SOYBEAN RESPONSE TO STARTER NITROGEN AND *Bradyrhizobium* INOCULATION ON A CERRADO OXISOL UNDER NO-TILLAGE AND CONVENTIONAL TILLAGE SYSTEMS[(1)]

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SUMMARY

In Brazil, *Bradyrhizobium* inoculation has successfully replaced the use of N fertilizer on soybean [*Glycine max* (L) Merr.] crops. However, with the expansion of no-tillage cropping systems in the Cerrados region, the idea that it is necessary to use small N rates at the sowing to overcome problems related with N immobilization has become widespread, mainly when soybean is cultivated after a non-legume crop. In this study we examined soybean response to small rates of N fertilizer under no-tillage (NT) and conventional tillage (CT) systems. Four experiments (a completely randomized block with five replicates) were carried out in a red yellow oxisol, during the periods of 1998/1999 and 1999/2000, under NT and CT. The treatments consisted of four urea rates (0, 20, 30 and 40 kg ha^{-1} N). All treatments were inoculated with *Bradyrhizobium japonicum* strains SEMIA 5080 and SEMIA 5079, in the proportion 1 kg of peat inoculant (1,5 x 10^9 cells g^{-1}) per 50 kg of seeds. In both experiments, soybean was cultivated after corn and the N fertilizer was band applied at sowing. In all experiments, N rates promoted reductions of up to 50 % in the nodule number at 15 days after the emergence. Regardless of the management system, these reductions disappeared at the flowering stage and there was no effect of N rates on either the number and dry weight of nodules or on soybean yields. Therefore, in the Brazilian Cerrados, when an efficient symbiosis is established, it is not necessary to apply starter N rates on soybean, even when cultivated under no-tillage systems.

Index terms: soybean, *Bradyrhizobium japonicum*, inoculation, zero-tillage, biological nitrogen fixation.

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RESUMO: *RESPOSTA DA SOJA À ADUBAÇÃO NITROGENADA NA SEMEADURA E INOCULAÇÃO COM* Bradyrhizobium *EM SISTEMAS DE PLANTIO DIRETO E CONVENCIONAL EM UM LATOSSOLO DA REGIÃO DOS CERRADOS*

No Brasil a inoculação com rizóbio tem substituído com sucesso o uso de fertilizantes nitrogenados no cultivo da soja. Entretanto, com a expansão do plantio direto na região dos Cerrados, novamente surgiram dúvidas, por parte de alguns agricultores, sobre a eficiência do processo de inoculação e sobre a necessidade, ou não, da utilização de doses de "arranque" de adubo nitrogenado na semeadura, visando superar possíveis problemas relacionados à imobilização do N principalmente quando a soja é cultivada após uma cultura não-leguminosa. Foram conduzidos, na Embrapa Cerrados, dois experimentos em áreas sob plantio direto (PD) e dois em áreas sob plantio convencional (PC) visando avaliar a resposta da soja a pequenas doses de N na semeadura. Os tratamentos consistiram de quatro doses de N (0, 20, 30, e 40 kg ha^{-1} de N) na forma de uréia, aplicada na semeadura no sulco de plantio. Em ambas as áreas a soja foi cultivada após milho. Todos os tratamentos foram inoculados com as estirpes SEMIA 5079 (CPAC 15) e SEMIA 5080 (CPAC 7), na proporção de 1 kg de inoculante (1,5 x 10^{9} células g^{-1}) para 50 kg de sementes. A adição de pequenas doses de adubo nitrogenado na semeadura da soja promoveu reduções significativas, de até 50 %, na sua nodulação inicial, avaliada aos 15 dias após a emergência. Apesar dessa redução, houve uma recuperação no número e massa de nódulos, na fase de floração. Independentemente do sistema de manejo, a adição de pequenas doses de N na semeadura não promoveu aumentos no rendimento de grãos da soja. Os resultados confirmam que, na região dos Cerrados, quando uma simbiose eficiente é estabelecida, não é necessário adicionar fertilizante nitrogenado na semeadura da soja, mesmo em áreas sob plantio direto.

Termos de indexação: soja, inoculação, Bradyrhizobium japonicum, *nitrogênio, plantio direto, fixação biológica.*

INTRODUCTION

Soybean is an annual crop of great importance in Brazil. In the Brazilian Cerrados, which is an edaphic type of savanna covering about 25 % of the country's area, six million hectares were cultivated with this crop in 1999/2000, with an average yield of 2582 kg ha^{-1}. Amongst the factors responsible for soybean expansion in Brazil, its ability to nodulate and fix N effectively, with *Bradyrhizobium* strains, plays a major role. Besides entailing a significant reduction in production costs by replacing the use of nitrogen fertilizer (around US$ 1 billion yearly), soybean inoculation also reduces the risk of environmental pollution.

