Biol Fertil Soils (2004) 40: 28–35
DOI 10.1007/s00374-004-0739-1

ORIGINAL PAPER

Iêda Carvalho Mendes · Mariangela Hungria · Milton Alexandre Teixeira Vargas

Establishment of *Bradyrhizobium japonicum* and *B. elkanii* strains in a Brazilian Cerrado oxisol

Received: 28 July 2003 / Accepted: 16 February 2004 / Published online: 19 March 2004

Abstract The competition with established soil populations of *Bradyrhizobium* able to nodulate soybean has been one of the major constraints to the introduction of more efficient strains in Cerrados soils. The effects of nodulation establishment and persistence of four serologically distinct strains of *Bradyrhizobium japonicum* (CPAC 15 and CPAC 7, belonging to serogroups USDA 123 and CB 1,809) and *B. elkanii* (29 W and SEMIA 587, belonging to serogroups 29 W and 587) were examined. These strains were introduced in a dark-red oxisol, without indigenous populations of soybean bradyrhizobia, and were evaluated for 6 years. The experimental design was a completely randomized block with four replicates. In the first year, besides the inoculation treatments, there was also an uninoculated control. In the second year, the main plots were split into three sub-plots and treatments consisted of an uninoculated control, CPAC 7 and CPAC 15. In the third year, the entire area was inoculated with CPAC 7. In the fourth and sixth years, the plots were planted with soybean without inoculation, and in the fifth year the plots were left fallow. The strains introduced in the first year influenced nodule occupancy by strain CPAC 7 until the third successive growing season. By the fourth and sixth years, as a consequence of the dispersal of strains serologically related to serocluster 123 in the entire experimental area, this serogroup dominated the nodulation, occurring, on average, in more than 50% of the nodules of the treatments where it had never been inoculated.

I. C. Mendes (✉)
Centro de Pesquisa Agropecuária dos Cerrados, Embrapa,
Caixa Postal 08223, CEP 73301-970 Planaltina, DF, Brazil
e-mail: mendesi@cpac.embrapa.br

M. Hungria
Centro Nacional de Pesquisa de Soja, Embrapa,
Caixa Postal 231, CEP 86001-970 Londrina, PR, Brazil

M. A. T. Vargas
Bioagri Laboratórios,
Caixa Postal 08287, CEP 73301-970 Planaltina, DF, Brazil

Keywords Reinoculation · Competitive ability · Soybean · Biological nitrogen fixation

Introduction

The Cerrados region is one of the most important soybean [*Glycine max* (L) Merryll] producing areas in Brazil. In 2002/2003, 10 million hectares were cultivated with this crop in the Cerrados, with an average yield of 2,677 kg ha^{-1} (CONAB 2003). One of the factors responsible for the successful expansion of soybean in Brazil is its capacity to nodulate and fix N_2 effectively with *Bradyrhizobium* strains, resulting in a yearly economy of U.S. $ 2.5 billion.

As a result of the rapid expansion of soybean in the Brazilian Cerrados, populations of *Bradyrhizobium japonicum* and *B. elkanii* are now established in these soils, which originally were void of these bacteria (Peres and Vidor 1980; Vargas and Suhet 1980). For this reason, competition with established soil populations of *Bradyrhizobium* able to nodulate soybean has been one of the major constraints to the introduction of new and more efficient strains, raising doubts about the benefits of reinoculation.

Since 1986, it has been observed that strains from serogroup 123 occur in up to 70% of the soybean nodules growing in the Brazilian Cerrados, even in areas where it had never been inoculated (Vargas et al. 1993). The occurrence of strains from serogroup 123 in these areas is attributed to contamination by seeds and agricultural machinery originating from older soybean growing areas in southern Brazil, where inoculants containing strain SEMIA 566 (serologically related to the 123 serogroup) were used until 1978. More recently, the presence of strains serologically related to this serogroup was even reported in soils of the Amazon, which might be associated with contamination by rain and wind (Ferreira and Hungria 2002). The presence of the 123-serocluster strains in areas where they have never been introduced is evidence of a high saprophytic capacity.

Strain USDA 123 was isolated in 1960 from a soybean nodule in Iowa (Keyser and Cregan 1987). Since then strains serologically related to the 123 serogroup have been reported as being the most competitive of the indigenous *B. japonicum* strains from the midwestern United States, characteristically occupying 60–80% of the nodules formed (Damirgi et al. 1967; Kvien et al. 1981). The occurrence of this serogroup has also been reported in Canada (Semu and Hume 1979), and in Korea (Kang et al. 1991). The term serocluster 123 includes the three closely related, immunologically cross-reactive serotypes 123, 127 and 129 (Schmidt et al. 1986).

Despite the importance of soybean production in Brazil, little is known about the establishment of different populations of *B. japonicum* and *B. elkanii* in native Cerrados soils, their influence on soybean yields, and on the occurrence of inoculated strains. In this study, we examined the effects of nodulation establishment and persistence of four serologically distinct populations of *B. japonicum* and *B. elkanii*, introduced in a native Cerrado soil void of indigenous populations of soybean bradyrhizobia.

Materials and methods

Inoculum preparation

Single-strain peat inoculants were prepared from pure cultures of *B. japonicum* strains CPAC 7 (= SEMIA 5,080) and CPAC 15 (= SEMIA 5,079); serogroups CB 1,809 and USDA 123; (Boddey and Hungria 1997) and *B. elkanii* strains 29 W (= SEMIA 5,019) and SEMIA 587 (serogroups 29 W and 587) grown in yeast mannitol broth. Broth cultures were applied to sterilized peat (whose pH had been previously raised to 6.5 with $CaCO_3$) to reach about 50% moisture. The mixture was allowed to mature at room temperature for 30 days, reaching 1.8×10^8 to 2.0×10^9 cells g^{-1} peat (MPN counts). Immediately before planting, the seeds were inoculated by preparing a peat slurry with a 25% sucrose sticker solution, at a rate of 1 kg inoculant per 50 kg seeds, giving approximately 10^6 cells $seed^{-1}$.

