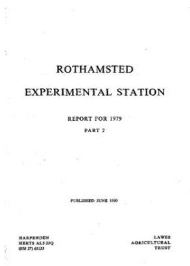
Rothamsted Experimental Station Report for 1979 Part 2


ROTHAMSTED
EXPERIMENTAL STATION

[Full Table of Content]

Persistence of Nodule Bacteria in Soil Under Long-term Cereal Cultivation

P. S. Nutman and Rosemary Hearne

P. S. Nutman and Rosemary Hearne (1980) *Persistence of Nodule Bacteria in Soil Under Long-term Cereal Cultivation ;* Rothamsted Experimental Station Report For 1979 Part 2, pp 77 - 90 **- DOI: https://doi.org/10.23637/ERADOC-1-34290**

Persistence of Nodule Bacteria in Soil under Long-term Cereal Cultivation

P. S. NUTMAN and ROSEMARY HEARNE

Abstract

The effects of continuous cereal farming and associated practices on the soil populations of rhizobia were assessed at Rothamsted and Woburn and three nearby commercial farms. For comparison, counts were made from soils under leguminous crops, under arable rotations (with and without legumes) and under continuous clean fallow. In the presence of their hosts the populations of *Rhizobium trifolii* and *R. leguminosarum* were of the order of 10^5–10^6 cells g^{-1} dry soil.

Intensive cereal farming with liming (and without undersowing), and with normal levels of fertiliser reduced numbers at an approximately exponential rate to, for example, about 10^4 g^{-1} after 10 years for clover rhizobia. Rates of reduction appeared to be unaffected by soil type, methods of cultivation or straw disposal but were slightly accelerated by the higher rates of fertiliser application and by aldicarb, especially if used with other biocides. In some combinations of treatments numbers were reduced 1000-fold over this period.

Surviving populations of *R. trifolii* and *R. leguminosarum* did not differ from the original populations in symbiotic effectiveness, but the small surviving populations of *R. meliloti* (< 10 g^{-1}) were largely ineffective in fixing nitrogen, and were almost entirely supplanted by the strain added as a seed inoculant, which reached *c.* 10^5 cells g^{-1}. *R. trifolii* and *R. leguminosarum* under continuous fallow were reduced after 18 years to *c.* 10 g^{-1} and 10^2 g^{-1} at Rothamsted and *c.* 10^2 g^{-1} and 10 g^{-1} at Woburn respectively; reductions were even greater for *R. meliloti*.

The declining numbers of soil rhizobia under cereals is discussed in relation to possible changes in farming systems.

Introduction

Earlier studies on the distribution of nodule bacteria in the soils of the classical experiments at Rothamsted showed appreciable numbers on Broadbalk Field where wheat has been grown without a break crop for more than 130 years (Nutman, 1969). Over this period leguminous weeds were reduced or eliminated by cultivation, by the use of nitrogenous fertilisers and latterly by herbicides. *Rhizobium leguminosarum* occurred in larger numbers than *R. trifolii; R. lupini* and *R. meliloti* were very sparse or absent. Over all plots in which rhizobia were counted the numbers (log most probable number (MPN) g^{-1} of dry soil) averaged 4·50, 2·68, $<1·15$ and $<0·79$ respectively. Numbers were slightly larger in plots not given herbicide and there was no consistent effect of fertiliser N used at rates up to 145 kg N ha^{-1} as ammonium salts, or 85 kg N ha^{-1} as nitrate. High rates of ammonium sulphate caused some of the plots to become acid prior to the chalking regime which began in 1954 (Johnston, 1969). This will have severely reduced numbers before 1954. There is, however, no evidence today of smaller surviving populations of rhizobia in these plots, suggesting that the present populations were derived from nodule bacteria introduced casually on farm implements, etc., rather than remnants of an autochthonous flora. This was also concluded from a study of other classical arable fields and from counts made over a number of years of the long-term clean fallows. Numbers of rhizobia under permanent grassland given different fertiliser treatments (Park Grass) and experiments sown to legumes showed a strong correlation with the

https://doi.org/10.23637/ERADOC-1-18

occurrence of a leguminous crop or weed. Whenever the use of nitrogenous fertiliser lowered pH the numbers of rhizobia declined and their symbiotic effectiveness was sometimes reduced (Nutman & Ross, 1970).

The amount of fertiliser applied in the foregoing arable and grassland experiments was generally much below that used today and this paper examines the effects of modern intensive cereal farming on residual populations of rhizobia, mainly *R. trifolii* and *R. leguminosarum*, which are most likely to be important should alternate husbandry or the growing of field beans as a break crop be increased. Parallel studies on grassland used for intensive forage production showed a decline in rhizobia when high rates of nitrogenous fertiliser (>200 kg N ha^{-1}) were used, but not to a vanishing point, and without affecting the symbiotic effectiveness of the survivors, provided that the soil did not become acid (Nutman, Dye & Davis, 1978).

Methods

R. trifolii, *R. leguminosarum* and *R. meliloti* were counted by the most probable number method (MPN, Vincent, 1970) in which measured dilutions of the soil sample were added to sterile-grown host plants (red clover, *Trifolium pratense*—hairy vetch, *Vicia hirsuta*—or lucerne, *Medicago sativa*) and the number and symbiotic effectiveness of nodulated plants recorded. From this the numbers of rhizobia g^{-1} dry weight in the original sample were calculated. When one or more samples failed to nodulate at the lowest dilution the MPN estimate in the tables is prefixed by '<' and when both replicated test plants nodulated at the highest dilution a '>' sign is used. Samples were taken at two depths (surface 0–5 cm and deep 10–15 cm) using four 2-cm auger cores at each site or plot. Surface and deep samples for each site were bulked, mixed and sub-sampled. Two replicated test plants were inoculated with the soil suspension at each dilution. Plants nodulating at the highest dilution (viz. by strains most abundantly represented in the sample) were scored as effective, intermediate or ineffective in nitrogen fixation. In some experiments strains were also isolated from these plants and their effectiveness examined in open pot culture using an axenic sand/gravel/vermiculite mixture (Nutman, 1980).

