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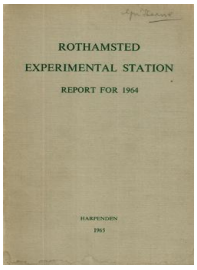

Soil Microbiology Department

P. S. Nutman

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SOIL MICROBIOLOGY DEPARTMENT

P. S. NUTMAN

R. Cooper left to join the staff of the Hatfield College of Technology. Dr. Zora Saric of the Agricultural Faculty, Novi Sad University, Jugoslavia, was in the Department for three months studying antibiosis by rhizosphere micro-organisms. Miss Cristal König and Mr. K. O. Lippert, of the Institute of Microbiology, Göttingen, assisted for some weeks with the work on nitrification and decomposition; their visit was arranged under the scheme for the exchange of students to gain technical experience. N. Walker attended the Symposium on "Enrichment Cultures and Selection of Mutants" organised by the Deutsche Gesellschaft für Hygiene und Mikrobiologie at Göttingen. P. S. Nutman was a delegate at the First I.B.P. General Meeting at Paris. Barbara Mosse attended the 10th International Botanical Congress in Edinburgh and Margaret Brown the 8th International Congress of Soil Science at Bucharest. J. Darbyshire was awarded the Ph.D. degree of the University of London.

On R. Cooper's departure, work on nodule metabolism was suspended; all other lines reported on in previous years were continued. Those of direct agricultural importance include studies on interaction between plant and soil microbes, on the nitrogen cycle and on some processes of decomposition occurring in soil. Three kinds of plant–microbe association were investigated: the vesicular–arbuscular type of mycorrhiza, which is symbiotic and probably of benefit to the host plant; the symbiosis between bacteria and legumes in root nodules which is important because it fixes nitrogen; and the flora of the rhizosphere, with particular reference to the effect on plant growth of establishing Azotobacter in the root zone.

Work on decomposition was concerned with the fate of organic chemicals, such as herbicides, that enter soil in increasing quantities, and anaerobic cellulose decomposition. The microbial changes in spoiled hay responsible for farmer's lung disease were also studied in collaboration with other departments and institutes.

Inactivation of a Rhizobium bacteriophage by ultra-violet radiation of different wavelengths. Rhizobium bacteriophage was much more susceptible to inactivation by ultra-violet radiation than tobacco mosaic or tobacco necrosis virus or even their ribose nucleic acids when freed from their proteins. The fact that at wavelengths between 240 and 290 mμ its action spectrum for inactivation resembles the absorption spectrum of thymus deoxyribose nucleic acid (abstract 4.3) can be taken as evidence that in this range of wavelengths inactivation is caused by the ultra-violet radiation affecting the phage DNA. (Kleczkowska)

Genetic studies with *Rhizobium trifolii*. Studies on the transformation of nitrogen-fixing effectiveness in clover nodule bacteria were continued,

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combining treatments with bacteriophage and transforming DNA obtained from effective and ineffective bacterial strains. Three bacterial strains were used; an effective strain A, an ineffective variant f12 of strain A and an unrelated ineffective strain HKC. All strains were isolated from single colonies and were known from long experience to be stable with respect to effectiveness. The proportion of phage-resistant cells in all these strains was about 0·001%. Ineffective variants were readily isolated from a culture of strain A after treatment; for example, when transforming DNA from an ineffective strain was used the treated culture contained 15% of cells that were ineffective in fixing nitrogen, and after lysis with phage, 68% of the phage-resistant colonies were ineffective. Most variants came from treating the effective strain with both phage and transforming DNA, when 85% of isolated cells were changed in their effectiveness.

By contrast, the symbiotic properties of the ineffective strains f12 and HKC were much more stable. Effective cells did not develop in cultures treated with transforming DNA of the effective strain A. Of the colonies of strain f12 produced by survivors from phage treatment, only 75% were changed in symbiotic properties and could fix nitrogen; all phage-resistant colonies of strain HKC remained ineffective. Treating f12 with both phage and DNA produced fewer effective variants than exposure to phage alone. Thus, the change from effectiveness to ineffectiveness seems largely irreversible. The bacteria remaining after DNA and phage treatment are at first all phage-resistant, but when subcultured on phage-free medium the cultures tend to regain susceptibility to phage, without affecting their nitrogen-fixing properties. (Kleczkowska)

Rhizobium culture collection. All but a few of our own strains have been tested and dried, and gaps in our collection are being filled by cultures from collections abroad.