Field trials

The field experiments were conducted at the Brazilian Cerrados Research Center (Embrapa Cerrados), in Planaltina, DF, Brazil. The study was initiated in 1993 and was carried out for six successive years, until 1997. The soil was a clay Dark-red oxisol that had never been inoculated, and was void of indigenous *Bradyrhizobium* able to establish an effective symbiosis with soybean.

The chemical analysis of the soil before planting showed the following characteristics: pH (H_2O) 4.6; 0.95 $cmol_c\ dm^{-3}$ Al, 0.45 $cmol_c\ kg^{-1}$ Ca + Mg, 0.9 mg kg^{-1} P, 21 mg kg^{-1} K and 30.2 g kg^{-1} soil organic matter. Ca, Mg and Al were extracted with 1 N KCl and determined through atomic absorption (Ca and Mg) and titration with 0.025 M NaOH (Al); P and K were extracted using the Mehlich 1 method (0.0125 M H_2SO_4 + 0.05 M HCl), and determined through flame spectrophotometry (K) using the blue-Mo method (P); soil organic matter was determined using the Walkley and Black method. All procedures are described by EMBRAPA (1997). Soil pH was corrected with the addition of 5 ton ha^{-1} dolomitic limestone. In the first year, 300 kg P_2O_5 ha^{-1}, 150 kg K_2O ha^{-1} and 60 kg FTE ha^{-1} were applied to correct P, K and micronutrient deficiencies. In all the other experiments, P and K were added to the soil with yearly band applications of 500 kg ha^{-1} of the mixture 0-20-20.

The experiment was arranged in a completely randomized block with three replicates. In the first year, the main plots were 17×4 m and treatments consisted of an uninoculated control (cultivated with rice), and soybean inoculated with four strains belonging to serologically distinct serogroups: 29 W, 587, CPAC 7 and CPAC 15 (serogroups 29 W, 587, CB 1,809 and USDA 123, respectively). In the second year, the main plots were divided into three sub-plots of 5×4 m cropped with soybean and the treatments consisted of an uninoculated control, CPAC 7 and CPAC 15. In the third year, the entire experimental area was cropped with soybean inoculated with strain CPAC 7. In the fourth and sixth years, the plots were planted with soybean without inoculation, and in the fifth year, the plots were cultivated with corn, cultivar BR 205. The experimental set up is presented in Table 1. The inoculation treatments of the first, second, third, fourth and sixth years will be specified in this order (e.g. 29 W/CPAC 15/CPAC 7/uninoc./uninoc.).

The experiments were performed with soybean cultivar Doko, planted 50 cm apart, with 17 seeds m^{-2}. Soybean seeds were sown 5 cm deep in moist soil on 22 November 1993, 11 November 1994, 17 November 1995, 18 November 1996, and 8 December 1998. In the first year, in the uninoculated plots cultivated with rice, the cultivar used was Rio Paranaiba, planted 50 cm apart, with

Table 1 Experimental set up (*1st year* 1993/1994, *2nd year* 1994/1995, *3rd year* 1995/1996, *4th year* 1996/1997, *5th year* 1997/1998, *6th year* 1998/1999)

Treatments[a]					
1st year	2nd year[b]	3rd year	4th year	5th year	6th year
SEMIA 29 W	Uninoculated	CPAC 7	Uninoculated	Corn	Uninoculated
	CPAC 7				
	CPAC 15				
SEMIA 587	Uninoculated	CPAC 7	Uninoculated	Corn	Uninoculated
	CPAC 7				
	CPAC 15				
CPAC 7	Uninoculated	CPAC 7	Uninoculated	Corn	Uninoculated
	CPAC 7				
	CPAC 15				
CPAC 15	Uninoculated	CPAC 7	Uninoculated	Corn	Uninoculated
	CPAC 7				
	CPAC 15				
Uninoculated	Uninoculated	CPAC 7	Uninoculated	Corn	Uninoculated
	CPAC 7				
	CPAC 15				

[a]In the first year the main plots consisted of an uninoculated control cultivated with rice and soybean inoculated with four strains. In the second, third, fourth and sixth year all plots were cropped with soybean

[b]In the second year, the main plots were split into three sub-plots

20 seeds m^{-2}. With the exception of the second year, when all plots were sown by hand, all experiments were sown by a hand seed machine.

The plots were laid out 1.0 m apart, to prevent contamination and, from the second crop on, the plots were disturbed as little as possible. Land preparation was minimal, consisting of reforming seed beds, to avoid the dispersal of bradyrhizobia.

Nodulation was evaluated in the experiments conducted in the second, third, fourth and sixth years (1994/1995, 1995/1996, 1996/1997 and 1998/1999, respectively). At the pre-flowering stage, six plants of the second and seventh rows of each plot were collected to determine the number and dry weight of nodules, and to perform serological analyses. The root systems were rinsed with tap water, the nodules detached, dried at 72°C for 72 h, weighed and counted.

At harvest, a 4.0-m section was removed from the four central rows of each plot. Harvested seeds were cleaned and weighed and yields were adjusted to 13% moisture.