Sites for counting (Rothamsted and Woburn field experiments and commercial farms

TABLE 1

Sites and descriptions of field experiments

	Code number of experiment	Years under cereal	Date of sampling
Clover or bean crops or rotations and permanent old grassland			
Grass/clover/lucerne trial at Rothamsted Pastures	R.CS.200	—	Oct. 78
Ditto at Woburn, Butt Furlong	W.CS.200	—	Oct. 78
Clover field, Rothamsted Pastures	R.CS.208	—	Aug. 78
Rothamsted beans, Long Hoos	R.BE.12	—	Oct. 78
Woburn beans, Butt Furlong	W.CS.99	—	Oct. 78
Rothamsted Ley Arable, Highfield	R.RN.1	—	Sept. 79
Woburn Ley Arable, Stackyard	W.RN.3	—	Sept. 79
Woburn old pasture	—	—	July 78
Caddington, chalky natural grassland	—	—	Dec. 78
Intensive cereal sites at Rothamsted and Woburn			
Rothamsted, wheat bulb fly experiment, Great Harpenden	R.RN.10	15	May 78
Rothamsted, PK and take-all, West Barnfield	R.24	11	June 78
Rothamsted, cultivation for cereals, Meadow	R.CS.90	7	Aug. 78
Rothamsted, Chemical reference experiment, Long Hoos, V(3)	R.CS.140	7	Aug. 78
Rothamsted, straw disposal experiment, Blackhorse II	R.CS.157	9	May 78
Woburn, continuous barley, Butt Furlong field	W.CS.40	9	July 78
Woburn, intensive cereal experiment, wheat, Stackyard	W.RN.13	2, 13	April 78

https://doi.org/10.23637/ERADOC-1-18

outside) were chosen to give a wide range of fertiliser use, soil type and prior treatment, including length of continuous cereal cropping, different cultivations, pest and weed control and straw disposal. Soil pH was determined at sampling. Table 1 lists sites, descriptions and identification codes of the Rothamsted and Woburn experiments.

Results

Sites not used for intensive cereal production. For comparison counts were made from fields and experiments under clover, field beans (*Vicia faba*) or lucerne, from the Rothamsted and Woburn long-term fallows and from sites alongside the intensive cereal experiments. These are summarised in Tables 2 and 3 and form the basis on which to assess the effects of intensive cereal farming on soil populations of rhizobia.

The log MPN counts in Table 2 refer to selected plots of a field experiment set up a year before sampling to examine the effects of fertiliser, irrigation, pest control and frequency of cutting on the productivity of ryegrass (cv. S23), grass/clover (cv. Blanca) mixtures, clover alone and lucerne (cv. Vertus) at Rothamsted (Pastures field, clay loam, Batcombe series) and Woburn (Butt Furlough, sandy loam, Cottenhoe series). Both sites had previously carried ley and arable crops including cereals and clovers (and *Vicia faba* field beans at Rothamsted). All plots examined were cut the same number of times.

Numbers of rhizobia were generally greater in the Rothamsted than Woburn soil. In the grass plot the numbers of *R. leguminosarum* exceeded those of *R. trifolii* and were in general depressed by fertiliser nitrogen especially at the heavier rate; but these effects failed to reach a level of statistical significance. The residual populations of *R. meliloti* were very small or absent and unaffected by N fertiliser; there was no history of lucerne having been sown at either site. At both sites the presence of clover (alone or with grass) increased its homologous rhizobia 10–100-fold, irrespective of fertiliser N. Lucerne (which was seed-inoculated) sustained populations of *R. meliloti* that were more than 1000-fold greater than those originally present.

The surface/deep, +/− irrigation and +/− aldicarb counts are not shown separately in the table. In the grass plots clover rhizobia were usually less numerous in the surface than in the deeper soil, especially when heavily fertilised, and more so at Rothamsted than at Woburn. Average counts at the two depths at Rothamsted were 5·60 and 6·09 and at Woburn 4·74 and 4·86; these means may be compared separately using the maximum SE in each section of the table as a guide. A strikingly opposite effect was observed in plots sown to clover; with a single exception surface counts exceeded deep counts at both sites irrespective of N fertilising. Thus at Rothamsted the average surface and deep populations under clover were 6·73 and 5·51 respectively; corresponding values at Woburn were 6·99 and 5·69.

Numbers under grass were unaffected by irrigation but were generally depressed by aldicarb whether or not clover or lucerne were present; *R. meliloti* seemed to be more sensitive to aldicarb than clover rhizobia in the Woburn soil but was not affected in the Rothamsted soil. Mean counts for *R. trifolii* with and without aldicarb at Rothamsted were 6·70 and 6·06 and at Woburn they were 6·42 and 5·90; for *R. melioloti* at Woburn, counts with and without aldicarb were 2·56 and 4·99.

Counts were also made from five equidistant sites in a neighbouring 0·8 ha plot of white clover (cv. Huia) sown in 1978, not fertilised with nitrogen, irrigated and cut as required. Previously the area had been used for arable crops after ploughing from ley in 1963. The average log MPN of *R. trifolii* in the surface samples was $6{\cdot}82 \pm 0{\cdot}261$ and in the deep samples $5{\cdot}70 \pm 0{\cdot}259$. *R. leguminosarum* was less abundant beneath the clover (mean log MPN 4·98) and showed no effects of sampling depth.