Native Cerrado soils do not present indigenous bradyrhizobial populations able to nodulate soybeans (Peres & Vidor, 1980; Vargas & Suhet, 1980). Therefore, inoculation with nitrogen fixing bacteria is important in order to obtain good yields with low production costs. In the early seventies, when the first soybean fields were established in the Brazilian Cerrados, several limiting factors restricted the success of inoculation. Among them was the absence of commercial inoculants, containing strains adapted to the weathered acid soils of the region, capable of efficiently nodulating the soybean cultivars used during this period. In the late seventies, as a result of several studies (Peres, 1979; Peres & Vidor, 1980; Vargas & Suhet, 1980), *Bradyrhizobium elkanii* strains 29W and SEMIA 587 were released for the production of commercial inoculants. The strain selection program continued and, in 1992, *Bradyrhizobium japonicum* strains SEMIA 5079 and SEMIA 5080 were recommended for inoculant production in Brazil (Peres et al., 1993; Vargas et al., 1993). These four strains allow the cultivation of soybean in the Brazilian Cerrados without the use of nitrogen fertilizers (Peres, 1979; Peres & Vidor, 1980, Vargas & Suhet, 1980, Vargas et al., 1982, 1993; Peres et al., 1993).

However, even after the release of the first strains adapted to Cerrado soils, some farmers insisted on the use of starter nitrogen rates. There were concerns that inoculation alone would be insufficient to provide all the nitrogen required for optimal soybean yields. The main argument was that in newly opened Cerrado areas, the incorporation of plant residues with large C/N ratios into the soil would promote nitrogen immobilization, reducing the amount of soil N available for plants. Vargas et al. (1982) showed that the addition of starter N was not necessary, even in areas where large amounts of plant residues (26 t ha^{-1}) had been incorporated in the soil. Similar results were observed in the South of Brazil (Hungria et al., 1997) and other parts of the world (Sij et al., 1979; Koutroubras et al., 1998).

In 1998, three million hectares were cultivated under no-tillage (NT) in the Cerrados region (Freitas, 1999). With the expansion of NT cropping systems, there has been a revival of the idea that it is necessary to use small N rates at sowing to overcome problems related to N immobilization, mainly when soybean is cultivated after a non-legume crop. In this study we examined the response of inoculated soybean to starter N rates under no-till (NT) and conventional tillage (CT) systems.

MATERIALS AND METHODS

Field trials

Four field experiments were carried out in adjacent areas, two under no-till (NT) and two under conventional tillage (CT) systems, in the Brazilian Cerrados Research Center (Embrapa Cerrados), in Planaltina, Distríto Federal, Brazil. The soil was a clay-loam Red Yellow Oxisol with an established *Bradyrhizobium* population, able to nodulate soybean, estimated at 4.0 x 10^4 cells g^{-1} of soil. The areas were cultivated under CT for three years from 1978 on, and then left fallow until 1993, when cultivation under no-tillage and conventional tillage began. The experiments were conducted in 1998/1999 and in 1999/2000. Chemical analyses of soil samples collected from the four areas before planting, at depths of 0 to 5 cm and 5 to 20 cm, are presented in table 1.

Phosphorus and potassium were band applied (250 kg ha^{-1} of the mixture 0-20-20 in the experiment conducted in 1998/1999 and 400 kg ha^{-1} of the same mixture in 1999/2000). In all experiments, soybean was cropped after corn cv. Cargill C-101, which had received 500 kg ha^{-1} of the mixture 4-30-16 at planting and 125 kg ha^{-1} of N, as urea, split in two applications (20 and 33 days after germination). The amount of corn residues produced in the NT and CT areas was around 8,0 tons of dry matter ha^{-1}. In the CT areas, corn residues were plowed into the soil immediately after harvesting, whereas in the NT areas they were left on the soil surface.

In the NT areas, weeds were controlled with glyphosate (2.5 L ha^{-1}) before soybean planting. In the CT areas, soil management and weed control consisted of moldboard plowing followed by two runs of a disk harrow, and incorporation of trifluralin (1.5 L ha^{-1}) and imazaquim (1.5 L ha^{-1}). Thirty five days after emergence (DAE), all sites received additional post emergence applications of the mixture phluoziphop-P-butil + phomasaphen (1.5 L ha^{-1}).

