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Seasonal Dynamic, Host Range and Symbiotic Efficiency of Native Rhizobial Populations in Three Soil Horizons of Four Contrasting Savanna Sites

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Abstract

A strong seasonality was detected in the size of the native rhizobial populations (NRP) located in three soil horizons of four savanna sites with contrasting soil and vegetation features. Within individual soil sites the increased size of NRP during the rainy season was attributed to the improved soil water content and lower temperatures brought about by rains. A highly significant negative and positive correlations were detected between the size of NRP and the temperature and water content of soils, respectively. On the other hand, differences in the size of NRP among sites and soil horizons at both seasons was ascribed to the deleterious effect excerted by the soil chemical properties on the native rhizobia. The size of NRP was significantly positive correlated with the soil pH and cation exchange capacity and significantly negative correlated with the soil exchangeable acidity regardless of the season. There was also a significant positive correlation between the size of NRP and the legume density in the 4 savanna sites. In the savanna, the highest rhizobial number were detected in the rhizosphere of native legumes. Among them, *Chamaecrista tetraphila*, *Desmodium barbatum*, *Galactia jussieuana*, *Indigophera lespedezioides*, *Phaseolus gracilis*, *Stylosanthes* sp. and *Zornia curvata*, widely distributed among savanna sites, proved to be important reservoirs of NRP during the dry season.

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NRP were composed by *Bradyrhizobium* sp. (*Vigna*), *Bradyrhizobium* spp. (*Centrosema*, *Leucaena*, *Psophocarpus* and *Stylosanthes*), *Rhizobium tropici* and *R. etli*. Whereas, *R. leguminosarum* bv *viceae* and *B. japonicum* were absent in all soil sites. Under controlled conditions the NRP proved to be symbiotically inefficient in whole soil inoculated *Centrosema pubescens*, *Leucaena leucocephala*, *Psophocarpus tetragonolobus* and *Phaseolus vulgaris* regardless of the site and month of soil collection. A growth promotion and a relative ureide content similar to that elicited by the commercial *Bradyrhizobium* sp. (*Vigna*) strain I–125 (Hup^+) were only obtained in *Vigna unguiculata* plants inoculated with whole soils or with several efficient native *Bradyrhizobium* sp. (*Vigna*) isolates, all found to be Hup^-. There was an apparent non-relationship between the cultural characteristics of the native isolates and the displayed efficiency on *V. unguiculata*.

Keywords: native rhizobial populations, savanna, efficiency, seasons

1. Introduction

The reduced size of native rhizobial populations (NRP) in tropical areas has been attributed mainly to the prevalent high Al levels, low pH and high temperature characteristics of the soils (Rice et al., 1977; Munns and Keyser, 1981; Mulongoy and Ayanaba, 1986; Richardson et al., 1988; Flis et al., 1993; Evans et al., 1993). Nevertheless, in savannas showing a strong seasonality in water availability nodulation in native legumes is a common phenomenon, with several species displaying relatively high N_2 fixation rates even during the driest months of the year (Sicardi de Mallorca and Izaguirre-Mayoral, 1994).

Preliminary studies conducted in the upper soil horizons (0–20 cm) of a *Trachypogon* Savanna showed NRP to be positively correlated with the soil cation exchange capacity (CEC) and Al content, and negatively correlated with the % of sand, the maximum number of rhizobia being detected in soils with pH between 4.2 and 4.8 (Izaguirre-Mayoral et al., 1992). However, there are no reports on the size, seasonal trends, species composition, symbiotic effectiveness and distribution along soil horizons of NRP in a neotropical savanna. Therefore, the present study was conducted to monitor the NRP existing in four contrasting savanna sites as affected by climatic conditions, soil depth and soil physico-chemical properties. The effectiveness of NRP and of selected native *Bradyrhizobium* isolates on commercially cultivated legumes was also determined.

2. Materials and Methods

Climate, soil and vegetation analyses in the savanna

Four undisturbed sites with contrasting soil and vegetation features were chosen in a *Trachypogon* savanna located in the Estación Experimental La Iguana, Guárico State (8° 25′N, 65° 24′ W), Venezuela. In the area under study about 81 mm of rainfall was recorded between February and May. The onset of the rainy season took place in June and a total of 1139 mm of rain fell on the savanna during the following four months. Air temperatures oscillated between 38 ± 2°C at the dry season and 30 ± 1°C during the rainy months.