Counts of *R. leguminosarum* in neighbouring sections of Long Hoos V showed similarly

https://doi.org/10.23637/ERADOC-1-18

TABLE 2

Numbers of clover, field bean and lucerne rhizobia in plots sown with grass, clover or lucerne and cut for hay. Mean log MPN g^{-1} dry soil

Nitrogenous fertiliser	*Rhizobium* counted	Rothamsted rye grass	Rothamsted rye grass/clover	Rothamsted clover	Rothamsted lucerne	Rothamsted SE max.	Woburn rye grass	Woburn rye grass/clover	Woburn clover	Woburn lucerne	Woburn SE max.
None	*R. trifolii*	6·64	7·65	6·03	—	0·312	4·86	6·29	6·33	—	0·290
	R. leguminosarum	>4·98	—	—	—		5·02	—	—	—	
	R. meliloti	<2·52	—	—	5·44		<1·18	—	—	4·59	
Medium*	*R. trifolii*	5·63	6·96	5·85	—	0·273	4·50	6·54	5·37	—	0·346
	R. leguminosarum	>6·21	—	—	—		5·39	—	—	—	
	R. meliloti	<0·59	—	—	—		<0·97	—	—	—	
High†	*R. trifolii*	5·27	6·33	—	—	0·307	4·58	5·03	—	—	0·436
	R. leguminosarum	>4·84	—	—	—		4·79	—	—	—	
	R. meliloti	<1·89	—	—	—		<1·35	—	—	—	

* 200 kg N ha^{-1} for grass/clover and clover, 400 kg N ha^{-1} for grass
† 400 kg N ha^{-1} for grass/clover mixture and clover, 600 kg N ha^{-1} for grass

https://doi.org/10.23637/ERADOC-1-18

large stimulatory effect of its host. Where beans had last been grown 5 years previously, counts of *R. leguminosarum* at six sampling points averaged 4·58 and 3·79 in the surface and deep samples, whereas under beans (Table 3) numbers had increased at least 100-fold, irrespective of irrigation or fertiliser nitrogen at 100 kg N ha^{-1}. Table 4 shows numbers

TABLE 3

Numbers of field bean rhizobia in soil under beans or barley after beans. Mean log MPN g^{-1}

	R. leguminosarum	SE max.
Rothamsted		
Long Hoos V(8)		
Unirrigated No nitrogen	>5·90	±0·273
Unirrigated +nitrogen	6·92	
Irrigated No nitrogen	6·14	
Irrigated +nitrogen	>5·90	
Woburn		
Butt Furlong		
Beans in rotation	6·63	±0·269
Barley after beans	4·39	±0·290

TABLE 4

Numbers of rhizobia in old pasture and natural grassland. Mean log MPN g^{-1}

	R. trifolii	SE max	*R. leguminosarum*	SE max.
Woburn old pasture				
Surface	2·73	0·290	<0·92	0·311
Deep	4·39		3·29	
Caddington. Natural grassland on chalk escarpment				
Surface	5·74	0·290	6·42	0·273
Deep	5·64		>4·98	

of rhizobia in a non-experimental cattle-holding area of permanent grass/clover given nitrogen at Woburn and a chalky natural grassland area near Caddington. Both species were rather sparse at the acid Woburn site, especially in the surface sample and abundant beneath natural grassland at Caddington.

Counts were made in the ley–arable experiments at Rothamsted (Highfield, Batcombe series) and Woburn (Stackyard, Cottenham and Stackyard series) comparing plots carrying clover/grass mixture for 3 or 15 years at Rothamsted and 3 or 8 years at Woburn. Third year lucerne plots were sampled at Rothamsted and wholly arable sequences without legumes at both sites. The 1979 crops were potatoes at Rothamsted and barley at Woburn. The forage crop soil samples were taken from plots not given nitrogen.

The surface and deep counts did not differ in the plots in the arable phase and these are combined in Table 5. They are also combined for the lucerne plot which had been cut and ploughed immediately before sampling.

R. trifolii was again more abundant at Rothamsted than Woburn, and was considerably stimulated at both sites by its host, either when present in the current sward or ploughed in 2 or 3 years previously. Under existing swards numbers were significantly greater in the surface than deep samples. *R. leguminosarum* which was more abundant on Stackyard than Highfield, appeared to be slightly stimulated by the presence of clover but no differences could be attributed to depth of sampling or sequence in the rotation. *R. meliloti* were very numerous in the soil of the lucerne plots.

The populations of *R. trifolii* in the long-term fallows were estimated in 1977 from four

https://doi.org/10.23637/ERADOC-1-18

TABLE 5

Ley-arable rotations. Numbers of R. trifolii *and* R. leguminosarum. *Mean log MPN* g^{-1}

	R. trifolii		*R. leguminosarum*	
Rothamsted, Highfield				
Arable rotation without legumes	5·82	SE max. 0·300	2·76	SE max. 0·436
Potatoes after 3 years grass/clover ley	6·20		3·44	
Grass/clover since 1962/63				
surface	6·47		2·37	
deep	4·63		3·03	
	R. meliloti			
Third year lucerne	6·44	0·301		
Woburn, Stackyard				
Arable rotation without legumes	>4·98	0·276	3·85	0·300
Barley following wheat after 3 years grass/clover ley	5·75		6·06	
Grass/clover ley in 5th year				
surface	7·19		5·03	
deep	6·48		5·53	

bulked samples at each site. Because of the wet season the sites had become weedy (non-legumes only). The mean log MPN for the Woburn Orchard (Woburn series) and Stackyard fallow (Cottenham and Stackyard series) and the Rothamsted Fosters and Highfield fallows (Batcombe series) were respectively 3·33, 3·29, 3·58 and 2·18 g^{-1}; populations similar to those previously estimated at these sites.