The following are now in the dried form:
Rhizobium trifolii, 156 strains; *Rh. meliloti*, 14 strains; *Rh. leguminosarum*, 16 strains; *Rh. lupini*, 4 strains; *Rh. japonicum*, 6 strains; *Rh. phaseoli*, 3 strains; *Rh.* spp. (Lotus group), 3 strains; *Ph.* spp. (Cowpea group), 9 strains.

Thirty-one cultures (mostly dried) were sent on request to Universities and Research Institutes and 312 cultures on agar to growers, mostly for inoculating clovers, peas and lupins. Only the culture for lucerne can now be obtained commercially.

Strains of *Rh. trifolii* dried in 1963 and stored for a year at 2° C retained their viability and their symbiotic properties were unchanged. To test bacterial strains, plants with small seeds are grown aseptically on an agar medium in test-tubes; plants with larger seeds are grown in pots, with precautions to exclude contamination. Aseptic pot cultures are difficult to maintain for long periods, and experiments are in progress on the culture in boiling tubes of some legumes with large seeds from which the cotyledons are first removed. The time the cotyledons are removed is critical; when too early or too late, nodulation and plant growth are impaired; when too late, the seedling growing in a boiling tube may derive too much nitrogen from the cotyledons to show a response to nitrogen

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fixation by nodules. With peas (var. "Meteor") the best time to remove cotyledons is 5 days after germination. Nodules appear on such plants after 3 weeks and yield differences between control and effectively nodulated plants become statistically significant (using three replicate cultures) after a further 2 weeks, though it is advisable to leave the plants for longer. (S. Bascomb)

Other micro-organisms we use are also being dried. *Azotobacter chroococcum*, strain A6, survives better in ampoules at 2° C than at 26° C; 10% sucrose, with or without 5% peptone, are equally satisfactory as a suspending fluid. (S. Bascomb and Jackson)

Varietal differences in the nodulation of subterranean clover (*Trifolium subterraneum L.*) The detailed study of the inheritance of patterns of nodulation in subterranean clover, started during the tenure of a C.S.I.R.O. Senior Research Fellowship at Canberra from 1953 to 1956, was completed in 1964. The time when nodules first appear, and their number and size, were examined in fifteen varieties of subterranean clover and their hybrids. Autotetraploid lines and normal diploids of five varieties were also compared.

The time the first nodules form differs little, but significantly, between varieties. The earliest varieties to form nodules, "Wangaratta" and "Yarloop", did so 2–3 days before the latest nodulating varieties, "Dwalganup" and "Northam First Early". Within a variety all plants nodulated at almost the same time, and ploidy did not affect the time of initial nodule formation. This uniformity in subterranean clover contrasts with the large variation in this characteristic shown by red clover (*Trifolium pratense*), occasional plants of which do not nodulate until 4 weeks after nodules form on the early plants. Bacterial strains also differ by a few days in the time they first nodulate subterranean clover; these strain and host differences are independent.

F_1 plants of crosses between varieties form their first nodules about 12 hours earlier than their parents, and the mean initial nodulation time of F_2 plants is intermediate between that of the parents. Attempts to select for earliness and lateness beyond the parental limits failed, except in one cross ("Tallarook" × "Morocco") which segregates dwarf plants that are also late in nodulating. In this hybrid these characteristics are inherited as a simple recessive. In others many genes probably affect the time of initial nodulation.

The varieties differ widely in the number of nodules formed. For example, "Wangaratta" has more than six times as many as "Pink Flower". Differences in nodule number between plants of a variety are small (S.E. 1–4% of mean), but some varieties (for example, "Dwalganup") are consistently more variable than others (for example, "Mount Barker"). The autotetraploid varieties form a third fewer nodules than their diploids. In hybrids the habit of abundant nodulation is dominant over sparse nodulation, and the range of nodule number in F_2 plants is usually greater than in the parents. In all hybrids studied, selection for nodule abundance in subsequent selfed generations increased mean nodule number above that of either parent. In only one hybrid ("Cranmore" × "Tallarook") was

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selection successful in decreasing nodule number below that of the sparse parent; in others, the number of nodules on the sparse lines and sparse parents were the same. These results indicate that nodule number is inherited polyfactorially. Dwarfs segregating in the "Tallarook" × "Morocco" cross formed about half as many nodules as normal plants; the simple factor for dwarfness presumably overshadows the other factors that also affect nodule number in this cross. No selection for nodule number was practised in this hybrid.