The experiments were arranged in a completely randomized block with five replicates. Treatments consisted of four urea rates: 0 (control), 0, 20, 30 and 40 kg N ha^{-1}, band applied at sowing. All treatments were inoculated. Plots were 5.0 m long and consisted of nine rows drawn 45 cm apart, with 17 seeds m^{-1} of the soybean cultivars Celeste (experiments carried out in 1998/1999) and EMGOPA 316 (experiments carried out in 1999/2000). *Bradyrhizobium japonicum* strains SEMIA 5080 (= CPAC 7, same serogroup as CB 1809 and USDA 110) and SEMIA 5079 (= CPAC 15, same serogroup as

Table 1. Chemical soil properties of soil samples collected from the four sites where the experiments were carried out. Results from a composite soil sample collected before planting(1)

Area	pH H_2O	Al	H + Al	Ca + Mg	P	K	SOM
		mmol$_c$ dm^{-3}			mg dm^{-3}		g kg^{-1}
				0 to 5 cm depth			
NT(2) (1998/99)	6,1	0,3	46,8	3,97	10,7	384	34
NT (1999/00)	5,9	0,1	48,8	3,79	18,5	335	38
CT(3) (1998/99)	5,7	0,2	49,6	2,74	7,8	296	28
CT (1999/00)	5,8	0,7	54,6	2,46	6,9	298	30
				5 to 20 cm depth			
NT (1998/99)	5,4	1,1	55,4	2,51	5,0	76	28
NT (1999/00)	5,4	0,9	50,2	2,01	2,5	88	29
CT (1998/99)	5,8	0,4	51,0	2,70	6,3	128	29
CT (1999/00)	5,4	1,1	50,4	2,42	3,8	101	28

(1) Ca, Mg and Al were extracted with 1 mol L^{-1} KCl and determined by atomic absorption (Ca and Mg) and titration with NaOH 0.025 mol L^{-1} (Al). P and K were extracted using the Mehlich-1 (H_2SO_4 0.0125 mol L^{-1} + HCl 0.05 mol L^{-1}) method, and determined by flame photometry (K) and spectrophotometry using the blue-Mo method (P), SOM = soil organic matter by the Walkley-Black method (EMBRAPA, 1979). (2) NT = no-tillage. (3) CT = conventional tillage.

USDA 123) were applied as a double-strain powdered peat inoculant. The inoculant was prepared from a pure culture of these strains, grown in yeast mannitol broth. The broth culture was applied on sterilized peat (whose pH had been previously raised to 6.5 with $CaCO_3$), to reach about 50 % moisture. The mixture was stored for 30 days to mature at room temperature. Plate counts showed 1.5×10^9 cells g^{-1} of peat and MPN counts 5×10^8 cells g^{-1}. Immediately before planting, the seeds were inoculated with a peat slurry in a 25 % sucrose sticker solution, at a rate of 1 kg of inoculant per 50 kg of seeds.

Twelve plants per plot were collected at 15 DAE (days after emergence) to evaluate the number of nodules. At the pre-flowering stage, six plants per plot were collected with a hoe (20 cm deep) to determine the number and dry weight of nodules and also for serological analyses. In these samplings, consecutive plants were removed from near the ends of the third and seventh rows of each plot. The root systems were rinsed with tap water, the nodules detached, dried at 72 ºC for 72 h, weighed, and counted.

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

Nodule serotyping

Nodule serotyping was carried out by immuno-agglutination (Vincent, 1970) and antisera prepared against the strains 29W (= SEMIA 5019), SEMIA 587, CB 1809 (SEMIA 586) and SEMIA 566 (same serogroup as USDA 123) as described by Somasegaran & Hoben (1985). Strains 29W and SEMIA 587 have been used in Brazilian commercial soybean inoculants since 1979, and strains SEMIA 5079 and SEMIA 5080 since 1992. More information about these serogroups has been described earlier by Boddey & Hungria (1997). Nodule suspensions were prepared in sterile 8,5 g kg^{-1} NaCl and boiled for 1 hour at 100 °C. For each nodule, agglutination was realized in microtiter trays. A sample of 300 µL of the nodule preparation (antigen) was mixed with one drop of the appropriate antiserum, besides the antigen control which contained only the antigen in saline solution. Positive reactions observed through a light source were determined by the formation of white precipitates. Recovery percentages for serogroups 29 W, SEMIA 587, CB 1809 and SEMIA 566 were determined by serotyping 50 nodules selected at random from each plot.

Statistical analyses

Data were submitted to analyses of variance using the general linear procedure provided by the SAS software package (SAS, 1996). Percentage data were subjected to arc sin $\sqrt{x}$ transformations prior to analysis and the retransformed means were presented. The treatments were compared by the Dunnet test at a probability level of 5 %.