The evolution of the bradyrhizobia soil population up to the fourth year (1996/1997) was followed with most probable number (MPN) counts, with a serial dilution plant infection method (Vincent 1970), using soybean cv. Doko as a test plant. The soil samples for the MPN counts were collected 1 month after seed germination in all treatments, according to the experimental set up of the first year.

Nodule serotyping

Serotyping of nodules was done by immuno-agglutination (Vincent 1970) and antisera were prepared against strains 29 W (serogroup 29 W), 587 (serogroup 587), CPAC 15 (serogroup USDA 123) and CPAC 7 (serogroup CB 1,809) as described previously (Somasegaran and Hoben 1994; Vargas and Suhet 1980). Strains 29 W and 587 have been used in Brazilian commercial soybean inoculants since 1979, and strains SEMIA CPAC 15 and SEMIA CPAC 7 since 1993. More information regarding these serogroups was given previously (Boddey and Hungria 1997). Recovery percentages for serogroups 29 W, 587, CB 1,809 and 123 were determined by serotyping 50 nodules selected at random from each plot.

Statistical analyses

The experiments were submitted to analyses of variance using the general linear procedure provided by the SAS software package (SAS Institute, Cary, N.C.). Percentage data were subjected to arcsin$\sqrt{(x)}$ transformations prior to analysis and retransformed means are presented. Comparison among treatments were made by the Duncan test.

Results and discussion

Nodulation and MPN counts

Although differences were not statistically significant, in the second year the treatments which had been inoculated in the previous year with strain 587 tended to present the highest number of nodules, whereas those not inoculated in the first year, and those inoculated with strains CPAC 15 and CPAC 7 tended to present the lowest number of nodules, regardless of the inoculation treatment applied in the second year (Table 2). No significant differences were observed in the dry weight of nodules. Since uninoculated soybean seed counts revealed the absence of bradyrhizobia, the presence of nodules in the uninoculated/uninoculated treatment might be attributed to

Table 2 Effects of inoculation history on nodule number and dry weight and soybean yield in the second (1994/1995) and third (1995/1996) years of experiment

Inoculation treatments		2nd year			3rd year[a]		
1st year	2nd year	Nodulation		Yield (kg ha^{-1})[c]	Nodulation		Yield (kg ha^{-1})
		Number	Weight[c](mg)		Number	Weight (mg)	
29 W	Uninoculated	110 ab[b]	196	2,213	117 abc	243 ab	2,784
29 W	CPAC 7	132 ab	255	2,127	110 abc	252 ab	2,776
29 W	CPAC 15	113 ab	198	2,139	108 abc	222 ab	2,806
587	Uninoculated	150 ab	175	2,185	128 ab	277 a	2,772
587	CPAC 7	166 a	250	2,218	95 abc	204 ab	2,718
587	CPAC 15	154 ab	227	2,162	134a	271 a	2,680
CPAC 7	Uninoculated	96 ab	196	2,173	83 bc	174 b	3,205
CPAC 7	CPAC 7	95 ab	192	2,128	81 bc	175 b	2,937
CPAC 7	CPAC 15	79 bc	164	2,159	102 abc	243 ab	2,790
CPAC 15	Uninoculated	99 ab	191	2,072	100 abc	239 ab	2,665
CPAC 15	CPAC 7	95 ab	164	2,192	70 c	172 b	2,808
CPAC 15	CPAC 15	92 ab	167	2,185	83 bc	223 ab	2,854
Uninoculated	Uninoculated	43 c	220	2,073	126 ab	274 a	2,943
Uninoculated	CPAC 7	95 ab	280	2,007	76 c	200 ab	2.945
Uninoculated	CPAC 15	94 ab	292	2,176	98 abc	207 ab	2,971
Coefficient of variation (%)		32.5	26	8.0	24.4	21.5	9.0

[a]In the third year all plots were inoculated with strain CPAC 7

[b]Means in columns *followed by different letters* were statistically different at $P < 0.05$ (Duncan's test)

[c]For these variables the differences among values were statistically non-significant

Table 3 Effects of inoculation history on nodule number and dry weight and soybean yield in the fourth (1996/1997) and sixth (1998/1999) years of experiment

Inoculation treatments[a]		4th year			6th year		
1st year	2nd year	Nodulation		Yield[c] (kg ha^{-1})	Nodulation		Yield[c](kg ha^{-1})
		Number	Weight (mg)		Number[c]	Weight[c] (mg)	
29 W	Uninoculated	91 abc[b]	116 a–d	3,612	71	200	1,914
29 W	CPAC 7	77 a–e	111 a–d	3,268	53	178	2,026
29 W	CPAC 15	70 b–e	89 bcd	3,550	73	210	2,175
587	Uninoculated	99 a	127 ab	3,507	61	209	2,085
587	CPAC 7	78 a–e	140 a	3,189	42	131	1,978
587	CPAC 15	88 a–d	122 abc	3,191	67	187	2,088
CPAC 7	Uninoculated	85 a–d	123 abc	3,224	50	181	1,807
CPAC 7	CPAC 7	81 a–e	123 abc	3,082	59	204	1,969
CPAC 7	CPAC 15	69 b–e	112 a–d	3,326	52	179	1,943
CPAC 15	Uninoculated	64 c–e	115 a–d	3,220	83	231	1,954
CPAC 15	CPAC 7	58 e	89 bcd	3,380	60	179	2,089
CPAC 15	CPAC 15	73 a–e	103 a–d	3,586	55	145	2,200
Uninoculated	Uninoculated	95 ab	114 a–d	3,153	61	189	2,117
Uninoculated	CPAC 7	62 cd	81 d	3,354	62	189	2,094
Uninoculated	CPAC 15	66 c–e	87 cd	2,978	61	151	2,169
Coefficient of variation (%)		18.0	18.0	8.9	32.4	32.0	10.3

[a]In the third year all plots were inoculated with strain CPAC 7. In the fourth and sixth years the plots were left uninoculated

[b]Means in columns *followed by different letters* were statistically different at $P < 0.05$ (Duncan's test)

[c]For these variables the differences among values were statistically non-significant

plot-to-plot contamination by rain, wind and seed bed preparation, inevitable in this type of experiment. In spite of the small number (43 nodules per plant), these nodules were of good size, therefore no differences were observed in the dry weight.

