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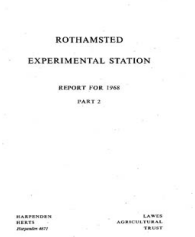
Rothamsted Experimental Station Report for 1968 - Part 2



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Symbiotic Nitrogen Fixation: Legume Nodule Bacteria

P. S. Nutman

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to support the idea that fallowing stimulated nitrogen fixation for the first crop after fallow. Azotobacter were few in sections under the 4th successive wheat crop and in the soil being fallowed, but increased greatly in the first wheat crop after the fallow. The increase in Azotobacter was greatest in the plots where the increased yield from fallow was greatest (Table 9·4). Plots given ammonium sulphate every year had fewer Azotobacter than the other plots (Meiklejohn, 1965).

However, Azotobacter in the Broadbalk soils seemed too few to fix enough nitrogen to affect the crop growth. Although an improved technique (Brown, Burlingham & Jackson, 1962) increased the count, the largest population found was only 9400 cells/g dry soil, which again is too few for Azotobacter to affect crop growth through nitrogen fixation. Nevertheless the Azotobacter numbers indicate that conditions are suitable for nitrogen fixation by other species, such as the anaerobe *Clostridium pasteurianum*, which occurs all over Broadbalk in much larger numbers than Azotobacter, ranging up to 100 000 cells per gram dry soil (Meiklejohn, 1956). The possibility that an anaerobe is the active fixing agent in Broadbalk soil is supported by laboratory observations of nitrogen fixation by soil from Broadbalk plot 3 incubated anaerobically with straw (Barrow & Jenkinson, 1962).

TABLE 9·4

Effect of fallow on Azotobacter numbers in 1962

		Largest number (cells/g soil)	Mean
Plots without added nitrogen			
3	Fallow	137	53 ± 16
	1st crop after fallow	1194	430 ± 135
5	Fallow	282	166 ± 30
	1st crop after fallow	894	400 ± 121
Plots with annual ammonium sulphate			
7	Fallow	410	134 ± 49
	1st crop after fallow	1429	631 ± 190
10	Fallow	555	129 ± 76
	1st crop after fallow	260	146 ± 27
Other nitrogen treated plots			
16	Fallow	4524	1003 ± 594
	1st crop after fallow	3782	1803 ± 435
17	Fallow	2620	580 ± 343
	1st crop after fallow	9406	2442 ± 1276

Symbiotic Nitrogen Fixation: Legume Nodule Bacteria

By P. S. Nutman

Four species of the genus *Rhizobium*, which form nodules on the roots of leguminous plants occur in Broadbalk soils, but in populations that are much smaller than in neighbouring fields.

Nodule bacteria were counted in samples of soil taken in March 1968

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from plots 3, 5, 8 and 16 in sections I, II, III, IV and VA, using dilutions of soil to inoculate sterile-grown seedlings of the test legumes, viz. *Vicia hirsuta* to test for the presence of *Rhizobium leguminosarum*, *Trifolium pratense* for *R. trifolii*, *Lotus corniculatus* for *R. lupini* and *Medicago sativa* for *R. meliloti*. The test seedlings form nodules and grow only if the inoculum contains the appropriate species. Samples were also taken from plots 2, 11 and 18 to count *R. leguminosarum* only.

Table 9·5 shows the logarithms of estimated numbers per gram dry soil. (Nutman, 1962.)

TABLE 9·5

Rhizobium counts on Broadbalk, March 1968

Log estimated no./g dry soil

Section	Herbicides since	*R. leguminosarum* Plot 3	5	16	8	2	11	18	*R. trifolii* 3	5	16	8
I	1957	5·12	5·14	4·83	5·16	—	—	—	2·64	2·10	3·12	>4·08
II	1964	4·42	3·48	4·29	4·55	4·15	3·81	4·20	2·95	1·26	2·29	>3·97
III	1964	4·89	4·50	3·87	4·02	—	—	—	2·94	1·96	1·41	3·55
IV	1964	3·68	4·59	2·97	4·89	—	—	—	3·32	2·53	1·85	>4·00
VA	none	4·52	5·07	4·20	5·70	5·48	3·99	>5·52	3·32	1·38	1·75	>3·20

Section	Herbicides since	*R. lupini* Plot 3	5	16	8	*R. meliloti* 3	5	16	8
I	1957	1·02	2·38	1·33	<0·51	<0·32	2·32	<0·59	<0·87
II	1964	<0·33	0·61	<0·96	1·06	<1·18	0·96	<0·45	<0·27
III	1964	<0·47	1·58	0·75	2·53	0·89	1·71	1·30	<0·42
IV	1964	0·75	<0·49	1·20	<0·15	0·97	<0·56	0·68	<0·15
VA	none	2·13	1·16	1·19	1·98	<0·67	0·47	<1·04	<0·15

Rhizobium leguminosarum is the most abundant species in Broadbalk. The average of all counts was 28000/g dry soil, and ranged from 1800/g dry soil in one sample of plot 11 to >87000 in plot 18. In most plots numbers varied by less than a hundred-fold, and no significant differences were noted between sections or plots. All *R. leguminosarum* cells nodulating the test plant were effective in fixing nitrogen.