Soil sampling was carried out during the dry (February and May) and rainy (August and October) seasons. In each site triplicate soil samples were collected at random at 0–20 cm, 20–40 cm and 40–60 cm depth with a 20 cm long and 10 cm diameter core borer. The soil located within 5 cm distance from the main root of selected native legume species was also collected and defined as rhizosphere. Soil samples were placed in plastic bags and kept on ice for transport to the laboratory. The soil water content was determined at the three soil horizons of the savanna site. For this purpose soil subsamples were immediately weighed upon collection and dried in a ventilated oven at 60°C until constant weight. The chemical properties and texture of soils were analyzed in air dried subsamples crushed to pass a 2 mm screen as described in Izaguirre-Mayoral et al. (1992). Soil temperatures in the two uppermost soil horizons (0–20 cm and 20–40 cm) of each savanna site were recorded in the late afternoon.

Vegetation measurements during the dry and rainy seasons and at each of the four savanna sites were carried out in 10 m long transects using a point intercept method of 1 m intervals along the transect. The importance value index (IVI) of individual plant families was calculated as in Sicardi de Mallorca and Izaguirre-Mayoral (1994). Native legumes were harvested and analyzed in terms of nodulation and type of roots.

Rhizobial determinations

The plant infection technique (Vincent, 1970) was used to estimate the most probable number (MPN) of native rhizobia in triplicate soil samples collected at different depths and sites during the rainy and dry seasons. Four-fold dilutions with four replicates were prepared from the equivalent of 10 g of dry soil and aliquots of 1 ml were applied to tubes containing sterile N-free agar and pregerminated seeds of *Macroptilium atropurpureum*. Results are expressed as the $\log_{10}$ of MPN of rhizobia g^{-1} soil dry wt.

The host range of NRP located in the upper soil horizon (0–20 cm) was determined on *Centrosema pubescens* var CIAT-5126, *Glycine max* var DPA-2 (Diproagro), *Leucaena leucocephala* var CIAT-17492, *Macroptilium atropurpureum*, *Phaseolus vulgaris* var Tacarigua, *Pisum sativum* var Meteor, *Psophocarpus tetragonolobus*, *Stylosanthes capitata* CIAT-106 and *Vigna unguiculata* var Tuy. All seeds were germinated in 1.5% water-agar petri dishes and transplanted into growth pounches (Northrup King Co., Minneapolis, MN) containing 20 ml of sterilized N-free Farhaeus solution, pH 6.8 and 1 ml of the soil water suspensions. Before sowing seeds of *M. atropurpureum*, *L. leucocephala* and *C. pubescens* were sterilized for 10 min with concentrated sulphuric acid, while seeds of *G. max*, *P. vulgaris*, *P. sativum*, *P. tetragonolobus* and *V. unguiculata* were sterilized for 10 min with 1% mercuric chloride. Thereafter, seeds were rinsed six times with sterile distilled water. The plants were grown under controlled conditions at 28/24°C day/night temperature and a 12 hr photoperiod. The irradiance provided by fluorescent lamps was of 120 mol m^{-2} s^{-1}.