In the third year, the lowest number and dry weight of nodules were observed in the treatments CPAC 15/CPAC 7/CPAC 7, CPAC 7/CPAC 7/CPAC 7 and CPAC 7/uninoc./CPAC 7 (Table 2). In the fourth and sixth years, when the entire area was planted without inoculation, the number and dry weight of nodules were smaller (Table 3) than those observed in the second and third years (Table 2), when the plants were inoculated. In the fourth year, the smallest and the greatest number of nodules were observed in the treatments CPAC 15/CPAC 7/CPAC 7/uninoc. and 587/uninoc./CPAC 7/uninoc., respectively, whereas the smallest and the greatest nodule dry weights were found in the treatments uninoc./CPAC 7/CPAC 7/uninoc. and 587/CPAC 7/CPAC 7/uninoc., respectively (Table 3). In the sixth year, there were no statistical differences in nodulation among treatments (Table 3).

The results of MPN counts are shown in Table 4. Since the soil was free of indigenous populations of *B. japonicum/B. elkanii* prior to the first planting, initial MPN counts were zero (data not shown). In the second growing season, soybean bradyrhizobia populations were on average 1.3×10^3 cells g^{-1} in the treatments inoculated in the previous year (Table 4). The MPN count was zero in the uninoc./uninoc treatment. Since the soil samples for MPN counts were collected 1 month after seed germination, the presence of nodules in this treatment (Table 2) shows that the plot-to-plot contamination occurred after this period.

In the third season, soil *B. japonicum/B. elkanii* populations doubled to an average of 3.0×10^3 cells g^{-1}, even in the uninoculated plots (Table 4). By the fourth season, these populations reached an average of 6.9×10^5 cells g^{-1}. Due to the large confidence intervals associated with the MPN counts (data not shown) differences among treatments were significant only in the second growing season (uninoculated × inoculated treatments). The presence of a large soil population of bradyrhizobia by the sixth year did not prevent the reductions in the number and dry weight of nodules observed in that experiment.

Table 4 Soil populations of bradyrhizobia able to nodulate soybean in the second, third and fourth years. Soil samples were collected according to the experimental set up of the first year (*MPN* most probable number)

Treatments in the 1st year	MPN counts (cells g^{-1} dry soil)		
	2nd year	3rd year	4th year
29 W	0.87×10^3	1.98×10^3	19.20×10^5
587	0.97×10^3	2.46×10^3	7.30×10^5
CPAC 7	1.23×10^3	5.20×10^3	2.34×10^5
CPAC 15	2.12×10^3	0.96×10^3	3.05×10^5
Uninoculated	Zero	4.5×10^3	2.69×10^5

The increase in the bradyrhizobia soil populations over time has been reported by several authors (Brockwell et al. 1987; McLoughlin et al. 1990) and is mostly associated with the release of rhizobia from nodules as they disintegrate. However, as observed by Brockwell et al. (1987), these increases do not occur indefinitely, since edaphic factors limit the size of the populations that can develop in a soil resulting in an equilibration of soil populations.

Grain yield

Strains CPAC 15 and CPAC 7 were released for the production of Brazilian commercial inoculants in 1993, and in several field experiments, conducted in soils without indigenous populations of bradyrhizobia, there was an improvement in N fixing efficiency in comparison with the former inoculant strains 29 W and 587 (Peres et al. 1993). However, there was no reinoculation effect with strains CPAC 7 and CPAC 15 on soybean grain yields in the second season (Table 2). Although in the United States the lack of soybean response to reinoculation has been reported by several authors (Abel and Erdman 1964; Elkins et al. 1976; Klubek et al. 1988), in Brazil positive responses to reinoculation have been reported quite frequently (Hungria et al. 1996, 1998, 2001; Mendes et al. 1994; Vargas et al. 1994, 2002). In the present study, the lack of significant grain yield responses in the second growing season might be associated with the low nodule occupancy by the inoculated strains, and also with the low yield performance of soybean cultivar Doko, as compared to other cultivars such as cv. Cristalina which are more responsive to reinoculation (Vargas et al. 2002). No differences were observed in grain yields among treatments in the third, fourth and sixth years (Tables 2, 3).

As shown in Tables 2 and 3, some of the treatments inoculated with strains CPAC 7 and CPAC 15 presented lower nodule numbers and dry weights, though this was not reflected in soybean grain yields. These data confirm previous studies showing that strains CPAC 15 and CPAC 7 present a higher N_2 fixing efficiency, i.e. they are able to fix higher rates of N_2 with a lower nodule mass or number (Freire et al. 1983). Similar results for strain CB 1,809 serologically related to strain CPAC 7 were also reported (Neves et al. 1985; Nishi and Hungria 1996; Nishi et al. 1996).

Dynamics of nodule occupancy

All strains inoculated in the first year presented a successful establishment in soybean nodules, with occurrence percentages of 100% (data not shown). Similar results were obtained in a Wisconsin soil with low indigenous populations of bradyrhizobia (McLoughlin et al. 1990).