In general, varieties that form many nodules also form many lateral roots, but the connection between rooting habit and nodulation is not so close as in red clover. In some hybrids selection for nodule abundance also produces plants with many lateral roots, and vice versa, but in others selected lines forming many or few nodules have intermediate numbers of lateral roots.

The proportion of nodules that form on lateral roots is small (25%) when nodules are very few (less than 10). Plants with 20 nodules have about 40% of their nodules on lateral roots, and further increase to 100 nodules and above only increases this proportion to about 45%. On sparsely nodulating plants most nodules form on the main root because the lateral roots which start to form at about the same time have to grow and form root hairs before they can become infected.

All varieties and lines were also examined with strains of bacteria less effective than SU 297, and with a strain that was totally ineffective (C1F). These strains also differed widely in the numbers of nodules they produced; host and strain differences were generally not independent.

Nodule number and size are inversely related in subterranean clover, as in many other legumes. This relation was studied not only by measuring nodule length on a wide range of material but also in more detail with some hosts and bacterial strains by measuring the length and diameter of nodules and some internal features of nodule structure.

The relation between average nodule length and number was always hyperbolic in form (i.e., aggregate nodule length tends towards a constant value that is characteristic of a particular combination of host and strain). With effective strains of bacteria, whatever their origin, the hyperbolae are host-dependent. After crossing, the data for nodule length and number for the F_1 and F_2 generations give points that lie between the parental hyperbolae, and those for abundant and sparse lines of later generations give hyperbolae that extend the curve of one or other of the parents towards its asymptote. Nodule diameter is highly correlated with nodule length, and has a hyperbolic relation with number.

Some strains of poorly effective and ineffective bacteria give hyperbolae relating length and number that coincide with those for effective strains, but with others they are different. For example, the ineffective strain C1F forms nodules that are both more numerous and much larger than those produced by effective strains. One such instance of divergent behaviour was of special interest because the strains concerned formed the same number of nodules, but of different sizes, on one of two varieties that were later hybridised ("Mount Barker" and "Northam Second Early" inoculated with strains SU 297 and SU 220). Selected lines after hybridisation gave

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two distinct hyperbolae. About equal numbers of plants gave points on each curve, suggesting that this characteristic, determined by both nodule size and number, is simply inherited; no other example of this kind was met.

Whereas nodule number was found to vary several-fold, aggregate nodule length varied less than two-fold and total nodule volume, calculated from measurements of length and diameter (assuming a nodule to be cylindrical with hemispherical ends), varied less than half; abundantly nodulating selections have the larger total nodule volume.

When the volumes of the different parts of the nodule were calculated from measurements of median longitudinal sections of nodules, the total amount of bacteroid tissue formed on a plant by an effective bacterial strain was substantially the same, irrespective of the number of nodules formed. In the conditions used, this was 51·5 units (volume $\times$ duration) in sparsely nodulating lines and 49·5 units in abundantly nodulating lines; the average rate of nitrogen fixation per mm^3 of bacteroid tissue was 22 μg nitrogen per day.

These results indicate that the plant determines the amount of nitrogen fixed by controlling the volume of bacteroid-containing tissue, and that the efficiency of a unit of this tissue in the plants examined is constant. Earlier physiological work suggested that the mechanism by which the plant controls nodule number and size acts through meristem initiation and inhibition, and is self-regulatory. (Nutman, P. S. (1958) in *Nutrition of the legumes*, ed. E. G. Hallsworth, London: Butterworths, pp. 87–107.)

