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Autecology of *Bradyrhizobium japonicum* in soybean-rice rotations

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Abstract

The effect of rice culture on changes in the number of a strain of soybean root-nodule bacteria, (*Bradyrhizobium japonicum* CB1809), already established in the soil by growing inoculated soybean crops, was investigated in transitional red-brown earth soils at two sites in south-western New South Wales. At the first site, 5.5 years elapsed between the harvest of the last of four successive crops of soybean and the sowing of the next. In this period three crops of rice and one crop of triticale were sown and in the intervals between these crops, and after the crop of triticale, the land was fallowed. Before sowing the first rice crop, the number of *Bradyrhizobium japonicum* was 1.32×10^5 g^{-1} soil. The respective numbers of bradyrhizobia after the first, second and third rice crops were 4.52×10^4, 1.26×10^4 and 6.40×10^2 g^{-1} soil. In the following two years the population remained constant. Thus sufficient bradyrhizobia survived in soil to nodulate and allow N_2-fixation by the succeeding soybean crop. At the second site, numbers of bradyrhizobia declined during a rice crop, but the decline was less than when the soil was fallowed (400-fold cf. 2200-fold). Multiplication of bradyrhizobia was rapid in the rhizosphere of soybean seedlings sown without inoculation in the rice bays. At 16 days after sowing, their numbers were not significantly different ($p < 0.05$) from those in plots where rice had not been sown. Nodulation of soybeans was greatest in plots where rice had not been grown, but yield and grain nitrogen were not significantly different ($p < 0.05$). Our results indicate that flooding soil has a deleterious effect on the survival of bradyrhizobia but, under the conditions of the experiments, sufficient *B. japonicum* strain CB1809 survived to provide good nodulation after three crops of rice covering a total period of 5. 5 years between crops of soybean.

Introduction

Soil used for the production of rice (*Oryza sativa* L.) under a paddy system undergoes physical, physiochemical and biochemical changes as a result of immersion (Bacon and Cooper, 1985; De Datta, 1981). The changes are often sustained and may affect growth of following crops (Bacon and Cooper, 1985). Likewise, these changes may create environments hostile to aerobic, chemo-heterotrophic organisms such as *Bradyrhizobium japonicum*. Soybean (*Glycine max* (L.) Merr.), which is frequently grown in rotation with rice on irrigated soils in Australia, is dependent on symbiosis with *B. japonicum* to fix atmospheric N_2. Under optimum conditions for N_2 fixation, high-yielding crops of soybean can fix their own nitrogen (N) requirements and contribute substantially to N reserves in the soil (Bergersen et al., 1985; Chapman and Myers, 1987; Hughes and Herridge, 1989). Any detrimental effect of cropping rice on populations of *B. japonicum* may adversely affect nodulation of soybeans and the N economy of soybean-rice rotations.

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B. japonicum does not occur naturally in Australian soils (Diatloff and Brockwell, 1976) and strain CB1809 has been used as the sole Australian commercial inoculant strain for soybean since 1966 (Date, 1969). It is now widely distributed in fields used for soybean cropping, and is the dominant and usually the only strain of *B. japonicum* present (Brockwell and Roughley, unpubl. data). There has been little or no loss of symbiotic characters among field populations of CB1809 (Gibson et al., 1990).

These circumstances present a unique opportunity for field studies of the autecology of a single strain of *B. japonicum*. In this communication, we report two experiments in which numerical trends in soil populations of *B. japonicum* were followed when rice was grown in fields which had previously grown well-nodulated crops of soybean. In the first experiment, rice was grown for four successive years followed by two periods of fallow, separated by one crop of triticale (× *Triticosecale* sp.), before the land was resown with soybean. In the second, rice was grown for only one season before resowing with soybean.

