# Soybean yield and nodule occupancy as a function of yearly inoculation in the Brazilian Cerrados.

Mendes, I.C.[1*], Reis Junior, F.B.[1], Hungria, M.[2]
[1] Embrapa Cerrados. Planaltina, Distrito Federal, Brazil. [2] Embrapa Soja, Londrina, Paraná, Brazil.
[*] ieda.mendes@embrapa.br

## ABSTRACT
The effects of yearly inoculation of three serologically distinct strains of *Bradyrhizobium japonicum* (SEMIA 5079= CPAC-15), *B. diazoefficiens* (SEMIA 5080= CPAC-7*)* and *B. elkanii* (SEMIA 587) have been examined in a long-term field experiment in the Cerrado Region of Brazil. By the second year even though nodule occupancy by strains CPAC-7 and CPAC-15 decreased, soybean yield increases of 6,6% were observed in the treatments inoculated with these strains, showing the importance of reinoculation yearly with more efficient strains.

## INTRODUCTION
One of the factors responsible for the soybean successful expansion in Brazil is its capacity to nodulate and fix $N_2$ effectively with *Bradyrhizobium* strains, resulting in an economy of about US$ 9 billion/year. As a result of the rapid expansion of soybean in the Brazilian Cerrados, populations of soybean *Bradyrhizobium* strains are now established in these soils, which originally were void of these bacteria (Vargas and Suhet, 1980). For this reason, the 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.
A former study carried out by our group (Mendes *et al*., 2004) showed that different strains introduced in the first year influenced nodule occupancy by strain CPAC-7 until the third successive growing season (when CPAC-7 was inoculated yearly). In the 4$^{th}$ and 6$^{th}$ years, when soybean was sown without inoculation, strains serologically related to serocluster 123 (the same as strain CPAC-15) dominated the nodulation, occurring, on average, in more than 50% of the nodules of the treatments where it had never been inoculated.
However, further studies, with more responsive soybean cultivars are still necessary to evaluate to what extent reinoculation could prevent nodulation by very competitive strains, and the outcome of this practice for soybean yields.
In the present study we report the effects of yearly inoculation of three serologically distinct strains of *Bradyrhizobium japonicum* (CPAC-15), *B. diazoefficiens* (CPAC-7*)* and *B. elkanii* (SEMIA 587) in a long-term field experiment in the Cerrado Region of Brazil, which is now in its second year.

## MATERIAL AND METHODS
A field experiment at the Brazilian Cerrados Research Center (Embrapa Cerrados), in Planaltina, DF, Brazil, was initiated in 2009 and will be carried out for six successive years, until 2016. The soil is a clayey Dark-red oxisol that had never been inoculated, and was void of indigenous *Bradyrhizobium*. The experiment is arranged in a completely randomized block with four replicates. In the first year, the main plots were 17 m x 4 m and treatments consisted of uninoculated controls without and with N-fertilizer (200 kg N.ha$^{-1}$, split at sowing and flowering) and inoculation with three strains belonging to serologically distinct serogroups: 587 (*B. elkanii*), CPAC-7 (*B. diazoefficiens*, Delamuta *et al.*, 2013, same serogroup as CB 1809) and CPAC-15 (*B.*

*japonicum*, same serogroup as USDA 123). From the second year, the main plots were divided into three sub-plots of 5 m x 4 m. For the uninoculated control and for plots inoculated in the first year with strains CPAC-7 and CPAC-15, the treatments from the second year on consisted of an uninoculated control, and yearly inoculations with CPAC-7 and CPAC-15. For the plots inoculated in the first year with strain SEMIA 587, the treatments consisted of an uninoculated control and yearly inoculation with strain 587.

The experiments were performed with soybean cultivar Raimunda, planted 50 cm apart, with 17 seeds $m^{-2}$. Soybean yields (adjusted to 13% moisture) were determined by harvesting a 4.0 m section from the four central rows of each plot.

Recovery percentages for serogroups 29W (also present in Brazilian soils, *B. elkanii*), 587, CB 1809 and 123 were determined by serotyping 48 nodules selected at random from each field plot. Serotyping of nodules was done by immuno-agglutination.

## RESULTS AND DISCUSSION

In the first year soybean yield responses to inoculation with strains CPAC-15 and CPAC-7 were of 4908 and 4905 $kg.ha^{-1}$ respectively, and were similar to the nitrogen control (4801 $kg.ha^{-1}$). In relation to the control treatment (4438 $kg.ha^{-1}$) no yield increase was observed in the treatment inoculated with strain SEMIA 587 (4400 $kg.ha^{-1}$). Nodule occupancy by the inoculated strains in this first growing season was of 97%, 82% and 77% for strains 587, CPAC-7 and CPAC-15, respectively.

In the second growing season, soybean yields in the treatments without inoculation were on average 3928 kg $ha^{-1}$. In relation to their respective control treatments, reinoculation with strains CPAC-7 and CPAC-15, promoted yield gains of 260 and 261 kg $ha^{-1}$, respectively (corresponding to 6,6%), as opposed to reinoculation with strain 587, for which no yield gain was observed. In relation to the first growing season, nodule occupancy in the second year by strains CPAC-7 and 587 decreased to 32% and 70%, respectively. Nodule occupancy by CPAC-15 in the second year did not differ as compared to the first growing season.

Although in the USA there has been a lack of soybean response to reinoculation (de Bruin *et al*., 2010), in Brazil positive responses to reinoculation have been often reported (Mendes *et al*., 1994; Peres *et al*., 1994; Hungria *et al*., 1998).

Our results corroborate these findings and showed the importance of reinoculation yearly with more efficient strains. For the less efficient strain 587, the lack of significant grain yield response in the second growing season was not associated with its nodule occupancy (70% of the nodules on average).

## ACKNOWLEGMENTS

This work was partially supported by the National Counsel of Technological and Scientific Development (CNPq Repensa, 562008/2010-1).

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