The measurements of nodule sections also showed that the relative proportions of the principal parts of the nodule are only slightly affected by selection for nodule number and are independent of bacterial strain. Thus, with the effective strain SU 297, the percentage of the nodule that is infected (whole nodule less meristem and cortex) was 34·5% in sparse lines and 32·5% in abundant lines. Even with the ineffective strain C1F, which produces a much larger aggregate nodule volume and a larger zone of degenerate tissue, the proportion of infected tissue was about the same, 38·0% and 37·5% respectively. (Nutman)

Metabolism of succinate by root nodules. The oxidation of succinate by oxygen or by 2,6–dichlorophenol indophenol was measured in fractions of pea nodules separated by low-speed centrifuging. With each hydrogen acceptor, the bacteroid fraction was very active and its endogenous reducing activity very small. However, the supernatant fluid had a large endogenous reducing activity (35–40% of the bacteroid activity with succinate), which was little affected by adding succinate. Thus, the powerful succinate-oxidising ability of these nodules resides mainly in their bacteroids, and the respiration of the supernatant fluid (which is mainly of host origin and contains haemoglobin) is largely independent of succinate. (Cooper)

Fluorescence-microscopy of clover roots and nodules. The fluorescent antibody technique was used to study the antigenic reactions of clover root-hair surfaces, and to try to identify the origin of the infection thread

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matrix. Antisera were prepared against two strains of *Rhizobium trifolii* (C1F and SU 297). The strains had no somatic antigens in common, but they shared a flagellar antigen. Root-hair surfaces of seedlings inoculated with either strain, and their attached rhizobia fluoresced strongly when treated with the homologous antiserum, but with the heterologous antiserum they showed only very small, but brightly fluorescent spots where bacteria were attached to the root surface, suggesting that attachment is at the flagellar end of the bacterial cell. The bacteria usually showed a polar attachment to the root surface. Flagellar attachment is also indicated by recent electron-microscope evidence (Dart and Mercer. *Arch. Mikrobiol.* (1964) **47**, 344). The root surface free from rhizobia gave a weak non-specific fluorescence, and the root-hair nuclei a somewhat stronger fluorescence. (Mosse)

Vesicular–arbuscular mycorrhiza. The conditions that induce *Endogone* spores to germinate and the changes in their fine structure during the induction period were studied in detail. Of particular interest is the origin of mitochondria that are evident in the germinated but not in the dormant spore. The factor, or factors, that induce spores to germinate in aseptic conditions is not volatile, and it diffuses into sterile agar or glass paper, which strongly adsorbs it. Solutions of the factor can be obtained by incubating soil-agar plates or small amounts of soil in a shallow layer of water and removing the soil and contaminating micro-organisms by high-speed centrifugation. Direct soil extracts did not induce germination, and *Endogone* spores placed on soil, either directly or with an intervening layer of cellophane, did not germinate.

Host specific reactions to infection by *Endogone* spores were found in two clover species grown aseptically in an agar medium. In *Trifolium parviflorum* the usual stages of a vesicular arbuscular infection developed and extended with the growing root, whereas the more numerous infections in *T. patens* remained confined to short lengths of root and were largely intercellular, i.e., very few arbuscules developed. The external mycelium in the agar was equally well developed with each host, but infected roots of *T. parviflorum* were longer than uninfected ones (*Rothamsted Report* for 1961, p. 80), whereas in *T. patens* they were not. Thus root elongation seems to be stimulated by the cellular phase of the infection, rather than the activity of the external mycelium. (Mosse)

Fungistasis in soil. Whether soil contains a fungistatic principle that inhibits the germination of fungus spores has been questioned by Lingappa and Lockwood (*J. gen. Microbiol.* (1961) **26**, 473–485; (1964) **35**, 215–227), who consider that inhibition reflects the activity of organisms stimulated by nutrients leached from the spores or derived from assay substrates. Lockwood (*A. Rev. Phytopath.* (1964) **2**, 341–362) further suggests that depletion of the spore nutrients may be a more important cause than inhibitory organisms in preventing fungus spores from germinating in soil.

Stained impression-preparations of spores of *Penicillium citrinum* and *Aspergillus niger*, added to the surface of natural soil, were made on cover

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slips and examined, but gave no evidence that micro-organisms multiplied near the inhibited spores; increased microbial activity without multiplication is unlikely.