Materials and methods

Experiment 1

This experiment was part of a commercial sowing at Coleambally, New South Wales, (34° 51'S, 146° 05'E) on a transitional red-brown earth, (Dr2.22; Northcote et al., 1975) typical of many soils used for growing rice in the region. Soil pH (1 : 5, soil : H_2O) 5.7; mineral N, 2.0 $\mu g\ g^{-1}$ NO_3 and 3.0 $\mu g\ g^{-1}$ NH_4 was measured before soybean was sown in 1990. Irrigated soybean cv. Chaffey was grown for four seasons from 1981/82 to 1984/85. The seed of each crop was inoculated with commercial peat inoculant containing *B. japonicum* strain CB1809. Rice was then grown for three seasons; 1985/86, 1986/87 and 1987/88. The field was then fallowed for six months until triticale (× *Triticosecale* sp.) was grown in winter 1989. After it was harvested in December 1989, the field was again fallowed until November 1990 when soybean (cv. Bowyer) inoculated with CB1809 was sown. A strip 3 m wide, running the length of the field, was sown with uninoculated seed.

Observations began in 1985. There were 10 sampling sites in the field arranged in grid formation at centres of approximately 30 m. These sites were precisely relocatable by reference to fixed coordinates. Soil populations of CB1809 were counted on seven occasions: in October 1985 before rice was sown; in August 1986, May 1987 and September 1988 after the harvest of each rice crop; in November 1989 after harvest of the triticale; in September 1990 during fallow and finally immediately before sowing soybean cv. Bowyer in November 1990. In 1990/91 nodulation was scored at R3 (early pod-fill) and R6 (just prior to physiological maturity) according to the soybean physiological development scheme of Fehr et al., (1971). Nitrogen in the crop was measured at R6 and the proportion of crop N derived from N_2 fixation was estimated at R3 and R6 (Peoples et al., 1995). Grain was harvested at maturity.

Experiment 2

This experiment, also on a transitional red-brown earth, was located at the Yanco Agricultural Institute, Yanco, N.S.W. (34° 36'S, 146° 25'E). The area (110 m × 50m) had been sown with inoculated soybean cv. Chaffey in 1988/89 which was harvested in April 1989. The area was not cropped until November 1989 when it was divided into nine equal plots in a fully randomised design, three to be sown with rice cv. Echuca, three with uninoculated soybean cv. Stephens and three left as bare fallow.

Two L ha^{-1} of pre-emergence herbicide Trifluralin and P as Mo superphosphate at 25 kg ha^{-1} were incorporated into all plots and 120 kg ha^{-1} of N as urea was drilled into the rice plots one week prior to sowing the rice. These are designated "1st crops". The integrity of these treatment plots was retained for the duration of the experiment; they are referred to as "original rice", original soybean" and "original fallow". Weeds were controlled in the rice plots with Ordram® at two L ha^{-1} applied at one week and Londax® at 60 g ha^{-1} applied at five weeks and in the bare fallow plots by scarification to a depth of 5 cm. The rice plots were flooded after two weeks and the soybean plots were furrow-irrigated at one and 14 days after sowing (DAS) and then as necessary to avoid moisture stress. After harvest of the "first crops", the trash was burned, the area levelled, cultivated, fertilised with 10 kg ha^{-1} of P as Mo superphosphate and sown with oats (*Avena sativa* L. cv. Cooba) in June 1990. The oats is designated the "2nd crop". The oats was harvested for hay in October 1990. The land was prepared, fertilised with 40 kg ha^{-1} of P as Mo superphosphate and sown with uninoculated soybean cv. Stephens, designated the "3rd crop", in December 1990.

Plant densities one month after emergence were in the range 2.5 – 3.0 × 10^5 plants ha^{-1} and 6.0 - 7.5 × 10^5 plants ha^{-1} for soybean and cereals, respectively.

