

# Seasonal dynamic of native rhizobial populations associated with *Arachis pintoii* in Cerrado soils

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## Introduction

Multi-use tropical forage legumes, such as *Arachis pintoii*, are of increasing importance because like other legumes, they form effective symbiosis with selected rhizobia strains and are able to establish in soils with little fertilizing nitrogen (Thomas, 1994). However, successful establishment requires knowledge of the ecology of appropriate root nodule bacteria.

An understanding of rhizobial ecology requires their enumeration in natural habitats, and the relationship between rhizobial numbers and legume nodulation response is of considerable practical importance for legume inoculation (Esiobu, 1994; Nazih and Weaver, 1994).

Low numbers of native rhizobial populations in tropical soils seem to be the result of, among other factors, low pH, high Al, high temperatures and low humidity, especially during certain times of the year (Evans et al., 1993; Flis et al., 1993; Mulongoy and Ayanaba, 1986; Munns and Keyser, 1981; Richardson et al., 1988).

This paper reports data on the occurrence of native rhizobia strains of *A. pintoii* over a 12-month period in three Cerrado soil sites. The purpose of the study is not only to verify numeric distribution, but also to document the influence of season and soil type. This study is part of a project to assess inoculation needs and to develop *A. pintoii* inoculants in Cerrado soils.

## Materials and methods

### Sites, soil sampling, and climate in Cerrado soils.

Three sites located at the Santa Rita experimental farm at the Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG) in the Cerrado region of the state of Minas Gerais, Brazil, were chosen for soil sampling. One site is typical virgin Cerrado (pH = 4.7; P = 13 ppm; Al = 60%; organic matter = 3.8%); one site has been cultivated for more than 15 years with different forage legumes (pH = 5.8; P = 10 ppm; Al = 0%; organic matter = 3.02%); and one site has been cultivated with *A. pintoii* for 3 years (pH = 5.4; P = 9 ppm; Al = 3.0%; organic matter = 2.68%). A complete description of these sites is presented in Pinto et al. (1999). Composite soil samples were collected monthly between December 1997 and December 1998 from the top 15-cm soil layer in transversal transects using a soil borer. Moisture content (%) was calculated after drying soil subsamples to constant weight at 80 °C. Pluviometric indicators and average temperatures were registered monthly throughout the collection period (Figure 1). The study sites had never been inoculated with any rhizobial species; therefore those found represent the indigenous population.

**Rhizobial identification.** The plant infection technique (Vincent, 1970) was used to estimate the most probable number (MPN) of rhizobia associated with *A. pintoii* ecotype Bra 031143. The seeds were pre-germinated and grown in aseptically in Leonard jars without nitrogen (Vincent, 1970). Serial 10-fold dilutions of soil samples were prepared and a 1-ml aliquot of appropriate dilutions was applied directly to the roots of the test legume in four replicates. After 30 days, nodule numbers for each of the dilutions and replicates were recorded. Rhizobial population estimates were determined using the 10-fold dilution MPN chart (Vincent, 1970). Results were expressed as the log 10 of MPN of rhizobia/g soil dry weight.

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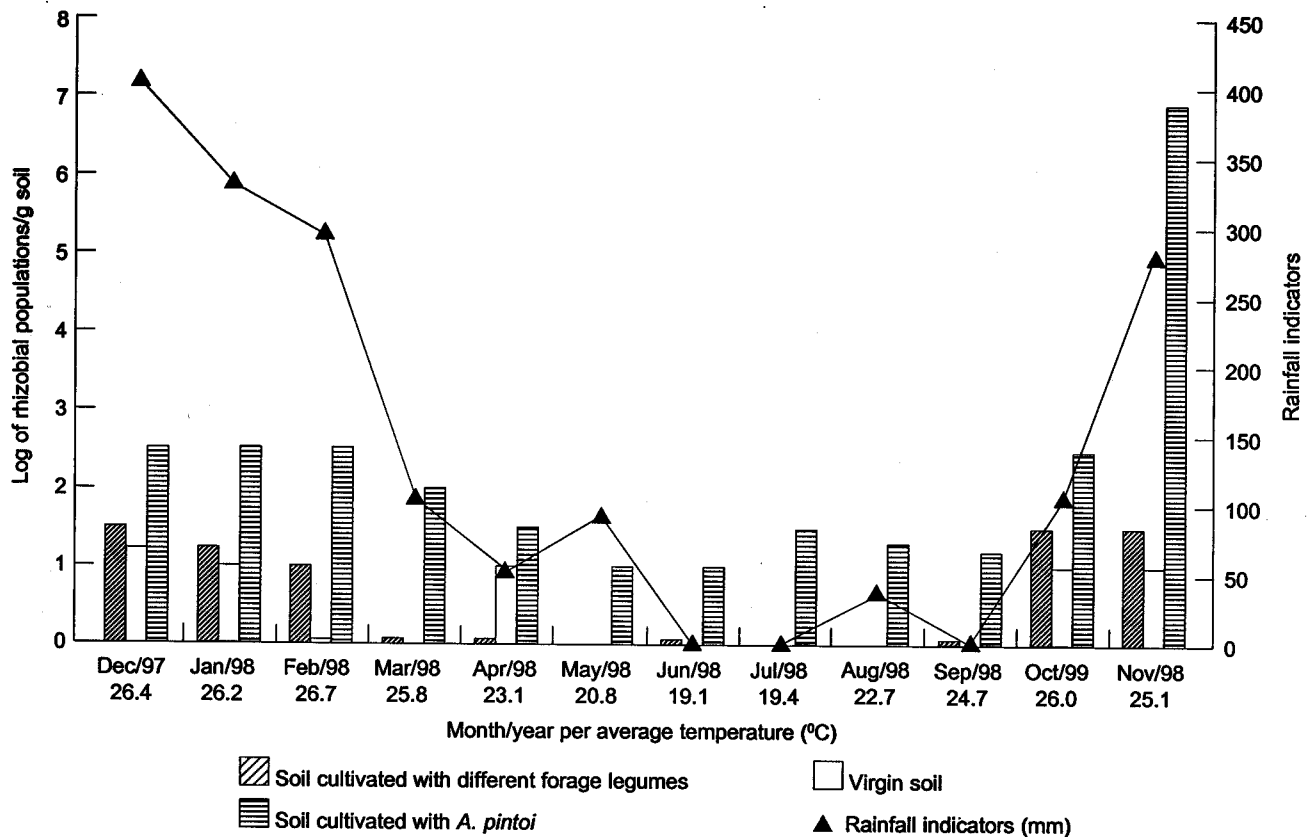