In the second growing season (Table 5), in the uninoc./uninoc. plots, serogroups 29 W, 587, 123 and CB 1,809 formed 45%, 30%, 19% and 2% of the nodules, respectively. In the treatments inoculated in the first year and left uninoculated in the second growing season, the strains that dominated the nodulation were those introduced in the first growing season, which formed 90–97%

Table 5 Effects of inoculation history on nodule occupancy by four strains of *Bradyrhizobium japonicum/B. elkanii* in the second (1994/1995) and third (1995/1996) years of the experiment

Inoculated strain		% Nodule occupancy in the 2nd year				% Nodule occupancy in the 3rd year[a]			
1st year	2nd year	29 W	587	CPAC 7	CPAC 15	29 W	587	CPAC 7	CPAC 15
29 W	Uninoculated	91 a[b]	0 d	1 d	5 d	43 a	3 b	33 cd	8 f
29 W	CPAC 7	72 b	0 d	25 d	0 d	43 a	1 b	44 c	10 ef
29 W	CPAC 15	69 b	0 d	0 d	26 c	35 a	2 b	36 cd	22 efd
SEMIA 587	Uninoculated	1 d	93 a	1 d	1 d	9 bcd	41 a	33 cd	7 f
SEMIA 587	CPAC 7	1 d	72 a	22 c	3 d	3 cd	34 a	43 c	18 efd
SEMIA 587	CPAC 15	3 d	78 a	0 d	13 cd	5 bcd	39 a	30 cd	22 efd
CPAC 7	Uninoculated	0 d	0 d	97 a	3 d	17 bc	2 b	69 ab	8 f
CPAC 7	CPAC 7	0 d	1 d	95 a	3 d	5 bcd	5 b	71 ab	15 efd
CPAC 7	CPAC 15	1 d	1 d	78 b	17 cd	5 bcd	2 b	60 b	31 cde
CPAC 15	Uninoculated	1 d	0 d	0 d	97 a	2 d	3 b	26 d	67 a
CPAC 15	CPAC 7	0 d	1 d	21 c	75 b	5 bcd	1 b	29 d	62 ab
CPAC 15	CPAC 15	0 d	1 d	0 d	97 a	0 d	1 b	25 d	72 a
Uninoculated	Uninoculated	45 c	30 c	2 d	19 cd	19 b	10 b	30 cd	35 bc
Uninoculated	CPAC 7	1 d	1 d	99 a	0 d	5 bcd	5 b	81 a	9 ef
Uninoculated	CPAC 15	6 d	3 d	0 d	90 ab	12 bcd	11 b	30 cd	45 bc
Coefficient of variation (%)		51	41	24	39	54	61	18	15

[a]In the third year all plots were inoculated with strain CPAC 7

[b] Means in columns *followed by different letters* were statistically different at $P < 0.05$ (Duncan's test)

of the nodules (Table 5). On the plots left uninoculated in the first year, inoculation with strains CPAC 15 and CPAC 7 resulted in a nodule occupancy of 90% and 99%, respectively. In the treatments CPAC 15/CPAC 7 and CPAC 7/CPAC 15, strains CPAC 7 and CPAC 15 formed 21% and 17% of the nodules, respectively. As shown in Table 5, in the treatments inoculated in the first growing season with 29 W and 587, inoculation in the second year with strains CPAC 15 and CPAC 7 resulted in 13% to 26% of nodule occupancy.

In the third growing season, the entire area was inoculated with strain CPAC 7 and Table 5 shows that its nodule occupancy was affected by the strain introduced in the first year. In the treatments inoculated with CPAC 7 in the first season (CPAC 7/unicoc/CPAC 7, CPAC 7/CPAC 7/CPAC 7 and CPAC 7/CPAC 15/CPAC 7) and in the one left uninoculated in the first year but inoculated with CPAC 7 in the second and third years (uninoc./CPAC 7/CPAC 7), nodule occupancy by CPAC 7 ranged between 60% and 81%. In the treatments 29 W/CPAC 7/CPAC 7 and 587/CPAC 7/CPAC 7, nodule occupancy by CPAC 7 in the third year (44% and 43%, respectively) was statistically superior to that observed in the treatment CPAC 15/CPAC 7/CPAC 7 (29%). This result shows that in areas where strains 29 W and 587 had been introduced, the establishment in nodules of strain CPAC 7 is easier than in areas with the dominance of strains serologically related to serocluster 123 (CPAC 15). A further increase in nodule occupancy by CPAC 7 in the third year in the treatment 29 W/CPAC 7/CPAC 7 (from 25% in the second year to 44% in the third) and 587/CPAC 7/CPAC 7 (from 22% in the second year to 43% in the third), shows that under these conditions (i.e. areas with established populations of serogroups 29 W and 587), reinoculation might improve nodule occupancy. In areas with established populations of serocluster 123, this response is less pronounced. Similar results have been reported before by Mendes et al. (1994).