The population of bradyrhizobia in the soil was estimated before sowing 1st crop soybean and rice. The populations which then developed in the soybean and rice rhizospheres and in the fallowed soil were estimated at 14, 35, 84, and 125 DAS, and also 26 days after the crops were harvested (175 DAS). The numbers in oat rhizospheres were estimated at 34 and 145 DAS. Populations were estimated in the soil on the day the 3rd crop soybean was sown and in soybean seedling rhizospheres at 16 DAS.

Grain produced by 1st crop soybean and rice was harvested at maturity. Growth of 2nd crop oats was excellent and uniform across all plots. Shoot N was determined at 145 DAS. The crop was then removed with a forage harvester before maturity to allow time to prepare the land for soybean. The biomass of oats was not measured. Nodulation of 3rd crop soybean was scored at 60 DAS; grain yield and N content of the grain were determined at maturity.

Data for this experiment were analysed separately between sampling times within each crop and also between crops within each sampling time by analysis of variance. The data for bacterial counts were subjected to $\log_{10}$ transformation before analysis.

Observations, assays and measurements

The most probable number (MPN) of *B. japonicum* in soils and rhizospheres was estimated using a dilution/nodulation-frequency, plant-infection test (Brockwell et al., 1975). Three cores of soil (ca. 10 cm deep × 5 cm diam.), from each of the 10 sampling points in Experiment 1 were combined, mixed and sub-sampled. In Experiment 2, samples for counting populations of *B. japonicum* in rhizosphere soil of rice consisted of the roots of 12 single rice plants dug on a diagonal transect across each plot. For soybean, 12 samples of five plants were dug at random using a spade at 14 days and of three plants at the other samplings when the plants and their root systems were larger. For oats, five samples each of four plants were dug from each plot. The roots were shaken to remove excess soil and transferred to a polyethylene bag. Soil in the fallow plots was sampled at 12 sites on a diagonal transect of each plot at a depth between 4-10 cm. Each soil sample was mixed, sub-sampled and transferred to a polethylene bag. The samples of roots and adhering soil and soil samples from the fallow plots were kept cool on ice until diluted for counting bradyrhizobia. The sub-samples of soil, or soil adhering to the plant roots, were suspended in sterile H_2O by agitation in a stomacher for 30 sec (Sharpe and Jackson, 1972). A 10-fold dilution series of each suspension was prepared and used to inoculate three test plants of *Glycine soja* Sieb. and Zucc. line Q10847 at each dilution level. The elapsed time in Experiment 2 between sampling and inoculation of the test plants never exceeded 6 hr. After diluting the soil for counting, the first dilution was evaporated to dryness and the dry weight of soil determined to enable the number of bradyrhizobia to be expressed on the basis of dry rhizosphere soil.

Nodulation was scored on a 0 – 5 basis (mean of 10 plants) taking into account nodule number, size, pigmentation and distribution (Sykes et al., 1988).

Extractable mineral N in soil was determined according to Turner et al. (1987). Procedures for handling samples of plant material from the field and their preparation for Kjeldahl analysis of N were as described by Bergersen et al. (1985).

In Experiment 1, N_2 fixation was estimated by ureide assay (Herridge and Peoples, 1990). Plants were sampled at R3 and R6 stages of development (Fehr et al., 1971). Xylem sap was recovered by vacuum extraction from four replicates of four plants selected at random from each of the inoculated and uninoculated areas within 10 min of removing plants from soil (Herridge et al., 1988). Sap samples were chilled on ice immediately after collection and later frozen until analysed for ureides (allantoin and allantoic acid), nitrate and amino-N (Peoples et al., 1989). The proportion of plant N derived from N_2 fixation, (P_{fix}), was calculated from the relative abundance of ureide N in xylem exudates (Herridge and Peoples, 1990).

Yield was expressed at moisture contents of 12% for soybean grain and 14% for rice paddy.