Symbiotic efficiency of native rhizobial populations

The symbiotic efficiency of NRP located in the upper soil horizon (0–20 cm) of the four savanna sites was determined on *C. pubescens*, *L. leucocephala*, *P. vulgaris*, *P. tetragonolobus* and *V. unguiculata* using the "whole-soil inoculation" technique (Brockwell et al., 1988) with the following modifications: sterilized seeds were sown in Leonard jars (Leonard, 1944), containing 0.8 l of N-free Farhaeus solution, pH 6.8, and 0.8 kg of a 2:1 mixture of sand sterilized at high vapor pressure for 2 hr and non-sterilized soil from each savanna site. The sand and soil in which rocks and roots had been previously removed, were thoroughly mixed and allowed to equilibrate for 2 days before planting. Six replicates were prepared for each species-soil site combination. In addition, three control treatments were included for each species: uninoculated (−N), uninoculated (+N) and inoculated with commercially recommended *Rhizobium* strains. The latter treatment included inoculation of *P. vulgaris* with *Rhizobium leguminosarum* bv *phaseoli* strain I-113 (CIAT 899) and *V. unguiculata* with *Bradyrhizobium* sp. (*Vigna*) strains I-700 (V.A.S.B., Africa, kindly supplied by Dr. S.K.A. Danso), I-125 (Tal-209, Niftal USA) or I-38 (CB756, Australia). Inoculation with the effective strains was carried out by adding 1 ml of inoculum containing 2×10^9 cells to the root system of 3-d-old seedlings. Uninoculated (−N) and *Rhizobium* inoculated plants were grown in Leonard jars with 0.8 kg of sterilized sand and 0.8 l of N-free Farhaeus solution, pH 6.8.

Uninoculated (+N) plants were supplied with 70 ppm of KNO_3. The surface of the jars was covered with 1 cm of sterile aquarium gravel.

For all treatments, plants were grown under the controlled conditions described above and harvested at the beginning of the flowering stage: *V. unguiculata*, *P. vulgaris*, *P. tetragonolobus* (45 d after germination), *L. leucocephala* and *C. pubescens* (60 d after germination). At harvest, plants were cut at the soil surface and dried at 60°C in a ventilated oven until constant weight. Roots were washed free of soil and nodules were removed for counting and dry weight determinations.

Rhizobial isolation and cultural characteristics

Rhizobial cells were isolated from nodules of *M. atropurpureum* inoculated with water suspensions of the 0–20 cm soil horizon of the four sites and from nodules of several native legumes collected at the dry season. The rhizobial isolation was carried out on yeast-mannitol agar at pH 5.5 or 7.0, with Bromocresol blue. Cultural characteristics of rhizobial isolates and nodulation in *C. pubescens*, *M. atropurpureum* and *V. unguiculata* were determined following the methodology described in CIAT (1988). From the 52 rhizobial isolates, 11 bradyrhizobial isolates obtained at pH 5.5 and capable of nodulating *V. unguiculata* were selected for further analysis. The origin of selected *Bradyrhizobium* isolates is indicated in Table 1. The qualitative analysis of hydrogenase (Hup phenotype) (Delgado et al., 1989) was performed in nodules of 35-d-old *V. unguiculata* plants inoculated at seedling with 1 ml (2×10^9cells ml^{-1}) of individual rhizobial isolates. The symbiotic efficiency

Table 1. Origin of selected *Bradyrhizobium* isolates. The collection of nodules from native legume species and of soil samples for the inoculation of *Macroptilium atropurpureum* were carried out in February.

Isolates	Isolated from one nodule of:
I-701	*Mimosa camporum* collected at site 1
I-703	*Chamaecrista rotundifolia* collected at site 2
I-706	*M. atropurpureum* inoculated with soil from site 2
I-707	*Chamaecrista calycioides* collected at site 1
I-709	*M. atropurpureum* inoculated with soil from site 3
I-711	*M. atropurpureum* inoculated with soil from site 4
I-714	*M. atropurpureum* inoculated with soil from site 4
I-715	*M. atropurpureum* inoculated with soil from site 1
I-717	*Stylosanthes guianensis* collected at site 4
I-718	*M. atropurpureum* inoculated with soil from site 4
I-719	*Desmodium barbatum* collected at site 1

of the 11 *Bradyrhizobium* isolates was determined in 45-d-old *V. unguiculata* plants. All plants were grown in Leonard jars as above.

Plant measurements

For all treatment combinations the growth and nodulation of plants was analysed in terms of the dry weight of aerial biomass, nodule number and nodule mass. The ureide (allantion plus allantoic acid), relative abundance of ureides (RAU) and chlorophyll concentration were determined as described by Sicardi de Mallorca and Izaguirre-Mayoral (1994).

Statistical analyses

For statistical analyses data of soil parameters were transferred to $\log_{10}$ and data in percentage were transformed to degrees by angular transformation. A simple correlation test was carried out to determine interactions between variables. Correlation analyses between the MPN of rhizobia and soil climatic and chemical properties were performed with data obtained at the three soil horizons of the four savanna sites.

