken the medium was not caused by any inhibitory action of the sulphite.

Hence, some nitrogen-fixing clostridia are missed in media designed for total counts of clostridia in environments other than soil.

Survey of *Clostridium botulinum* in soils. Total clostridial spores in 25 soils from Rothamsted, Suffolk and Oxfordshire were counted as part of a joint project with Dr. T. A. Roberts (A.R.C. Meat Research Institute, Langford, Bristol) on the distribution of these spores in soil. Parallel samples were analysed at Langford for aerobic bacterial spores and for the incidence of *Cl. botulinum*.

Spores were not numerous; numbers ranged from $4{\cdot}28 \times 10^3$/g dry soil for Broadbalk, Plot 19 to $6{\cdot}25 \times 10^5$/g dry soil for Broadbalk Plot 2B. Very dry soil during June, when most samples were taken, may explain the small counts. Total counts and organic matter content of the soils were correlated ($r = 0{\cdot}57$). Numbers of aerobic spores were remarkably constant in the 25 soils sampled, ranging only from $2{\cdot}1 \times 10^5$ to $1{\cdot}8 \times 10^6$/g dry soil. *Cl. botulinum* was not detected in any soil. (Skinner)

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Cellulose decomposition by aerobic bacteria. To investigate the rates at which cellulose is decomposed, enrichment cultures were prepared using mineral salts solution containing nitrate and cellulose and seeded with soil or with activated sewage sludge. Microbial attack on cellulose particles was slow in static or aerated flask cultures with inocula of soil, and the concentration of nitrate in solution declined slowly. With a small inoculum of sewage sludge, cellulose was attacked rapidly in the aerated culture. After 3 days the cellulose particles became sheathed in zoogloeal bacterial growth and aggregated into floccular masses that settled rapidly. After 6 days no nitrate was found in solution.

Because of interest in nitrate polluting water supplies, and the difficulty of removing it from solutions biologically, this relationship between sheathed cellulose particles and nitrate absorbtion was investigated further using aerated cultures seeded with sludge. At room temperature (18°–25°C), cellulose particles began to aggregate into flocs by 39 hours and most settled from suspension when undisturbed for 1 minute, leaving a fairly clear supernatant solution containing slightly less nitrate and total nitrogen than was present originally. After 63 hours almost all particles were sheathed in bacterial growth and most of the nitrogen had disappeared from the solution. The rapid settling or filtration of the cellulose with its attached micro-organisms effectively removed the assimilated nitrogen and most of the biomass.

At 22°C the pattern of events was similar, but the cellulose was attacked faster and the particles were uniformly coated with bacteria by 34 hours; nitrate disappeared from solution by 48 hours.

At 18°C zoogloea started to form early but floccular masses developed more slowly. Between 54 and 72 hours, when the rate nitrate was removed was linear, nitrate was assimilated at 1·76 μg/ml of culture filtrate/hour (7·79 p/m of nitrate ion/hour).

A small amount of soluble carbohydrate initially present, as a contaminant of the cellulose, remained fairly constant during bacterial development, presumably by diffusion of some products of cellulose digestion from the sheathed particles. When nitrate was exhausted, the concentration of soluble carbohydrates began to increase.

Washed cellulose particles with zoogloeal coatings were suspended in tap water containing 0·25 g of KNO_3/l and incubated at 22°C with aeration. The solution began immediately to lose its nitrate and this proceeded at a fairly linear mean rate of 0·89 μg/ml of NO_3-N/hour. Loss was much faster when washed pregrown cellulose floc was incubated in a stirred flask at room temperature. In all experiments total N in solution declined with nitrate; ammonia and nitrite were not found.

The cellulose was at first digested slowly but, after more than one week's incubation, cellulose had disappeared leaving empty zoogloeal sheaths. The identity and distribution of the organisms responsible for this type of cellulose digestion, and the conditions necessary to form stable zoogloeal growth, are not yet known. (Skinner)

Staff and visiting workers

N. Walker attended and read a paper at Ghent, Belgium, on 'Interactions of pesticides and herbicides with soil microflora and microfauna'. He also spent six weeks at the Biologisches Institut der Universität Freiburg, Germany. J. Day, P. J. Dart, J. Kleczkowska and P. S. Nutman presented papers at the IBP meeting on Nitrogen Fixation at Wageningen, Netherlands; Kleczkowska and Nutman also took part in the 1st International Symposium on Genetics of Industrial Microorganisms at Prague. Nutman contributed to the V Reunião Latino-Americana de Rhizobium at Rio de Janeiro and also lectured in Argentina, Uruguay, Eire and Portugal.

Visiting workers from overseas included Dr. H. Marecková, Institute of Plant Nutri-

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tion, Prague (under the auspices of IBP), Dr. L. Raicheva, Academy of Agricultural Sciences of Bulgaria, Sofia (British Council), Professor S. Soriano of the University of Buenos Aires (Ministry of Overseas Development), Dr. J. Döbereiner, I.P.E.A.C.S., Rio de Janeiro, Brazil (British Council), Mr. R. Islam of University of Dacca (Dawood Foundation), and Dr. I. Cacciari of the Laboratorio di Radiobiochimica ed Ecofisiologia vegetale, Rome.

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