Thus the log numbers of rhizobia expected to be present before the cereal sequences began would be as follows: *R. trifolii* at Rothamsted 5·26 in the absence of clover and 6·27 with clover, at Woburn 4·43 without clover and 6·18 with clover. The corresponding values for *R. leguminosarum* at Rothamsted and Woburn without or with beans were 4·73, 6·21 and 5·06, 6·63 and for *R. meliloti* <2·52, 5·94 and <1·18, 4·59.

Intensive cereal sites (Table 6 *et seq.*)

Rothamsted

Great Harpenden I. At this site, a clay with flints soil (Winchester and Batcombe shallow series) a two-course rotation of wheat (Cappelle, dressed 'Ceresan') and fallow was designed to study wheat bulb fly infestation; it was sampled in its 15th experimental year. Table 6(1) shows that compared with the populations at Rothamsted not in a cereal sequence (Tables 2 and 4) the wheat/fallow sequence considerably reduced numbers of both *Rhizobium* species; these were also lower than in the headland, the history of which was in doubt. Numbers of *R. trifolii*, but not of *R. leguminosarum* were less in the fallowed than cropped plots, but no consistent effects could be attributed to depth of sampling. The wheat and fallow areas were free of weeds.

West Barnfield II. This field examined the effects of P, K and N fertiliser on the yield of Cappelle wheat grown annually for 11 years on a clay with flints soil (Batcombe and Hook series) using standard cultivations with herbicides. Prior to 1968, the area was used for arable sequences. Weed grasses and annual chickweed (*Stellaria media*) were sparse; there were no leguminous weeds in the sampling areas. Counts were made on samples taken from plots given the lowest (50 kg N ha^{-1}) or highest (200 kg N ha^{-1}) levels of nitrogenous fertiliser ('Nitro-Chalk') and from neighbouring areas with different cropping histories. Table 6(2) gives mean log MPN values combining the surface and deep samplings which showed no differences. Under both fertiliser treatments the numbers of *R. trifolii* exceeded those of *R. leguminosarum*, possibly because clover was grown on the

https://doi.org/10.23637/ERADOC-1-18

TABLE 6

Effect of farming methods on numbers of rhizobia in soil under continuous cereals. Mean log MPN g⁻¹ dry soil

	R. trifolii	SE max.	*R. leguminosarum*	SE max.
1. Great Harpenden I. 2-course rotation; wheat/fallow. 15th year				
Wheat				
shallow	4·06		4·34	
deep	3·84		4·33	
Fallow				
shallow	3·54		4·09	
deep	2·58	0·290	4·43	0·350
Headland				
shallow	4·90		5·72	
deep	3·60		5·01	
2. West Barnfield II. PK* and take-all. 11th year				
P1K1N1				
plot 1	5·38		4·21	
plot 25	5·26		4·03	
P4K4N4				
plot 20	4·75	0·300	4·27	0·350
plot 22	4·25		4·27	
Neighbouring fields				
potatoes	4·25		4·42	
barley	5·00		4·35	
3. Meadow. Cultivation for cereals. 7th year				
Ploughed, disc drilled	5·07		4·49	
Paraquat, direct drilled	5·67	0·350	4·89	0·350
Lodraft chisel ploughed	>5·43		4·56	
4. Long Hoos V. 3 Chemical reference plots. 5th year				
Nil	>4·49		>4·83	
Benomyl	4·98		5·09	
Chlorfenvinphos	>4·98		>5·17	
Aldicarb	>4·37	0·193	>5·66	0·424
Chlortoluron	>4·78		>4·72	
All	>3·24		>4·08	
5. Blackhorse II. Effects of burning straw. 4th year				
Chopped and spread	>5·34		3·92	
Baled and removed	>5·33		4·43	
Burnt in swathes	5·43	0·300	4·34	0·299
Spread and burnt	5·44		3·43	
Headland	5·71		4·17	
6. Butt Furlong. Continuous barley				
9th year barley				
surface	5·02		4·94	
deep	5·09		4·71	
Barley after beans		0·302		0·290
surface	3·72		4·19	
deep	4·10		4·60	

* N1 = 50 kg N ha⁻¹, N4 = 200 kg N ha⁻¹

site in the years before the experiment whereas there is no record of field beans having been grown on this field. Applications of 200 kg N ha^{-1} reduced *R. trifolii*, but not *R. leguminosarum* counts below those for the low nitrogen plots (50 kg N ha^{-1}) and those from the neighbouring barley field.

Meadow. The cultivation treatments given to the seventh consecutive wheat (Maris Huntsman) crop following a ley are shown in Table 6(3); the soil is a flinty silt loam (Charity complex). Weeds were abundant and included black grass (*Alopecuris myosuroides*, wild oat (*Avena* spp.), knot grass (*Polygonum aviculare*), chickweed (*Stellaria*

https://doi.org/10.23637/ERADOC-1-18

media), poppy (*Papaver* spp.), fat hen (*Chenopodium album*) and buttercup (*Rununculus repens*). A single white clover plant was observed in the area sampled. Because there were no effects related to depth of sampling the surface and deep counts were combined. *R. trifolii* was more abundant than *R. leguminosarum*, again possibly reflecting previous cropping history. Paraquat with direct drilling appeared to raise the counts slightly for both species.