Results and discussion

Experiment 1

The soil population of *B. japonicum* in October 1985 following four successive crops of soybean was 1.32 × 10^5 g^{-1} soil (Fig. 1). During 1985/88, when rice was grown, the population declined by more than 99.5% to 6.40 × 10^2 g^{-1} soil. In the following two years which included a crop of triticale and a period of bare fallow, the population remained approximately constant. When soybean was sown in November 1990, it was

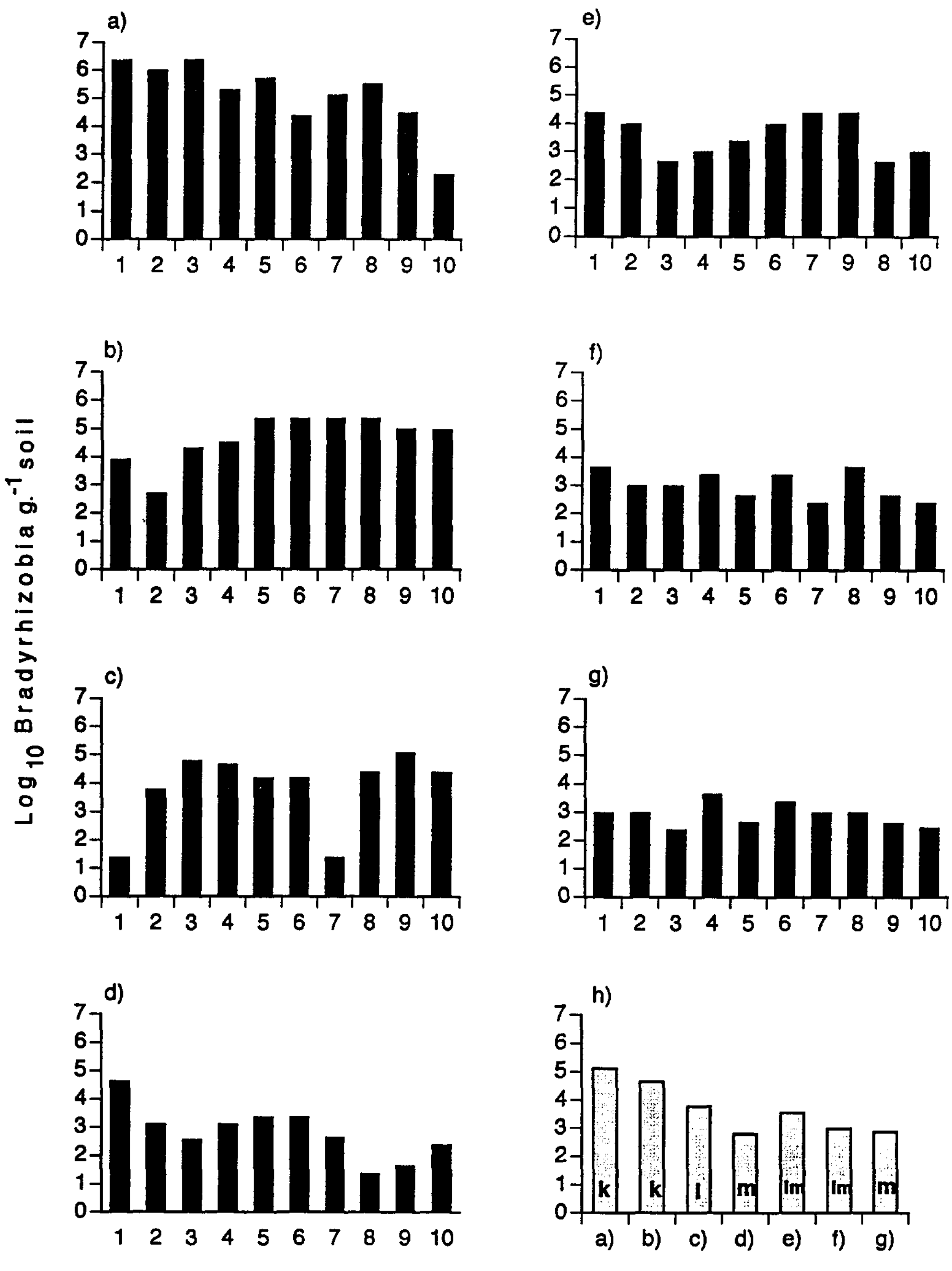

Fig. 1. Experiment 1. Changes in numbers (MPN) of *B. japonicum* at 10 relocatable sites (1–10) on a red-brown earth used for cropping at Coleambally, NSW, over the period October 1985 to November 1990. **a.** Before rice crop 1 was first sown: October 1985. **b.** Post rice crop 1: August 1986. **c.** Post rice crop 2: May 1987. **d.** Post rice crop 3 soil not cropped: September 1988. **e.** Post triticale: November 1989. **f.** During fallow: September 1990. **g.** Immediately before sowing soybean crop of 1990/91: November 1990. At any one time of sampling, the minimum significant difference (p =0.05) between individual sites is represented by 1.16 $\log_{10}$ units. **h.** Mean values for each time of sampling (a-g) are shown by stippled bars; stippled bars with a common letter are not significantly different at p =0.05.

9.50×10^2 g^{-1} soil. The ranked order of sampling sites, according to numbers of *B. japonicum* varied considerably between times of sampling (Fig. 1). This observation resembles the marked variation, in time and place, found in the size and effectiveness of soil populations

Table 1. Experiment 1. Nodulation, N_2-fixation and yield of Bowyer soybeans sown with and without inoculation in November 1990, at Coleambally, NSW

Variable	Inoculated	Uninoculated[a]
Nodule score at R3[b]	4.3	3.8
Nodule score at R6[b]	4.7	4.6
P_{fix}(%) at R3	77	72
P_{fix} (%) at R6	96	89
Crop N at R6 (kg ha^{-1})	348	337