Fungistasis was assayed by placing on the soil surface pieces of glass-fibre filter-paper inoculated with spores. *P. citrinum* spores failed to germinate, whereas two-thirds of similar spores on glass-fibre paper moistened with distilled water germinated within 16 hours. The measurement of fungistasis by indirect assay methods therefore is not dependent on substrate nutrients. In another experiment agar discs containing 0·5% peptone were placed on filter-paper inoculated by brief contact with moist unsterile soil. Germination on these dics was not impaired, but it was inhibited when the filter-paper remained in contact with soil during the incubation period. These results confirm earlier ones and show that fungistasis is not caused by local stimulation of inhibitory organisms. (Jackson)

Root surface fungi. As a preliminary to studying the colonisation of roots by introduced rhizosphere organisms the take-all fungus *Ophiobolus graminis* was grown in mixed cultures with a range of root-surface fungi. Wheat seed was inoculated with spore suspensions of fungi selected for their antagonism towards *O. graminis* and sown in small pots of unsterile quartz grit, with or without an inoculum of *O. graminis*. After 2–4 weeks' growth in the glasshouse, roots were examined microscopically after clearing and staining. Inoculating seed with either of two strains of *Cylindrocarpon radicicola*, or with a species of *Septonema*, decreased root necrosis by 68–100%. The degree of infection as indicated by the presence of *Ophiobolus* lignitubers was decreased correspondingly. However, when wheat seed inoculated with one of the isolates of *C. radicicola* was sown in soil naturally infested with *O. graminis* it failed to protect the seedlings from infection. (Jackson)

The effect of Azotobacter on plant growth. Tomato plants grown at the Lee Valley Experimental Horticultural Station were treated with Azotobacter cultures and effects on flower development observed. Plants of two varieties, "Ware Cross" and "Potentate", were inoculated at the cotyledon stage with 10 ml of 7-day-old Azotobacter culture placed around the base of each seedling, and then put in a large greenhouse, subdivided to give supplementary CO_2 in some sections. Azotobacter treatment did not advance flowering on the first truss of "Potentate", but without supplementary CO_2 the flowering of the 2nd, 3rd, 4th, 5th and 6th trusses was advanced by 1–3 days. With supplementary CO_2 the flowering of the 2nd, 3rd and 4th trusses was advanced by 1–5 days. Flowering of the variety "Ware Cross" did not respond to inoculation.

Azotobacter is one of the "bacterial fertilisers", and experiments at Rothamsted were directed towards finding how it affects crop growth. It may do so directly by producing growth factors, or indirectly by changing the balance and activity of the rhizosphere flora. Earlier work has excluded nitrogen fixation as a significant factor in promoting earlier development. Experiments with tomatoes in 1962 and 1963 showed that flowers and fruits are formed earlier by plants treated with Azotobacter at transplanting

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(root treatment). This was confirmed in 1964 when treating seed with culture. Both root and seed treatments also significantly increased stem height and leaf length of tomato seedlings. Inoculation affects stem and leaf growth most while plants are very young, and these effects decrease progressively with age until at anthesis of the first truss they have usually disappeared. One application of Azotobacter, either to the seed or root, affects stem and leaf growth as much as applications every 2 weeks to the soil around the base of developing plants. Azotobacter cells washed free from culture medium are more stimulatory than their corresponding cell-free filtrates.

Tomato stems and leaves elongate when treated with gibberellic acid (GA), which is produced by Azotobacter (Vancura, *Nature, Lond.* (1961) **192**, 88–89). The effects of this plant-growth regulator were therefore compared with those of Azotobacter inoculation. GA was applied to seeds or roots at 5, 0·5, 0·05, 0·005 μg per plant. Only the 5-μg application significantly increased stem and leaf extension. The extension was the same as produced by Azotobacter, but whereas Azotobacter advanced flowering, GA delayed it by 1–3 days. Other workers have found that the flowering of tomatoes is advanced by 25 μg of GA applied to the apex of young plants when the first true leaf appears. (Rappaport, *Pl. Physiol., Lancaster* (1957) **32**, 440–444.) In our experiments the GA was applied to seed or root, which presumably accounts for the different effect on flowering, but the effects of applying GA at different times and sites are being tested further. (M. Brown, Jackson and Burlingham)

Growth regulators produced by Azotobacter in culture media

(*a*) ***Auxins.*** Cultures of the Azotobacter strain (A6) used to inoculate tomatoes were assayed for indole acetic acid (IAA). Supernatant fluids of shake-cultures grown at 25° C in liquid medium deficient in N were extracted and subjected to paper chromatography. IAA at R_F 0·37 was detected by its colour reaction (purple) with $FeCl_3$ reagent, and confirmed by the Avena coleoptile test. The amounts of IAA formed increased from 0·05 μg/ml in a 2-day-old culture to 1 μg/ml in a 30-day-old culture. A coloured spot (pink) also formed at R_F 0·05–0·1 in *iso*propanol/ammonia/water (10:1:1) and eluates from parts of the chromatogram corresponding to this R_F value inhibited the growth of Avena coleoptile sections. Like IAA, the amount of this unknown inhibitor produced by Azotobacter increased with the age of the culture.