3. Results

Soil conditions

The temperature in the two uppermost soil horizons and the water content of the three soil horizons from each savanna site are summarized in Table 2. The highest soil temperature was recorded in May. At this time of the year a soil temperature of 41 °C was observed at site 3. During the dry season, the water content in the upper soil horizon was very low at sites 2 and 3, while relatively higher values were detected at sites 1 and 4. In February and May the water content at sites 1, 2 and 4 increased at deeper soil horizons, in contrast to the low soil water content up to a depth of 60 cm observed at site 3. The onset of rains improved the water content in the upper soil horizon at all sites, while interseasonal changes in the soil water content were less conspicuous at deeper horizons at sites 1, 2 and 4. In October the soil water content was lower than in August due to the progressive decrease in the amount of rains recorded after September.

Chemical characteristics and texture of soil horizons at the four savanna sites during the dry season are summarized in Table 3. The upper horizon (0–20 cm) of sites 1 to 3 were sandy soils (> > 80% sand) in contrast to the 67% sand detected at site 4. At deeper soil horizons (20–40 cm, 40–60 cm) the percentage of sand decreased at sites 1 and 3, remained constant at site 2 and

Table 2. Temperature and water content of soils collected at 0–20 cm, 20–40 cm and 40–60 cm in four savanna sites during the rainy and dry seasons. Values of soil water content represent the mean of three measurements ± standard error of the mean.

		February		May		August		October	
Soil sites	Soil depth	°C	% water content	°C	% water content	°C	% water content	°C	% water content
1	0–20	32.4	7.0±0.2	36.5	5.8±0.2	27.0	16.7±0.8	28.0	10.4±1.0
	20–40	31.5	12.2±0.1	34.3	10.0±0.1	27.5	16.0±0.8	29.0	13.2±0.6
	40–60	nd	13.6±0.4	nd	12.2±0.2	nd	17.0±0.9	nd	13.5±1.5
2	0–20	34.3	1.0±0.1	37.2	1.0±0.1	26.5	10.2±0.4	32.1	7.0±0.7
	20–40	33.3	7.1±0.1	37.6	6.0±0.1	27.0	10.6±0.2	28.5	8.8±1.0
	40–60	nd	15.1±0.2	nd	13.2±1.3	nd	13.0±0.7	nd	10.3±1.5
3	0–20	34.5	0.9±0.1	41.0	0.9±0.1	26.7	7.2±0.7	29.3	1.3±0.1
	20–40	34.0	1.0±0.1	40.6	0.9±0.1	28.0	5.9±0.1	29.0	1.9±0.3
	40–60	nd	1.3±0.1	nd	1.1±0.2	nd	5.3±0.1	nd	1.6±0.2
4	0–20	32.0	8.0±0.3	37.4	8.6±0.8	26.2	14.0±0.3	28.1	10.1±0.6
	20–40	31.6	10.2±0.2	37.0	10.0±0.4	27.0	11.4±0.1	29.2	8.0±0.7
	40–60	nd	10.0±0.1	nd	10.2±0.7	nd	11.5±0.1	nd	7.2±0.2

Februay and May, dry season; August and October, rainy season; nd, not determined

Table 3. Chemical properties and texture of soil horizons collected in four savanna sites during the dry season. Soil depth: 0–20 cm, 20-40 cm and 40–60 cm