Grain yield (t ha^{-1})	2.96	2.96

[a]No significant difference (p=0.05) between treatments.
[b]R3 (early pod-fill) and R6 (just prior to physiological maturity) (Fehr et al., 1971).

of *Rhizobium trifolii* in grazing fields in New South Wales and Victoria (Gibson et al., 1975).

When soybean was grown commercially in this field in 1990/91, no significant difference (p <0.05) between inoculated and uninoculated treatments was recorded for any index of nodulation, N_2 fixation or yield (Table 1). The population of *B. japonicum* remaining in the soil after 5.5 years without soybean was sufficient, without further inoculation, to provide excellent nodulation of the crop, and to allow a high reliance on N_2 fixation for growth.

Experiment 2

Large numbers of *B. japonicum* developed in the soil during and following the growth of the original soybean crop in 1988/89. Immediately before sowing the 1st crops in November 1989, the soil population (4–10 cm) was ca. 1.0×10^5 g^{-1} soil (Table 2). Numbers of *B. japonicum* in the 1st crop rice and fallow treatments during the 1989/90 season declined significantly (p <0.05). Immediately after harvest, populations in the soybean plots exceeded 2.2×10^5 bradyrhizobia g^{-1} soil, greatly outnumbering those in rice (3.78×10^2 g^{-1}) and in fallow plots (4.46×10^1 g^{-1}).

When the soil was cropped with oats (2nd crop, 1990) numbers of *B. japonicum* in the original soybean plots had declined markedly whereas those in the other plots were largely unchanged (Table 3). They did not multiply in the oat rhizospheres between 34 and 145 DAS so that on sowing day of the 3rd crop soybean (December 1990), populations of *B. japonicum* in the original soybean plot were only 0.33% of the numbers in these plots when counted at 175 DAS ($\log_{10}$ 2.88 Table 3 cf $\log_{10}$ 5.35 Table 2). Sixteen DAS soybean (3rd crop), numbers of bradyrhizobia in rhizosphere soil had increased >1600-fold (Table 3).