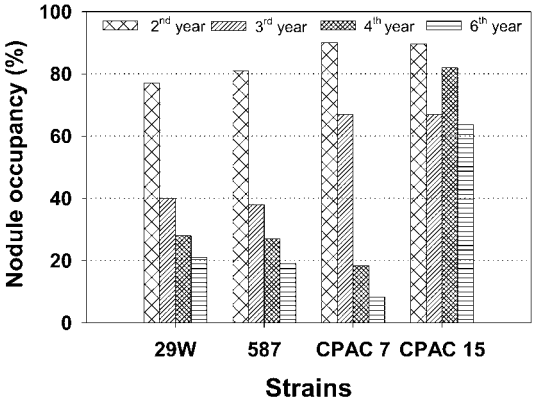

Fig. 1 Dynamics of nodule occupancy by four *Bradyrhizobium japonicum/B. elkanii* strains introduced in the first year, in a Cerrado soil void of soybean bradyrhizobia. Data represent the average nodule occupancy by each strain, over time, in the treatments where they were introduced in the first year

In order to have a better understanding of nodule occupancy by the different strains introduced in the first year, their average nodule occupancy over time is presented in Fig. 1. Strains 29 W and 587 had a very similar pattern of nodule occupancy with considerable

Table 6 Effects of inoculation history on nodule occupancy by strains of *Bradyrhizobium japonicum/B. elkanii* in the fourth (1996/1997) and sixth (1998/1999) year of the experiment

Inoculated strain[a]		% Nodule occupancy in the 4th year				% Nodule occupancy in the 6th year			
1st year	2nd year	29 W	587	CPAC 7	CPAC 15	29 W[c]	587[c]	CPAC 7[c]	CPAC 15
29 W	Uninoculated	32 a[b]	6 c	8 cde	54 cde	19	9	9	58 bcd
29 W	CPAC 7	32 a	8 c	12 b–e	47 e	24	14	11	45 cd
29 W	CPAC 15	27 a	8 c	5 e	55 cde	20	10	3	61 a–d
587	Uninoculated	5 b	29 ab	7 de	58 cde	9	18	2	65 ab
587	CPAC 7	3 b	34 a	4 e	59 b–e	11	21	4	57 bcd
587	CPAC 15	7 b	29 ab	4 e	60 b–e	11	18	7	55 bcd
CPAC 7	Uninoculated	4 b	11 c	17 bcd	68 a–e	28	12	8	48 cd
CPAC 7	CPAC 7	7 b	8 c	20 ab	63 b–e	13	14	8	54 bcd
CPAC 7	CPAC 15	7 b	5 c	18 abc	70 a–d	15	10	9	57 bcd
CPAC 15	Uninoculated	3 b	5 c	5 e	86 a	23	8	6	55 abc
CPAC 15	CPAC 7	1 b	8 c	10 b–e	80 ab	8	13	4	62 abc
CPAC 15	CPAC 15	6 b	6 c	7 de	80 ab	13	5	2	74 a
Uninoculated	Uninoculated	11 b	13 c	10 b–e	65 a–e	14	7	4	64 ab
Uninoculated	CPAC 7	12 b	12 c	28 a	49 de	21	12	7	51 bcd
Uninoculated	CPAC 15	11 b	11 c	2 e	74 abc	11	10	8	62 abc
Coefficient of variation (%)		53	73	51	17	55	56	91	14

[a]In the third year all plots were inoculated with strain CPAC 7. In the fourth and sixth years the plots were left uninoculated

[b]Means in columns *followed by different letters* were statistically different at $P < 0.05$ (Duncan's test)

[c]For these variables the differences among values were statistically non-significant

decreases in their occurrence in the third year after their introduction. CPAC 7 and CPAC 15 showed high nodule occupancies in the second and third years, however, in the following years a drastic decrease was observed for CPAC 7, while nodule occupancy by CPAC 15 (serogroup USDA 123) continued to be high.

The greater competitiveness of CPAC 15 was also evident in the never inoculated treatments where the strain occupied 4%, 14%, 40% and 55% of the nodules in the second, third, fourth, and sixth years, respectively (Tables 5, 6). An increase in nodule occupancy by SEMIA 566 (belonging to this serogroup), after the third year, had also been observed before in the Southern region of Brazil (Freire et al. 1983). In the United States, McLoughlin et al. (1990) also verified high nodule occupancy (>60%) by serocluster 123 after the second year.

It is possible that the delayed nodule occupation by the 123 serocluster in our study, and that of Freire et al. (1983) as compared to the data reported by McLoughlin et al. (1990), can be related to abiotic factors such as different climatic conditions (temperate × tropical) and soil types. However, it is likely that biotic factors, including the competitiveness of the strains introduced in the first year and differences in phenotypic/genotypic characteristics among Brazilian and North American bradyrhizobia populations, might have played a more important role. It also should be pointed out that our Cerrados soil was void of indigenous bradyrhizobia populations, whereas in the Wisconsin soil, the initial population was of 10 cells g^{-1}, increasing to 1.7×10^5 cells g^{-1} 100 days after the first soybean inoculation, with serogroup 110 forming all the nodules in those plots. In spite of the site-specific characteristics, the lack of reinoculation favored nodule occupancy by the 123 serocluster in both studies. Although inoculation in the first year was very successful in terms of the establishment of inoculated strains, in the absence of yearly inoculations, this effect was transitory with progressive replacement of inocula by naturalized strains.

By the third year, we observed the dispersal of strains serologically related to serocluster 123 throughout the entire experimental area, even though care was taken to avoid plot-to-plot contamination. What makes strains serologically related to the 123 serocluster so competitive has not been established yet. The tenacious competitive ability of this serogroup does not appear to be related to the size of its population in the rhizosphere (Moawad et al. 1984), its lectin-binding ability (Robert and Schmidt 1985a), its ability to reach the rhizosphere earlier or grow there more rapidly than competitor strains (Robert and Schmidt 1985b).