Long Hoos V3. This experiment examined the effects of long-term use of herbicides, pesticides and fungicides on the yield of barley (cv. Julia) on a clay with flints loam (Hook series). Prior to its start in 1974 the field grew oats, maize and sweet corn, treated with hormone weedkillers, atrazine and linuron. Unfortunately the chosen dilution series was too short for some samples to be counted so that most means had to be expressed as minimum values.

Table 6(4) shows that within this limitation no differences could be attributed to sampling depth, nor were benomyl (at 4 kg ha^{-1}), chlorfenvinphos (at 2 kg ha^{-1}), aldicarb (6 kg ha^{-1}) or chlortoluron (at 2 kg ha^{-1}) alone active in lowering numbers with either strain. When all biocides were used together at the above rates numbers of rhizobia appeared to be seriously reduced, especially *R. trifolii*. Except on herbicide-treated plots, weeds were fairly abundant and included convolvulus (*Convolvulus arvensis*), hearts ease (*Viola arvensis*), chickweed (*Stellaria media*), cleavers (*Galium apparine*), groundsel (*Senecio vulgaris*) and corn marigold (*Chrysanthemum segetum*); there were no legumes.

Blackhorse II. This experiment on a flinty silt loam of the Charity complex compared different methods of straw disposal: removal, ploughing in, burning in swathes or after spreading. The experiment was started in 1974 and sampled after nine consecutive cereal crops following a grass/clover ley; there is no history of cropping with field beans. Table 6(5) shows that counts of *R. trifolii* were greatest in the headland and larger than those of *R. leguminosarum*, again possibly reflecting the influence of clover ley ploughed in 13 years earlier. Otherwise no differences were attributable to treatment, method of straw disposal or depth of sampling.

Butt Furlong. Samples were taken from (i) the 9th year of continuous barley (Porthos) given basal fertiliser (100 kg N ha^{-1}) and weedkillers, with straw carted off, (ii) from a barley crop given 186 kg N ha^{-1} following field beans, fallow and five previous barley crops. The soils of (i) and (ii) were respectively a brown earth and a gleyed brown earth with sandy colluvium. The continuous barley plots had a fairly abundant weed flora comprising knotgrass (*Polygonum aviculare*) and some pineapple weed (*Matricaria matricaroides*); following beans the weeds were sparse—knotgrass, pineapple weed, speedwell (*Veronica persica*) and grass (*Poa annua*). Numbers of both species of rhizobia (Table 6(6)) were fairly high under barley but the previous crop of beans in (ii) had not stimulated the populations of *R. leguminosarum* or *R. trifolii*. Rhizobia were generally fewer in the surface than sub-surface layer, especially in the old grassland where repeated applications of ammonium sulphate had lowered the pH of the soil.

Woburn

Intensive cereal experiment. Plots of the experiment were sampled to examine the effect of 2 or 13 years continuous wheat (Flanders) with two levels of N fertiliser (63 or 252 kg N ha^{-1} as 'Nitro-Chalk'), with or without aldicarb (10 kg ha^{-1} in seedbed) using half replication (Table 7); the sandy loam derived from greensand was of the Stackyard series. Before the start of the experiment the field had grown wheat for 2 years after

https://doi.org/10.23637/ERADOC-1-18

TABLE 7

Effects of length of period of continuous culture of wheat, level of nitrogenous fertiliser and treatment with aldicarb on numbers of rhizobia in soil. Mean log MPN g^{-1}

Woburn Stackyard. Intensive cereals—wheat

Treatment								
	kg N ha^{-1}		Continuous wheat		*R. trifolii*		*R. leguminosarum*	
Aldicarb	63	252	2 years	13 years	surface	deep	surface	deep
−	+	−	+	−	4·68	4·31	4·47	3·36
−	−	+	+	−	4·87	3·79	5·00	4·08
+	+	−	−	+	3·79	2·94	5·12	3·94
+	−	+	−	+	3·97	2·36	5·27	3·65
+	−	+	+	−	3·81	2·89	4·52	3·29
+	+	−	+	−	4·04	2·81	4·42	3·22
−	−	+	−	+	3·23	3·23	5·17	4·02
−	+	−	−	+	3·84	3·32	3·80	4·04

ryegrass. *R. leguminosarum* was slightly more abundant throughout than *R. trifolii*, and with few exceptions surface counts exceeded deep counts. Aldicarb appeared to depress numbers (especially of *R. trifolii*) in combinations with other treatments, and in most comparisons fewer rhizobia were found in plots given the larger N fertiliser dressing and in those having grown wheat continuously for 13 rather than 2 years.

In the analysis of variance, tests of significance of main effects and first order interactions were made by assigning the second and third order interactions to error. Effects for depth, aldicarb and number of years under cereal, but not nitrogen fertiliser level, were significant at 5% for *R. trifolii*. First order interactions of aldicarb with number of years were significant at 5% and with depth at 10%, but the tables of means shows that these effects were not additive.

For *R. leguminosarum* the only significant single factor effect was depth but significant interactions appeared between aldicarb with depth (5%) and aldicarb with number of years of wheat (5%). The former was due to a larger reduction in the deep sample than expected from an additive model, whereas the latter appears to involve an absence of an aldicarb effect after the longer period of cereal culture, for which no explanation can be offered.