The next most abundant species of nodule bacteria is *Rhizobium trifolii* of which most were found in plot 3 (unmanured) and plot 8 ($N_3PKNaMg$). Maximum, minimum and average counts were <14500, 7 and 320/g soil and populations varied more than *R. leguminosarum*. No consistent differences were found between sections or plots. About 10% of the bacteria that nodulated *Trifolium pratense* when inoculated with the most dilute suspensions did not fix nitrogen or fixed very little on this host.

Rhizobium lupini occurs sparsely and sporadically; it was not found in seven plots (total of 10·4 g sampled) and in the others the average number was 310/g and the maximum 1620/g. Bacteria from all except one sample (plot 3, section II) were effective in fixing nitrogen in nodules of *Lotus corniculatus*.

Rhizobium meliloti occurs infrequently in low numbers. It was absent from nearly half the samples (total weight 19 g) and was few in the samples where it occurred; an average of 13/g and a maximum of 229/g. More than half of the strains were fully effective with *Medicago sativa*.

In fields such as Broadbalk where legume crops are not grown the nodule bacteria may be brought in from neighbouring fields or may persist on leguminous weeds. Whichever way they are introduced their distribution does not correspond with that of leguminous weeds.

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Medicago lupulina (black medick) is the commonest leguminous weed on the plots sampled, but its nodule bacteria is the rarest of the four species. Black medick is most frequent in plots 3 and 5, much less frequent on plot 16 and rarely occurs on plot 8, which also contains fewer *R. meliloti* than other plots. Herbicides have decreased numbers of *M. lupulina* in most years (see p. 197) but this has had no effect on the numbers of its nodule bacteria in the different sections.

The natural hosts of *R. leguminosarum* found in Broadbalk are common vetch (*Vicia sativa*) and meadow vetchling (*Lathyrus pratensis*), *Vicia sativa* being most frequent, particularly in certain years on plots 3, 5 and 18. *Lathyrus pratensis* occurs rarely and sporadically and very few of either legume occur on plot 16 and none on plot 8. There is no significant difference in numbers of *R. leguminosarum* between plots or between sections treated with herbicides and those not treated, in spite of the effect of herbicides in diminishing populations of *V. sativa* on all plots where it occurs.

Although *Rhizobium trifolii* is more widely distributed on Broadbalk and more abundant than *R. lupini* or *R. meliloti*, its natural host plants, the clovers, occur very rarely and not at all in most years.

No known host plant of *R. lupini* has been recorded on Broadbalk so that the occurrence of this species of *Rhizobium* cannot be related to weed history.

Numbers of *Rhizobium* in Rothamsted soil under leguminous crops are larger by several orders of magnitude than those found in Broadbalk. For example in Sawyers field (limed plots) under *Medicago lupulina*, tick beans and red clover, the average number of the specific rhizobia for these legumes were respectively 500 000, >2 000 000 and 700 000 per g/dry soil (Nutman, 1963). In the long term fallow experiment in Highfield the total Rhizobium population is small, but, as on Broadbalk, the clover and bean nodule bacteria are nevertheless more abundant than the lotus and medick bacteria. This suggests that the nodule bacteria found in Broadbalk are unlikely to have multiplied in the rhizospheres of the relatively few leguminous weeds but more likely to have been brought in from neighbouring fields.

Rhizosphere and Rhizoplane Fungi

By JUDITH ETHERIDGE

Take-all, caused by *Ophiobolus graminis*, is usually less severe on wheat on Broadbalk than on wheat grown successively in other fields at Rothamsted. In 1960 the fungi of the rhizosphere and rhizoplane of wheat from Broadbalk were compared with those from other fields in an attempt to identify any organism that might inhibit the development of take-all on Broadbalk. The rhizosphere fungi were studied by the dilution plate method using a glucose peptone medium (Johnson *et al.*, 1959) with 30 mg aureomycin per litre. After the rhizosphere soil had been washed away, the roots were washed in only two changes of sterile water, then cut into 0·5 cm

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and was especially rich in reticulate spores. Yellow vacuolate spores were most abundant in the FYM plot. Of the four types, laminate spores were fewest, whereas in the nearby field, Little Knott, they were the only ones found and were numerous in some plots, especially those not given nitrogen.

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