In Brazil, several studies with strains serologically related to the 123 serocluster have shown differences between the parental strain SEMIA 566 (isolated from a nodule of a plant that received a North American inoculant, without more specific information; Ferreira and Hungria 2002) and strain CPAC 15 (isolated in 1986 from a Brazilian Cerrados soil, in an area which had been inoculated a decade before with SEMIA 566; Peres et al. 1993). The adapted strain CPAC 15 is characterized by higher rates of N_2 fixation (Hungria et al. 1998) and higher competitive ability than the parental strain (Hungria et al. 1996, 1998; Scotti et al. 1997). Based on lipopolysaccharide electrophoretic profiles, Scotti et al. (1997) observed that the very competitive strain CPAC 15 presented an additional constitutive polysaccharide band of low molecular weight, when compared to SEMIA 566, the presence of which was correlated with competitive nodule dominance. It is possible that strains like CPAC 15, which are able to express such alterations in LPS structure, may be favored by successive soybean cultivation, resulting in a progressive population increase and enabling competition with serogroups 29 W and 587.

The persistence of introduced soybean *Bradyrhizobium* strains in subsequent growing seasons, based on nodule occupancy, can be extremely variable, depending mostly on the strains introduced and on the indigenous soil population (its size and composition). For this reason, local studies over a period of at least 3 years are necessary to better evaluate the performance of the strains inoculated in soil. For example, in Australia, where the only significant introduction of *B. japonicum* was of strain CB 1,809 (Gibson et al. 1990), Brockwell et al. (1987) observed the capacity of a background population of CB 1,809 in dominating subsequent inoculant strains. This superiority was a consequence of CB 1,809 being the first strain of *B. japonicum* introduced in that soil where, in the absence of competition from other naturalized bradyrhizobia, it soon developed into a very large and well-dispersed population. In the same way, Dunigan et al. (1984), in a Louisiana soil (United States) with an established population of bradyrhizobia estimated at 3.3×10^5 cells g^{-1}, and 3% of the nodules being formed by the 123 serocluster, reported the successful establishment in nodules of the non-indigenous *B. japonicum* strain 3I1b110, after 3 years of massive soil inoculation (up to 10^8 cells cm^{-1}).

Our results add more information regarding the higher competitive and saprophytic abilities of strain CPAC 15, serologically related to the 123 serocluster, in Cerrados soils. For this reason, soybean reinoculation strategies for the Brazilian Cerrados must take this fact into consideration. Strain CPAC 15 was isolated in 1986 when we first noticed the presence of strains from the 123 serocluster in soybean nodules growing in the Cerrados region. The idea behind the isolation of CPAC 15 was to select from that soil population a strain with good N_2 fixation capacity, associated with better competitive and saprophytic abilities. Therefore, the release of strain CPAC 15 for use in the commercial inoculant in Brazil was a strategy to prevent the dispersal of highly competitive strains with lower N_2 fixing efficiency in newly grown soybean areas. It is important to notice that in the present study, inoculation with CPAC 15 established an extremely unfavorable situation for the introduction of new strains. Even so, in the treatments where CPAC 15 was introduced in the first year, an average of 25% of the nodules were

occupied by strain CPAC 7 by the third year. Further studies, with more responsive soybean cultivars are still necessary to evaluate to what extent yearly reinoculations with strain CPAC 7 could prevent nodulation by very competitive strains belonging to the 123 serocluster, and the outcome of this practice for soybean yields.

Acknowledgements We thank Osmar Teago, Emilio Taveira, Maria das Dores Silva, Odete Justino and Vilderete Alves for their technical assistance. This work was partially financed by CNPq-PRONEX, Group of Excellence in Nitrogen Fixation (41.96.0884.00). M. Hungria (520396/96-0) acknowledges research fellowship from CNPq.

References

Abel GH, Erdman LH (1964) Response of Lee soybeans to different strains of *Rhizobium japonicum*. Agron J 56:423–424

Boddey LH, Hungria M (1997) Determination of characteristics of *Bradyrhizobium japonicum* and *B. elkanii* species in Brazilian strains which nodulate soybean. Biol Fertil Soils 25:407–415

Brockwell J, Roughley RJ, Herridge DF (1987) Population dynamics of *Rhizobium japonicum* strains used to inoculate three successive crops of soybean. Aust J Agric Res 38:61–74

CONAB (2003) Companhia Nacional de Abastecimento database. http://www.conab.gov.br. Cited 25 Jan 2004

Damirgi SM, Frederick LR, Anderson IC (1967) Serogroups of *Rhizobium japonicum* in soybean nodules as affected by soil types. Agron J 59:10–12

Dunigan EP, Bollich PK, Hutchinson RL, Hicks PM, Zaunbrecher FC, Scott SG, Mowers RP (1984) Introduction and survival of an inoculant strain of *Rhizobium japonicum* in soil. Agron J 76:463–466

Elkins DM, Chan CKY, Briskovich MA, Vandeventer JW (1976) Effect of cropping history on soybean growth and nodulation and soil rhizobia. Agron J 68:513–517

EMBRAPA (1997) Manual de análises de solo, 2nd edn. Serviço Nacional de Levantamento e Conservação do Solo, Rio de Janeiro

Ferreira MC, Hungria, M (2002) Recovery of soybean inoculant strains from uncropped soils in Brazil. Field Crops Res 79:139–152

Freire JRJ, Kolling J, Vidor C, Pereira JS, Kolling IG, Mendes NG (1983) Sobrevivência e competição por sítios de nodulação de estirpes de *Rhizobium japonicum* na cultura da soja. Rev Bras Cienc Solo 7:47–53

Gibson AH, Demezas DH, Gault RR, Bhuvaneswari TV, Brockwell J (1990) Genetic stability in rhizobia in the field. Plant Soil 129:37–44

Hungria M, Nishi CYM, Cohn J, Stacey G (1996) Comparison between parental and variant soybean *Bradyrhizobium* strains with regard to the production of lipo-chitin nodulation signals, early stages of root infection, nodule occupancy, and N_2 fixation rates. Plant Soil 186:331–341