(*b*) ***Gibberellins.*** Azotobacter strain (A6) was also assayed for gibberellin-like activity. Cultures grown for 7, 14 and 21 days in a liquid medium on a shaker at 25° C were divided into supernatant fluid, cells, filtrate and polysaccharide gum fractions, which were then extracted and chromatograms run in *iso*propanol/ammonia/water (10:1:1). The chromatograms were cut into sections and tested for gibberellin-like activity using the dwarf pea test, the lettuce and cucumber hypocotyl test and the barley endosperm test. Eluates from parts of the chromatogram of the culture filtrate and supernatant fluid extended the internodes of peas significantly.

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These sections corresponded to marker GA run at the same time (R_F 0·52). The supernatant fluid from cultures 14 days old also produced a substance at R_F 0·85–0·90 which extended internodes more than did eluates from the chromatogram sections corresponding to gibberellic acid. This unknown substance is present in supernatant fluids from 14 or more days old, but not in cultures 7 days old or in any filtrates.

Chromatogram sections of the culture filtrates and supernatant fluid from a position on the chromatogram corresponding to that of marker GA also significantly increased the lengths of lettuce and cucumber hypocotyls. The barley endosperm test indicated gibberellin activity in these same sections; α-amylase activity was stimulated and reducing sugar was released from the endosperm.

Gibberellin-like activity was not found in the polysaccharide gum, but cells from cultures of all three ages extracted with 70% acetone showed activity on dwarf peas at various R_F values. The pattern of gibberellin-like activity is irregular and experiments are in progress to determine the source of this variability, so that factors affecting the production of gibberellins can be assessed more critically. (Burlingham)

Effects of Azotobacter inoculation on seed coat and rhizosphere flora. Wheat seed was inoculated with Azotobacter and immediately sampled for bacteria and fungi. The inoculated seed was grown in either sterile nutrient culture or in unsterile potting compost. Samples were taken at regular intervals from these plants and from others treated only with the medium used to grow Azotobacter, without added sucrose; bacteria and fungi were isolated from the seed coat and developing roots. In nutrient solution the fungal flora was not affected by Azotobacter inoculation, and the seed coat fungi did not colonise the roots. Inoculation increased the chromogenic bacteria on the seed by 7 times, but did not increase numbers on the roots. It decreased the numbers of non-chromogenic bacteria on the seed and on the roots.

In unsterile potting compost (John Innes No. 1), inoculation did not affect the chromogenic bacteria on seeds or roots and non-chromogenic bacteria were not decreased significantly. It seems unlikely therefore that Azotobacter inoculation affects the microbial flora of seeds or very young roots when plants are sown in a potting soil mixture. (M. E. Brown)

Longer-term experiments were also done with wheat ("Jufy 1") grown in soil from Great Field and tomato ("Money Maker") grown in potting compost. The plants were grown singly in pots, watered automatically by capillarity and given supplementary fluorescent lighting to provide an 18-hour day. The rhizosphere and soil were separately sampled for total bacteria, chitinolytic micro-organisms (mostly Actinomycetes), glucose fermenters, anaerobes, cellulose decomposers and fungi. The fungi on the root surface were also examined using a modified Harley and Waid washing procedure, in which washed segments of root were plated out in a dilute Czapek medium containing aureomycin, from which isolates were made on to potato–carrot agar slants for identification. Azotobacter became established in the rhizosphere of both plants and promoted slightly earlier ear emergence in wheat and earlier truss formation in tomato.