Nodulation of the 3rd crop soybean at 60 DAS was significantly better (p <0.05) in the "original soybean" treatment than in the "original rice" and "original fallow" (Table 4) but yield of grain and its % N were not significantly higher than produced in those treatments (p >0.05). Substantial amounts of N were mineralised during the fallow as indicated by the relatively high N content of oats grown on the "original fallow" plots in 1990 (Table 4). Residual nitrate in these plots may have inhibited symbiotic development in the 1990/91 3rd crop soybean resulting in reduced grain N (cf. Bergersen et al., 1989).

General discussion

Experiment 1 shows that soybean bradyrhizobia persisted in the soil in the absence of the specific host for periods up to 5.5 years during which time three rice crops were grown and the soil flooded for extended periods.

A striking feature of the results was the dynamic nature of the soil populations of *B. japonicum*. Despite uneven distribution of root nodule bacteria in soil and inherent errors associated with the method of their estimation, changes in the size of their populations were sufficiently large to be statistically significant (p =0.05). At both sites, estimates of the population of bradyrhizobia within any one plot indicated differences which often exceeded 1000-fold. Large numbers developed during soybean cropping which persisted for a short time after crop maturity and harvest. In the absence of soybeans, populations tended to decline slowly at first (Table 2) and then later remain almost constant in bare soil and in oat rhizosphere soil at approximately $\log_{10}$ 2.0 g^{-1} (Table 3). A similar response occurred in Experiment 1 although here the basal population was approximately 10-fold higher.

The resultant population of bradyrhizobia in the rice and fallow plots is dependent on different stresses. The decline in numbers in the fallow plots we attribute partly to stress from low soil moisture, but more importantly to higher soil temperatures during summer. The mean maximum shade temperature between 35 and 84 days when numbers declined from $\log_{10}$ 6.0 to 1.72 was 32.3°C, with 12 days > 37°C and one day of 45°C. In the rice plots the roots were shaded and the

Table 2. Experiment 2. Numbers of *B. japonicum* in soil (log_{10} bradyrhizobia g^{-1} soil) growing either rice or soybeans or lying fallow at Yanco, NSW in 1989/90 and following soybean grown in 1988/89

Day of sampling[z]	1st crop rice[y]	1st crop soybean[y]	Fallow[y]
0	5.18 a A	5.04 a B	5.00 a A
14	3.06 b B	4.94 a B	4.45 a b A
35	3.72 b B	6.74 a A	6.00 a A
84	4.11 b A	6.45 a A	1.72 c B
125	3.43 B	n.d.[x]	n.d.
175	2.58 b C	5.35 a B	1.65 c B

[z]Sampled at intervals between day of sowing (day 0) and 26 days after final harvest (175 DAS). All samples except those from the fallow plots and at day 0 (sowing day) and 175 (after harvesting) from the rice and soybean plots were of rhizosphere soil.
[y]Data analysed separately for the different original treatments and for the different times of sampling; each value is the mean of 4 MPN estimates. Values in each **row** followed by the same lower case letter are not significantly different (p=0.05). Values in each **column** followed by the same upper case letter are not significantly different (p=0.05).
[x]n.d., not done.

Table 3. Experiment 2. Numbers of *B. japonicum* in Yanco soil (log_{10} bradyrhizobia g^{-1} soil) in the rhizosphere of oats 1990, in the seedbed for soybeans sown in 1990 and in their rhizosphere when grown in plots which originally (1989/90) grew either rice or soybean, or lay fallow

Time of sampling	Original rice plot[Z]	Original soybean plot[Z]	Original fallow plot[Z]
2nd crop - oats (1990)			
34 DAS[Y]	2.23 b B	3.26a B	1.22 c C
145 DAS	2.25 b B	3.01 a B	1.88 b B
3rd crop - soyhean (1990/91)			
Immediately before sowing	2.23 b B	2.88 a B	2.27 b B
16 DAS	5.82 a A	5.88 a A	5.36 a A

[Z]Data analysed separately for the different original treatments and for the different times of sampling. Values in each **row** followed by the same lower case letter are not significantly different (p =0.05). Values in each **column** followed by the same upper case letter are not significantly different (p =0.05).
[Y]Days after sowing.

water temperature approximated the air temperature. The decline in numbers resulting from rice culture is likely to be a response to direct and indirect changes attributable to flooding. The final stable populations probably reflect the base sustainable humber in these soils at the prevailing carbon levels until the equilibrium is perturbated by cultivation, fertilisation and root development.