Commercial Farms (Table 8)

Caddington. The field chosen for study was on chalky escarpment and had been in cereals since 1958, except for linseed in 1977; no legumes had been sown during this period. Compound fertiliser providing 74 kg N ha^{-1} was used for each crop. Weeds were controlled by herbicides and the straw was burnt on site. Except for volunteer flax, weeds were very sparse. Samples were taken from the lowest (No. 1) to the highest point (No. 3), and also from a nearby natural grassland on the chalk escarpment (see Table 4). For the cereal site the surface and deep samples did not differ and are combined. At the three cereal sites *R. trifolii* and *R. leguminosarum* were about equally abundant and fewer than under grassland, where the surface populations exceeded the deep populations (Table 4).

East Hyde Home Farm. The area sampled was ploughed from permanent grass in 1962, limed, and used for continuous cereal production with normal cultivations and herbicides. Residual straw was burnt on the field. Five multiple samples were taken

https://doi.org/10.23637/ERADOC-1-18

TABLE 8

Effects of intensive cereal production on the numbers of rhizobia in the soil of commercial farms. Mean log MPN g^{-1} soil

		R. trifolii	SE max.	*R. leguminosarum*	SE max.
Caddington. Mr. T. E. Dickenson's Farm					
Highland Field	Sample 1 (low)	4·63		>3·98	
20 years in cereals	Sample 2	5·16	0·300	>4·48	0·273
	Sample 3 (high)	4·76		>3·98	
East Hyde, Mr. T. E. Dickenson's Farm					
Doll Field	Site 1 surface	4·63		<0·58	
	deep	5·22		2·88	
	2 surface	4·46		3·15	
	deep	5·46		2·87	
	3 surface	4·28		2·12	
	deep	4·69	0·290	2·49	0·294
	4 surface	4·75		3·77	
	deep	5·26		3·71	
	5 surface	4·27		3·30	
	deep	4·44		2·43	
Thrales End Farm, Mr. J. W. Pigott's Farm					
Redbourn land	Site 1	4·90		4·90	
	2	4·92		4·61	
	3	4·20	0·300	4·20	0·290
	4	5·53		4·48	
	5	5·72		4·19	

across the field which had a slight dell in its centre (sample 3). The soil (clay with flints) of samples 1 and 2 were gravelly and those of 4 and 5 heavier. Nitrogen fertiliser was applied annually at 90 kg ha^{-1}. In 1972 100 t ha^{-1} of Luton sewage sludge was applied and the area was dunged in 1977. In contrast to the other arable sites examined there were consistently fewer *R. trifolii* in the surface soil but the counts showed no trends related to topography.

The numbers of *R. leguminosarum* were unusually low, in one sample less than one cell per gram of dry soil.

Thrales End Farm. This site had grown spring barley for 15 years, using minimal cultivation in most years, but prior to sampling it had been deeply ploughed. The soil was a heavy clay with flints. Normal rates of fertiliser were used with herbicides and periodic liming. Half the straw was removed from the field and half burnt. Samples were taken on an E–W line across the centre of the field and because of recent deep ploughing the surface and deep samples were bulked. *R. trifolii* was somewhat more numerous than *R. leguminosarum* but no trends in numbers were seen across the field.

Effect of number of years under continuous cereal

Fig. 1 shows that the decline in numbers were broadly related to the length of the continuous cereal sequences (except for *R. leguminosarum* at Woburn). Also shown (on the zero ordinate) are the mean values found at Rothamsted and Woburn for both *Rhizobium* species in the presence of their respective hosts and (as separate histograms) the values found in the long-term fallows. Data were insufficient to determine the regressions of the log MPN on years under cereal but the figure suggests that after an initial rapid decline there is a simple linear relationship appropriate to a slow exponential decline in numbers, similar to that previously demonstrated for long-term fallow, but at a lower rate.