RESULTS AND DISCUSSION

In all four experiments, regardless of the management system, a significant decrease was observed in the number of nodules 15 DAE in treatments receiving N (Tables 2 and 3). Compared to the control treatment, reductions ranged from 15 to 50 %, confirming reports that N fertilizer reduces or delays nodule formation (Allos & Bartholomew, 1955; Weber, 1966; Beard & Hoover, 1971; Koutroubras et al., 1998). Mineral N affects several steps of the nodulation process, e.g., Zhang et al. (2000) observed that N application reduces isoflavonoid concentration of soybean root systems, probably playing a regulatory role in soybean nodule formation. However, in spite of this initial reduction, nodulation was recovered and, as shown in tables 2 and 3, at the pre-flowering stage, no differences in nodulation were observed among the treatments.

Table 4 presents the serogroup distribution in nodules. Since there was no treatment without inoculation, it is not possible to evaluate the effects of reinoculation on the occurrence of the inoculant strains in the nodules. However, in all experiments, with the exception of the experiment under CT conducted in 1999/2000, concurrently with the increase in N starter rates, there was a downward trend there was a downward trend in serogroup CB 1809 ocurrence in nodules (this serogroup is the same of the inoculant strain SEMIA 5080). Although this effect was not statistically significant, further research is necessary to determine if this effect is related to a higher sensitivity of this serogroup to mineral N. Similar results were observed by Vargas et al. (2000) in dry beans fertilized with N and inoculated with *Rhizobium tropici* strain CIAT 899.

In the NT and CT areas, there was no effect of starter N rates on grain yields (Tables 2 and 3), showing that the N in the seed cotyledons and soil N were enough to provide the necessary N for soybean seedlings to meet their requirements until the beginning of biological N fixation. However, it should be mentioned that, after cotyledon consumption, inoculated soybean presented a temporary leaf yellowing when grown without starter N. This yellowing disappeared by the fourth week after emergence, and had no effect on soybean yields (Tables 2 and 3). Since the seedlings of the treatments with N seemed more vigorous, this is likely to be one of the reasons for farmers' concerns regarding the need of starter N rates, and their belief that the use of small rates of N at sowing may

Table 2. Effects of starter N rates on nodulation per plant (nodule number, NN; nodule dry weight, NDW), and on grain yield of the soybean cultivar Celeste grown under no-tillage (NT) and conventional tillage (CT) systems, in 1998/99

N rates[1]	No-tillage				Conventional tillage			
	Nodulation			Yield[2]	Nodulation			Yield[2]
	15 DAE	Pre-flowering[2]			15 DAE	Pre-flowering[2]		
	NN	NN	NDW		NN	NN	NDW	
$kg\ ha^{-1}$	— Nº $plant^{-1}$ —		mg	$kg\ ha^{-1}$	— Nº $plant^{-1}$ —		mg	$kg\ ha^{-1}$
0	24 a	92	343	3728	16 a	54	235	3284
20	17 b	77	306	3730	11 b	51	200	3006
30	12 b	90	341	3759	12 b	51	207	3217
40	13 b	75	294	3805	10 b	57	195	3229
C.V. (%)	24	35[2]	26[2]	9,0[2]	17,2	22[2]	27[2]	6,5[2]

[1] All treatments were inoculated before planting. [2] Non significant differences were observed among treatments.

Table 3. Effects of starter N rates on nodulation per plant (nodule number, NN; nodule dry weight, NDW) and on grain yield of the soybean cultivar Emgopa 316 grown under no-tillage (NT) and conventional tillage (CT) systems, in 1999/2000

N rates[1]	No-tillage				Conventional tillage			
	Nodulation			Yield[2]	Nodulation			Yield[2]
	15 DAE	Pre-flowering[2]			15 DAE	Pre-flowering[2]		
	NN	NN	NDW		NN	NN	NDW	
$kg\ ha^{-1}$	— Nº $plant^{-1}$ —		mg	$kg\ ha^{-1}$	— Nº $plant^{-1}$ —		mg	$kg\ ha^{-1}$
0	58 a	118	359	3224	46	144	425	3514
20	47 b	95	289	3320	39	119	443	3495
30	47 b	129	373	3305	40	117	413	3578
40	40 b	96	274	3337	40	115	366	3646
C.V. (%)	13	22[2]	16[2]	6,0[2]	10[2]	32[2]	25[2]	5,5[2]

[1] All treatments were inoculated before planting. [2] Non significant differences were observed among treatments.

improve soybean yields. Lack of responses to starter N in grain yields were also reported by Sij et al. (1979), in Texas; Vargas et al. (1982), in Brazilian Cerrados; Hungria et al. (1997), in the southern part of Brazil, and Papakosta & Veresoglou (1989) and Koutroubras et al. (1998) in Greece.