The lowest population of 17 bradyrhizobia g^{-1} of rhizosphere soil (Table 3), occurred under "2nd crop" oats growing in the original bare fallow plot. Less than six months later and only 16 days after sowing soybeans, populations of *B. japonicum* in the rhizosphere soil of these plots had increased to 2.29×10^5 g^{-1} (Table 3). Thus despite extensive mortality in some instances, the residual population was invariably suf-

Table 4. Experiment 2. Yield, nodulation and nitrogen indices of grain yield for three crop sequences at Yanco, NSW

Crop and time of sampling for	Original rice plot	Original soybean plot	Original fallow plot	LSD (p=0.05)
1st crop (1989/90)				
Grain yield (t ha^{-1})[b]	10.30	3.58	0.00	n.a.[a]
2nd crop -oats (1990)				
Crop N (%) 145 DAS[c]	0.80	0.95	1.08	0.14
3rd crop - soybean (1990 91)				
Nodule score (0-5) 60 DAS	1.90	2.64	1.94	0.47
Grain yield (t ha^{-1})	3.58	3.49	3.10	n.s.[d]
Grain N (%)	6.51	6.48	6.44	n.s.
Grain N (kg ha^{-1})	233	226	200	24

[a]n.a. not applicable.
[b]Of rice and soybean in the original rice and soybean plots respectively.
[c]Days after sowing.
[d]n.s., not significant.

ficient, without seed inoculation, to induce abundant nodulation and N_2 fixation by the crop. The rapid multiplication of the naturalised population highlights the advantage, possibly as a result of acclimation, that resident rhizobia may have relative to those recently introduced by inoculation. Such an advantage was demonstrated previously in studies of competition between different spontaneous mutants of this strain CB1809 in another soil (Brockwell, Roughley and Herridge, 1987).

Our results in Experiment 1 are also consistent with those of Osa-Afiana and Alexander (1979), who reported a strain of *B. japonicum* survived in flooded soil in relatively constant numbers, irrespective of size of the population, when the soil was first flooded. Weaver et al. (1987) also found that growing rice after soybean did not substantially reduce populations of *B. japonicum* in a number of major soil types in Texas. The decline in numbers they reported was of similar magnitude to those we observed during rice cropping in Experiment 1 rather than the more rapid effect we report in Experiment 2. It is important to note that they were dealing with a mixed population of strains which may have been better able to respond to changing conditions than the single strain used in our experiments.

It is obvious from the extensive work on the growth of soybean in saturated soil culture (e.g. Hartley et al., 1993; Lawn and Byth, 1989; Nathanson et al., 1984; Troedson et al., 1989; Wang et al., 1993) that inundation per se does not prevent *B. japonicum* introduced by seed inoculation from nodulating soybean. Soybeans grown in saturated soil culture nodulate abundantly and populations of bradyrhizobia in the saturated soil are frequently very large (Gemell and Roughley, unpubl. data).

However, it is prudent to strike two notes of caution. Firstly, our conclusions are based only on the performances of a single strain, CB1809, at 2 sites both of the same soil type. This strain has been reported by Peoples et al. (1995) to be sensitive to soil type, persisting so poorly in certain alkaline soils, that it cannot be detected within eight months from harvest of a well-nodulated soybean crop. Secondly, Boonkerd and Weaver (1982) observed various strains of bradyrhizobia for cowpeas and peanuts differed in their survival in flooded soil.

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