https://doi.org/10.23637/ERADOC-1-18 *pp 11*

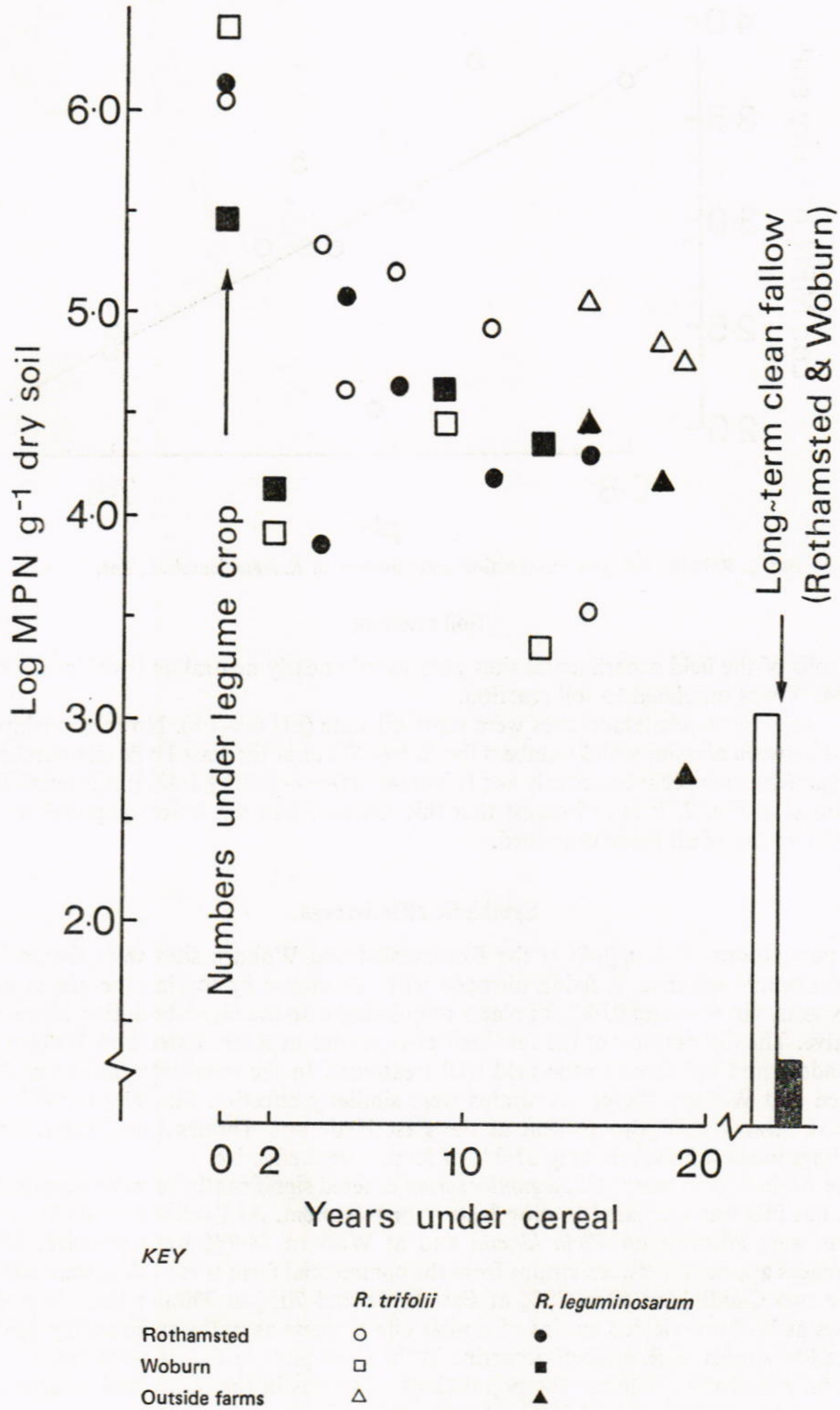


FIG. 1. The influence of continuous cereal farming on number of rhizobia in the soil.

https://doi.org/10.23637/ERADOC-1-18

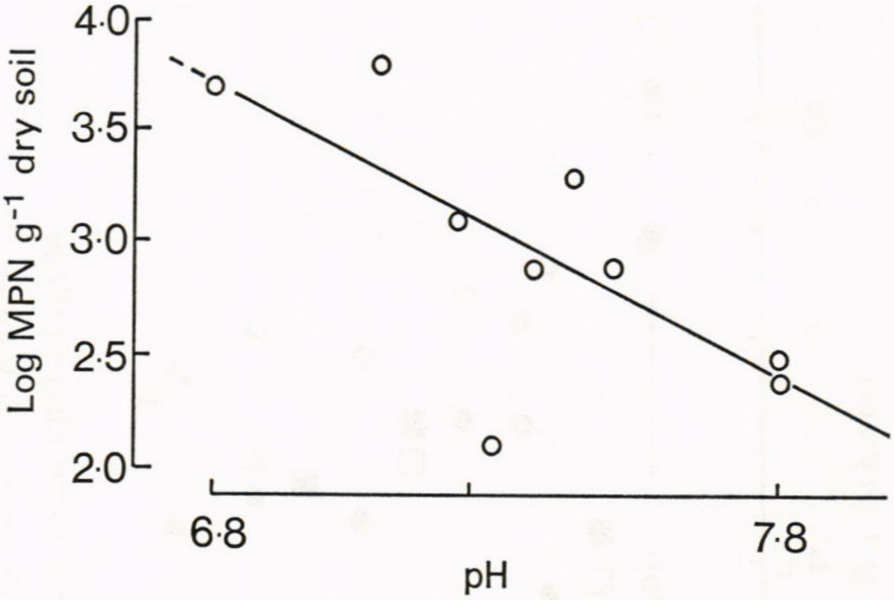


FIG. 2. Relation between soil reaction and numbers of *R. leguminosarum*, East Hyde.

Soil reaction

The soils of the field experimental sites were mostly nearly neutral and within this range log MPN was unrelated to soil reaction.

The soils of the chalkland sites were more alkaline (pH 6·8–7·8). No relationship was found between alkalinity and numbers for *R. trifolii* but at the East Hyde site numbers of *R. leguminosarum* declined linearly as pH increased ($r = -1{\cdot}803 \pm 1{\cdot}407$); this relationship is plotted in Fig. 2. It is of interest that this site also had the lowest population of *R. leguminosarum* of all those examined.

Symbiotic effectiveness

The populations of *R. trifolii* at the Rothamsted and Woburn sites were shown to be predominantly effective in fixing nitrogen with red clover by scoring the plants in the MPN tests, viz. 95·0 and 93·8% of plants nodulating with the highest dilution of soil were effective. The distribution of the few ineffective strains at Rothamsted and Woburn was at random and unrelated to the field trial treatment. In the long-term fallows at Rothamsted and Woburn the clover strains were similarly effective. Similar proportions of effective strains were also present at the East Hyde and Thrales End Farms. At the Caddington sites, however, only 27·0% of strains were effective.

The strain effectiveness of *R. leguminosarum* differed significantly between experimental sites, but this was unrelated to crop history or treatment. At Rothamsted 94·5% of the strains were effective on *Vicia hirsuta* and at Woburn 84·9% were effective. Larger differences appeared between strains from the commercial farm sites; 100% were effective at the two Caddington sites, 88% at East Hyde and 70% at Thrales End. Long-term fallows at Woburn yielded strains of similar effectiveness as in the surrounding fields.

The few strains of *R. meliloti* occurring in the grass plots at Rothamsted and Woburn were fully ineffective whereas the populations of strains in the inoculated lucerne plots were largely effective; 75 and 100% effective respectively.