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As usual, bacterial groups studied were more abundant in the rhizosphere than elsewhere, but the rhizosphere population was unaffected by Azotobacter inoculation. Species of Penicillium, Fusarium and an unidentified dematiaceous fungus were the dominant fungi in the rhizosphere and on the root surface. In the wheat experiment at the time of the second sampling (at 8 weeks) the dematiaceous fungus was significantly more numerous and the Penicillium isolates less abundant in the rhizosphere of inoculated plants than in the rhizosphere of control plants. These differences were noted at one time of sampling only and were not further considered. The microbial populations of the wheat and tomato rhizospheres differed quantitatively and qualitatively. (Patel)

Antibiosis against Azotobacter. Two strains of *Bacillus* and one of *Pseudomonas*, isolated from cereal rhizospheres, were tested for antibiosis against *Azotobacter chroococcum* growing in a nitrogen-deficient medium in Petri dishes. The culture fluids of all three strains had antibiotic activity; two of the strains but not the third (a *Bacillus*) were more active when grown on rich complex media than on a simpler, defined, medium.

Evaporation of the culture fluids by boiling destroyed much antibiotic activity, but partial lyophilisation preserved and concentrated the activity. Further attempts to isolate and characterise antibiotic agents were precluded by unexplained and progressive loss of clarity of the zones of inhibition of the test organism. (Dr. A. Saric and Cooper)

Fluorescent antibody studies on Azotobacter in soil. Azotobacter cells added to natural soils were detected after treatment with Azotobacter antiserum, prepared in rabbits, and with fluorescein-labelled sheep anti-rabbit globulin. There was much non-specific staining, but fluorescing Azotobacter cells could be distinguished by their characteristic morphology. (Jackson)

General growth of Azotobacter in culture. The growth, metabolism and morphology of Azotobacter were studied in a range of media. All cultures were grown at 25° C in 500-ml conical flasks on a rotary shaker. A maximum number of 10^9 cells per ml was produced in 3 days in media containing 5·0, 2·5 or 0·5% glucose or sucrose, regardless of whether the flasks contained 200, 150, 100, 50 or 10 ml of medium, indicating that aeration was not limiting growth. With 0·1% sucrose, the maximum of 4×10^8 cells per ml was reached in 24 hours. Cysts started to form in all media after the maximum number was reached, and pigment production was associated with this morphological stage. Except for the medium containing 5·0% carbohydrate, the sugar was consumed in 4 days and nitrogen fixation was fastest during the first 48 hours. With 5·0% sucrose, nitrogen fixation was greatest at 7 days and the sucrose was all used by 11 days; the Azotobacter cells in these cultures were large, pleomorphic and produced yellow pigment. (M. Brown)

Decomposition of aromatic compounds by bacteria. The strain of *Pseudomonas fluorescens* that decomposes β-naphthol was grown in con-

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tinuous culture and gentisic acid isolated from the culture fluid (yield about 10%). Critical tests, using small concentrations of substrate and plenty of young cells, failed to confirm earlier work suggesting there is a long pause in 0_2 uptake by β-naphthol-grown organisms with gentisic acid as substrate in Warburg respirometers (*Rothamsted Report* for 1963, p. 71). Spectrophotometric comparison of the maroon pigment appearing in β-naphthol cultures and that produced by air oxidation of dilute solutions of 2,3-dihydroxynaphthalene showed the two pigments to be different. Consequently, the bacterial dissimilation of β-naphthol probably proceeds through the intermediates, 1,2-dihydro-1,2,6-trihydroxynaphthalene and gentisic acid.

A *Nocardia* strain (probably *Nocardia corallina*) that can use toluene as the sole carbon source was isolated from soil. Washed organisms oxidise both 3- and 4-methylcatechol, so toluene metabolism in this organism may differ from that in the bacteria used previously.

Attempts to find micro-organisms able to decompose either simazine, 2,4,5-trichlorophenoxyacetic acid or *m*-chlorophenoxy-acetic acid have all failed.

Species of *Chlorella* were found to be sensitive to simazine and atrazine. and some progress was made towards standardising and simplifying conditions to use these organisms to assay triazine herbicides. (Walker)

Nitrifying bacteria. Two further strains of ammonia-oxidising autotrophs were isolated in pure culture from soil in Geescroft and Park Grass. Their properties are being studied and compared with those of previously isolated strains. (Walker)

Mouldy hay and farmer's lung disease. The development of "farmer's lung hay" (F.L.H.) antigen during self-heating was followed in moistened hay contained in Dewar flasks under different conditions of aeration and water content. Though the sequence in changes in temperature differed considerably with the type of hay and the duration of storage, the maximum temperature reached was correlated with water content. Hay of about 40% water content became hottest and invariably produced F.L.H. antigen.