Hungria M, Boddey LH, Santos MA, Vargas MAT (1998) Nitrogen fixation capacity and nodule occupancy by *Bradyrhizobium japonicum* and *B. elkanii* strains. Biol Fertil Soils 27:393–399

Hungria M, Campo RJ, Mendes IC (2001) Fixação biológica do nitrogênio na cultura da soja. (Embrapa Soja Circular Técnica 35/Embrapa Cerrados Circular Técnica 13) Embrapa Soja, Londrina

Kang UG, Somasegaran P, Hoben HJ, Bohlool BB (1991) Symbiotic potential, competitiveness, and serological properties of *Bradyrhizobium japonicum* indigenous to Korean soils. Appl Environ Microbiol 57:1038–1045

Keyser HH, Cregan PB (1987) Nodulation and competition for nodulation of selected soybean genotypes among *Bradyrhizobium japonicum* serogroup 123 isolates. Appl Environ Microbiol 53:2631–2635

Klubek, BP, Hendrickson LL, Zablotowicz RM, Skwara JE, Varsa EC, Smith S, Islieb TG, Maya J, Valdes M, Dazzo FB, Todd RL, Walgenback DD (1988) Competitiveness of selected *Bradyrhizobium japonicum* strains in midwestern USA soils. Soil Sci Soc Am J 52:662–666

Kvien CS, Ham GE, Lambert JW (1981) Recovery of introduced *Rhizobium japonicum* strains by soybean genotypes. Agron J 73:900–905

McLoughlin TJ, Hearn S, Alt SG (1990) Competition for nodule occupancy of introduced *Bradyrhizobium japonicum* strains in a Wisconsin soil with a low indigenous bradyrhizobia population. Can J Microbiol 36:839–845

Mendes IC, Suhet AR, Vargas MAT, Peres JRR, Vivaldi L (1994) Efeitos do trifluralin e da inoculação com rizobio na soja cultivada em solo de Cerrado. Rev Bras Cienc Solo 18:433–439

Moawad H, Ellis WR, Schmidt EL (1984) Rhizosphere response as a factor in competition among three serogroups of indigenous *Rhizobium japonicum*. Appl Environ Microbiol 47:607–612

Neves MCP, Didonet AD, Duque FF, Dobereiner J (1985) Rhizobium strain effects on nitrogen transport and distribution in soybeans. J Exp Bot 36:1179–1192

Nishi CYM, Hungria M (1996) Efeito da reinoculação da soja (*Glycine max* (L) Merrill) em um solo com população estabelecida com as estirpes SEMIA 566, 586, 5019, 5079 e 5080. Pesq Agropec Bras 20:147–162

Nishi CYM, Boddey LH, Vargas MAT, Hungria M (1996) Morphological, physiological and genetic characterization of two new *Bradyrhizobium* strains recently recommended in Brazilian commercial inoculants for soybean. Symbiosis 20:147–162

Peres JRR, Vidor C (1980) Seleção de estirpes de *Rhizobium japonicum* e competitividade por sítios de infecção nodular em cultivares de soja. Agron Sulriograndense 16:205–219

Peres JRR, Mendes IC, Suhet AR, Vargas MAT (1993) Eficiência e competitividade de estirpes de rizobio para soja em solos de cerrado. Rev Bras Cienc Solo 17:357–363

Robert FM, Schmidt EL (1985a) A comparison of lectin binding activity in two strains of *Rhizobium japonicum*. FEMS Microbiol Lett 27:281–285

Robert FM, Schmidt EL (1985b) Response of three indigenous serogroups of *Rhizobium japonicum* to the rhizosphere of preemergent seedlings of soybean. Soil Sci Soc Am J 17:579–580

Schmidt EL, Zidwick MJ, Abebe H (1986) *Bradyrhizobium japonicum* serocluster 123 and diversity among member isolates. Appl Environ Microbiol 51:1212–1215

Scotti MRMML, Carvalho-Silva DR, Vargas MAT, Neves MCP, Dobereiner J (1997) Changes in electrophoretic profiles of lipopolysaccharides from competitive strains of *Bradyrhizobium* spp. induced by soybean roots. J Appl Microbiol 83:552–560

Semu E, Hume DJ (1979) Effects of inoculation and fertilizer N levels on N_2 fixation and yields of soybeans. Can J Plant Sci 59:1129–1137

Somasegaran P, Hoben HJ (1994) Handbook for rhizobia: methods in legume-*Rhizobium* technology. Springer, New York Heidelberg Berlin

Vargas MAT, Suhet AR (1980). Efeitos de tipos e níveis de inoculantes na soja cultivada em um solo de Cerrado. Pesq Agropec Bras 15:343–347

Vargas MAT, Mendes IC, Suhet AR, Peres JRR (1993) Fixação Biológica do Nitrogênio. In: Arantes NE, Souza PIM (eds) Simpósio sobre a cultura do soja nos Cerrados. Potafos, Uberlandia, pp 159–182

Vargas MAT, Mendes IC, Suhet AR, Peres JRR (1994) Inoculation of soybean in Cerrado soils with established populations of *Bradyrhizobium japonicum*. Rev Microbiol 25:245–250

Vargas MAT, Mendes IC, Moreira AC, Burle ML, Hungria M (2002) Inoculação de leguminosas e manejo de adubos verdes. In: Sousa DMG, Lobato E (eds) Cerrados adubação e Correção do Solo. Embrapa Cerrados, Brasília, pp 97–127

Vincent JM (1970) A manual for the practical study of root-nodule bacteria. (IBP handbook 15) Blackwell Scientific, Oxford