The more discriminating pot tests of 33 isolated strains of *R. trifolii* confirmed their

https://doi.org/10.23637/ERADOC-1-18

general effectiveness. Average DM yields were 182 mg per plant, compared with 209 mg for strain RCR 5 and 183 for strain RCR 0403. A few of the better strains were significantly more effective than the worst; but strain differences were not related to site or treatment. The ineffective strains gave a mean plant dry weight of 49 mg.

Discussion

Counts of rhizobia made in the clover/grass leys indicated that in the presence of the host plant the numbers of *R. trifolii* in the surface soil attain average values of 6·56 at Rothamsted and 7·15 at Woburn, with somewhat lower values in the 10–15 cm horizon; viz. 5·08 and 6·10 respectively. Similarly large populations were recorded in the ley arable experiments at Rothamsted and Woburn and of *R. meliloti* under lucerne. For *R. leguminosarum*, neglecting possible small effects of sample depth, corresponding populations under field beans at Rothamsted and Woburn were >6·21 and 6·63. In contrast, surviving populations of rhizobia under a pure stand of grass was least in the surface layer, where the general rhizosphere effect might have been expected to be stimulatory. This reduction was similar to that noted in the intensive grassland studies already referred to (ibid.) and thought to be due to the recorded lowering of pH in this zone.

At the other extreme continuous fallow at the two sites at Rothamsted and Woburn reduced the *Rhizobium* populations to much lower levels. Those of *R. trifolii* fell to about 3·30 and 2·88 respectively and *R. leguminosarum* at Rothamsted to about 1·32; these do not differ appreciably from numbers recorded in these fields 14 years previously and which may have been maintained by transfer from nearby highly dense populations. *R. meliloti*, on the other hand, eventually virtually disappeared in the absence of its host.

The elimination of leguminous hosts by continuous cereal cultivation has clearly reduced the numbers of soil rhizobia below the levels attainable in the presence of their hosts. None of the fields under cereals had average counts approaching these values. The highest for *R. trifolii* was 5·39 in the Cultivations for Cereals experiment at Rothamsted and for *R. leguminosarum* a value of 5·09 was recorded in the 'Chemical Reference' plots; the overall averages were 4·77 at Rothamsted, 3·91 at Woburn and 4·88 on the outside farm sites. The corresponding overall averages for *R. leguminosarum* were 4·48, 4·37 and 3·79. These represent average reductions of more than 100-fold for *R. trifolii* and approaching 1000-fold for *R. leguminosarum* at both sites.

These overall effects were influenced by the levels of N fertiliser and pesticides (aldicarb) but more prominently by the previous cropping history as already noted for several experiments. In one instance this was related to the sporadic occurrence of volunteer clover in areas more recently ploughed from leys. Otherwise the small differences in the composition of the generally very sparse weed populations appeared to have no effects on numbers of surviving rhizobia. Nor were these affected by the depth or intensity of cultivation or methods of straw disposal.

This study provided no evidence that the symbiotic effectiveness of either species was affected by intensive cereal farming. The proportions of ineffective and poorly effective responses in the MPN tests were low throughout (viz. *c.* 5%). This was confirmed by the supplementary assessment of isolates taken from some of the experiments using pot culture.

Although the quantities of N fertiliser used in intensive cereal farming are much below those used for intensive forage grass production, the effects on the soil populations of rhizobia are similar, even if less severe. This may be because the cultivation and herbicide practices had an augmenting effect on the relatively moderate rates of N application in eliminating the legume (in this case weed legumes and volunteer clovers). Numbers of rhizobia decreased more than could be attributed to the fertiliser but not so much with

https://doi.org/10.23637/ERADOC-1-18

the heavier dressings of fertiliser and in forage grass production. It should also be borne in mind that under cereals the soil is less likely to become acid, even temporarily, than when very heavy dressings of nitrogenous fertiliser are used with alternating liming.

Numbers appear in general not to be reduced to levels that would prevent nodulation of clover or beans planted after long cereal sequences; nevertheless, populations may be sufficiently depleted to make it worthwhile to consider seed inoculation of clover and beans, either to bring numbers up to normal levels or to supplant the residual rhizobia with strains of greater effectiveness.

Acknowledgements

The authors thank Mr. T. E. Dickenson and Mr. J. W. Pigott for permission to take samples from their farms, Mr. R. P. White for undertaking the statistical analysis of the results and Mrs. Pauline Brown for help in the preparation of the typescript.

References

Johnston, A. E. 1969) Plant nutrients in Broadbalk soils. *Rothamsted Experimental Station. Report for 1968*, Part 2, 93–115.

Nutman, P. S. (1969) Symbiotic nitrogen fixation: legume nodule bacteria. *Rothamsted Experimental Station. Report for 1968*, Part 2, 179–181.

Nutman, P. S. (1980) Adaptation. *Proceedings. International Symposium on nitrogen fixation, Phytochemical Society of Europe*. Ed. W. D. P. Stewart. Academic Press. (In the press.)

Nutman, P. S., Dye, M. & Davis, P. E. (1978) The ecology of *Rhizobium*. In: *Microbial ecology*. Ed. M. W. Loutit & J. A. R. Miles. Berlin: Springer Verlag, pp. 404–410.

Nutman, P. S. & Ross, G. J. S. (1970) *Rhizobium* in the soils of the Rothamsted and Woburn Farms. *Rothamsted Experimental Station. Report for 1969*, Part 2, 141–167.

Vincent, J. M. (1970) A manual for the practical study of the root nodule bacteria. *IBP Handbook* No. 15. Oxford: Blackwell Scientific Publications, 164 pp.

https://doi.org/10.23637/ERADOC-1-18