Figure 1. Distribution of *A. pintoii* rhizobial population during 12 months in three different Cerrado soils.

## Results and discussion

The number of rhizobia associated with *A. pintoii* in Cerrado soils differed particularly by site and season. The values ranged from 0 to  $1 \times 10^3$  cells/g, the highest values were observed during the rainy summer months (October-January) at the site where *A. pintoii* has been cultivated for the last 3 years (Figure 1). The virgin soil site had the lowest values and depending on the season, no rhizobia were detected. These observations agree with several authors (Mahler and Wollum, 1981; Mallorca and Izaguirre-Mayoral, 1994; Rupela et al., 1987) who reported interseasonal variations in the size of rhizobial populations, which were attributed mainly to favorable soil temperature and water content levels resulting from fall.

On unexpected result was the low number of rhizobia at the site where various forage legumes have been cultivated for the last 15 years. Even though the presence and history of legume cover is considered one of the major influences on rhizobial numbers and the known symbiotic promiscuity of tropical forage, data obtained at this site confirm the specificity of *A. pintoii* reported by Thomas (1994).

The current results also agree with results we found in a study conducted in the summer of 1997 (Pinto et al., 1999) and other studies carried out in tropical soils (Esiobu, 1994; Thies et al., 1991).

Native rhizobial populations are also affected by a variety of physico-chemical factors such as pH, Al saturation, and percentage organic matter (Catroux and Amarger, 1992; Mallorca and Izaguirre-Mayoral, 1994). The levels of these parameters were very similar in the two sites under cultivation with either *A. pintoii* or other legumes, which indicates the variations in rhizobial numbers at the two locations are not the result of physico-chemical effects. However, low pH and high Al saturation in the virgin Cerrado site could be, together with factors mentioned earlier, responsible for the extremely low levels of rhizobia in this region.

Results indicate that seasonal variation and the type of vegetable cover crop were the most important factors related to the number of rhizobial populations associated with *A. pintoii* in Cerrado soils.

## Conclusions

The number of rhizobia associated with *A. pintoi* in Cerrado soils differed particularly by site and season. The values ranged from 0 to  $1 \times 10^3$  cells/g, the highest values were observed during the rainy summer months (October-January) at the site where *A. pintoi* has been cultivated for the last 3 years. The virgin soil site had the lowest values and depending on the season, no rhizobia were detected. Data obtained confirm the specificity of *A. pintoi*. Results indicate that seasonal variation and the type of vegetable cover crop were the most important factors related to the number of rhizobial populations associated with *A. pintoi* in Cerrado soils.

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## Resumen

El conocimiento de las relaciones entre el número de rizobios y la nodulación de las leguminosas es esencial en el desarrollo de programas de mejoramiento de fijación de N. Utilizando la técnica de infección, en el estudio se evaluó el número probable de rizobios asociados con *Arachis pintoi* durante un período de 12 meses en tres sitios de los Cerrados, Minas Gerais, Brasil. El número de rizobios varió entre 0 y  $1 \times 10^3$ , dependiendo del tipo de cobertura del suelo y de la época del año. En los sitios con suelos vírgenes se encontraron los valores más bajos y en algunas épocas no se encontraron rizobios. Los resultados confirman la especificidad de *A. pintoi*; igualmente que la variación estacional y el tipo de vegetación de cobertura son los factores que más influyen en el número de rizobios asociados con esta leguminosa en el Cerrado de Brasil.

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