In a study conducted in Minnesota by Lamb et al. (1990), grain yield responses to N, applied and incorporated before planting, were observed in 9 out of 12 field experiments where soil NO_3-N was less than 90 $kg\ ha^{-1}$. Simanungkalit et al. (1995) also observed positive responses to starter N rates (25 and 50 $kg\ ha^{-1}$) in a sandy clay Red Yellow Podzol in Indonesia. In both studies, the soils presented low populations of indigenous *B. japonicum* strains and the inoculation responses were small, indicating that perhaps with the use of more efficient strains, or higher rates of inoculum, responses to N could have been smaller or even null. In fact, in the study by Lamb et al. (1990), it was pointed out that although they used the double amount of inoculum recommended, there may not have been enough soybean bradyrhizobia applied on the seeds to provide a significant population. Starling et al. (1998) observed in Alabama's sandy soils that, under very specific conditions, such as those of late planted double-cropped soybean, starter N (50 $kg\ ha^{-1}$) was a viable input for increasing grain yield (150 $kg\ ha^{-1}$, on average).

Table 4. Effects of starter N and inoculation with SEMIA 5079 and SEMIA 5080 strains on serogroup distribution of soybean nodules. Plants were grown under no-tillage (NT) and conventional tillage (CT) systems(1)

N rates	NT 1998/1999				NT 1999/2000			
	29W	587	566	CB1809	29W	587	566	CB1809
kg ha^{-1}	Serogroup distribution in nodules, %							
0	6,6	2,1	69,5	15,6	15,9	11,4	41,4	17,8
20	4,2	1,5	79,3	10,3	9,7	6,9	62,7	10,2
30	5,2	3,6	76,0	9,2	11,0	14,8	50,0	10,7
40	5,7	3,6	74,0	10,8	7,8	11,8	60,9	9,7
C.V. (%)	47	67	9	23	20	40	13	31
	CT 1998/1999				CT 1999/2000			
	29W	587	566	CB1809	29W	587	566	CB1809
0	17,9	11,7	45,8	11,8	22,2	9,7	57,0	6,4
20	12,6	13,3	50,8	12,7	21,2	9,4	60,3	6,9
30	19,4	10,2	49,9	9,3	25,9	14,5	50,8	7,0
40	18,6	8,5	51,3	6,9	18,9	11,3	59,8	7,0
C.V. (%)	18	23	9	20	20	13	9	55

(1) Differences were not statistically significant.

Therefore, based on literature, it can be seen that soybean yield responses to starter N fertilization have been extremely variable, depending on the efficiency of *Bradyrhizobium* strains (Simanungkalit et al., 1995), soybean cultivars (Papakosta & Veresoglou, 1989; Starling et al., 1998), soil NO_3-N content (Lamb et al., 1990) and N rates. The combination of these factors make responses to starter N site-specific, showing that before drawing any conclusions on this subject, conditions under which results were obtained must be clearly specified, as well as any limiting factors. In the studies conducted by Koutroubras et al. (1998) in Greece, where the initial N status of the soil was sufficiently adequate to meet the needs of the plants, the seed yield of soybean cultivar Williams was not affected by N rates as high as 120 and 240 kg ha^{-1} (half applied before planting and half at full bloom), but plants did respond to *Bradyrhizobium* inoculation. Provided that mineral N was not the limiting factor, the authors suggested that the supremacy of symbiotic N vs. combined N was related to the fact that symbiotic N is already in the organic reduced form, and hence, more readily available for plant metabolism.

Our results proved once more that, in Brazil, rhizobium symbiosis can guarantee high yields without the need of supplying N fertilizer. It is noteworthy that if the initial delay in nodulation caused by N rates had occurred in a soil with low populations of soybean bradyrhizobia, a period of imbalance between N demand and N supply could have happened. Therefore, under adverse conditions (such as dry spells right after planting), it is likely that this reduction would have more serious consequences on soybean development and yields.

CONCLUSIONS

1. In this study, performed in an Oxisol of the Brazilian Cerrados, the use of starter N did not increase soybean grain yields, regardless of the soil management system. Therefore, under that condition, when an efficient symbiosis is established, it is not necessary to use starter N rates.

2. The addition of small N rates (up to 40 kg ha^{-1} N) at sowing reduced nodulation by up to 50 % at 15 DAE. However, soybean recovered nodulation by the pre-flowering stage.

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