Microbiological observations during the self-heating of good hay moistened to 40% water content showed that bacteria able to grow at 25° C increased from *c.* 10^6 to $3{\cdot}7 \times 10^9$ per g dry hay during the first day (temperature reached, 39° C). Almost all colonies were buff or orange-yellow, punctiform and consisted of gram-positive cocci referable to the genus *Micrococcus*. After 5 days (temperature down to 53° C after reaching a maximum of 63° C on the 4th day) the total number of bacteria declined, but the proportion forming filamentous colonies, mostly *Bacillus licheniformis*, increased. On plates incubated at 40° C these filamentous colonies formed a significant proportion of colonies from most samples. Thermophilic bacteria growing at 60° C occurred only in the last three samples (temperatures of 46°, 46°, and 39° C respectively), and were 21% of the total colonies in the last two. Bacteria growing at 60° C were also detected on Andersen Sampler plates, though actinomycetes growing

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readily at this temperature were not numerous in the early stages. Few *Thermoactinomycetes* (*Micromonospora*) *vulgaris* organisms were isolated at 60° C until the third day.

On the third day, *Thermopolyspora polyspora* was detected for the first time, and a trace of F.L.H. antigen appeared in the hay extract. Thereafter, strong and typical F.L.H. immuno-electrophoretic reactions were obtained, coinciding with extensive development of *T. polyspora*.

The persistence of F.L.H. antigen until the end of the sampling period was of interest. In baled hay (Gregory *et al.*, *J. gen. Microbiol.* (1964) **36**, 429–439) the F.L.H. antigen content decreased the longer the bale was stored, an effect tentatively ascribed to destruction of antigen by continued development of fungi. This hypothesis was tested using sterile ammonia-treated hay on which *T. polypora* had grown for several weeks at 40° C. The antigen-rich hay was distributed into four jars and re-sterilised with propylene oxide. Two jars were inoculated with a mixed inoculum of the thermophilic fungi, *Absidia ramosa*, *Aspergillus fumigatus*, *Humicola lanuginosa* and *Mucor pusillus*, and the third jar received an equivalent volume of sterile water. All jars were further incubated at 40° C. One of the fungi-containing jars was removed for analysis after 1 week, and the other two after 3 weeks. Prolonged incubation at 40° C with or without concomitant fungal growth did not alter the F.L.H. antigen content.

When unsterile hay at 40% water content was incubated at 40°, 45° and 50° C the samples quickly developed extensive microbial growth. Although actinomycetes were detected in several jars, few of the antisera reacted with extracts; and immuno-electrophoretic tests gave only weak lines caused either by fungi or by *Micromonospora vulgaris*. Prolonged incubation (up to 41 days) seemed to decrease the antigenicity of the samples slightly, although the pH values increased to well over 7, which should have favoured growth of *T. polyspora* and *M. vulgaris*. Conditions for growth of these organisms evidently depend on a sequence of events in the self-heating process not determined by temperature and water relations alone. (Skinner, in collaboration with Festenstein, Biochemistry Department, Lacey, Plant Pathology Department; Dr. J. Pepys and Dr. P. A. Jenkins, Institute of Diseases of the Chest, Brompton, London.)

Carbohydrate fermentations in soil. The anaerobic dissimilation of various carbohydrates in soils was studied under different conditions of moisture content, aeration and temperature. The soils were incubated in columns and sections examined for changes in the microbial populations, pH and volatile fatty acid content. Soils were incubated without amendment at 25° or 35° C anaerobically and aerobically. The pH of samples from five depths in anaerobic tubes ranged from 6·8 to 7·3, and in aerobic tubes from 7·3 to 7·8. The aerobic soils had the usual odour of fresh soil; the anaerobic soils, a sweetish foetid odour. The addition of cellulose to this soil had no detectable effect.

Adding 1% glucose to moist soil, followed by aerobic incubation at 25° C for 3 days, produced volatile acids, including butyric, and the pH changed from 7·4 to 6·7. Bacterial numbers were doubled in two days,

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because of a tenfold increase in large rod-shaped bacteria and a fivefold increase in Azotobacter-like cells. Anaerobes increased to a maximum after 6 days' incubation. The content of volatile fatty acids first increased, but later declined to the initial level. Whether these volatile metabolic products can serve as energy sources for nitrogen-fixing micro-organisms is being investigated. (Skinner and Walker)

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