	Soil site 1			Soil site 2			Soil site 3			Soil site 4		
Soil depth (cm)	0–20	20–40	40–60	0–20	20–40	40–60	0–20	20–40	40–60	0–20	20–40	40–60
pH (H_2O)	5.0	4.9	5.0	5.9	5.3	5.3	5.1	5.4	5.1	5.2	5.3	5.3
N (mg/g)	0.35	0.84	0.63	0.52	0.35	0.42	0.67	0.42	0.39	0.49	0.32	0.35
P available (μg/g)	0.23	0.16	0.16	0.23	0.16	0.16	0.23	0.23	0.16	0.16	0.16	0.19
Mg (meq/100 g)	1.00	0.68	0.26	1.20	1.11	0.91	0.90	0.33	0.06	0.80	0.63	0.36
K (meq/100 g)	0.02	0.01	0.01	0.08	0.06	0.05	0.02	0.01	0.00	0.06	0.02	0.00
Ca (meq/100 g)	0.20	0.00	0.00	2.02	0.43	0.20	0.68	0.09	0.00	0.31	0.14	0.00
Mn (μg/g)	0.03	nd	nd	0.01	nd	nd	0.006	nd	nd	0.003	nd	nd
Fe (μg/g)	0.24	nd	nd	0.12	nd	nd	0.117	nd	nd	0.117	nd	nd
Al (meq/100 g)	0.53	0.70	0.87	0.06	0.15	0.22	0.08	0.12	0.26	0.80	0.36	0.22
Exch. ac[1] (meq/100 g)	1.28	1.65	2.20	0.14	0.57	0.73	2.74	0.34	0.94	1.81	0.00	0.00
CEC[2]	1.74	1.38	1.14	3.36	1.74	1.38	1.68	0.54	0.33	1.97	1.14	0.59
$\sum$ bases	1.22	0.68	0.27	3.30	1.59	1.16	1.60	0.42	0.07	1.17	0.78	0.37
% organic matter	2.01	1.88	1.21	1.03	0.87	0.96	1.74	1.21	1.14	1.00	0.67	0.67
% sand	80.0	66.3	52.5	88.8	87.5	83.8	92.5	87.5	87.5	67.5	88.8	87.5
% clay	6.5	16.5	24.0	3.8	5.0	7.5	2.5	7.5	7.5	20.3	5.3	6.5
% slit	13.5	17.3	23.5	7.5	7.5	8.8	5.0	5.0	5.0	12.3	6.0	6.0
% Al sat[3]	30.3	50.7	76.3	2.4	8.6	15.7	4.8	3.5	78.8	40.6	31.6	37.3

[1], Exchangeable acidity; [2], Cation exchange capacity; [3], aluminium saturation.

increased at site 4. All savanna sites were characterized by a low nutritional status and by the increasing % Al saturation up to 76% with increasing soil depth at sites 1 and 3. At soil site 4 the % Al saturation remained constant at different soil horizons, and only site 2 showed relatively lower soil exchangeable acidity regardless of soil depth. The pH of soils ranged from 5.0 (site 1) to 5.9 (site 2), with no marked differences with increasing soil depth at each site. The onset of the rainy reason did not affect the texture and pH of soil sites, but decreased the % Al saturation in the 0–20 cm soil horizon at sites 2, 3 and 4 (not shown). At deeper horizons there were no apparent interseasonal changes in the soil properties. During the dry season the pH of the rhizosphere of *Desmodium barbatum* (site 1), *Eriosema crinitum* (site 2), *Aeschynomene* sp. and *Chamaecrista tetraphila* (site 3), *D. barbatum* and *Stylosanthes guianensis* (site 4) was similar to that of bulk soils from where plants were harvested. Acidification of the rhizosphere was observed in *Chamaecrista flexuosa* (site 1), while the highest pH of 6.5 was recorded in the rhizosphere of *Mimosa debilis* (site 2). The rhizosphere of all plants contained up to 3-fold more nitrogen, but lower Mg and CEC than bulk soils.

Vegetation analyses

All savanna sites were composed mainly by grasses and to a lesser extent by legumes and Cyperaceae species (Fig. 1A–D). In February, IVI of legumes was high at site 4 followed by sites 2> 3, and legumes were not detected at site 1. Between February and August there was a progressive decrease in the legumes IVI at site 4, while at sites 2 and 3, IVI of legumes decreased in May followed by an increase in August. The lowest legume IVI was detected at site 1 at all sampling times. The survey of the savanna sites during the rainy season revealed the presence of 12 native legume species at site 1, 21 at site 2, 13 at site 3 and 10 at site 4.

Visual observations of legumes collected in February showed a high frequency of individuals with underground xylopodia and a low frequency of individuals with active nodules (Fig. 2). A further decrease in the frequency of nodulated individuals took place in May and most of the legumes presented xylopodia. The opposite situation was observed during the rainy months with 82% of the individuals presenting active nodules. In the collected native legumes, nodules were formed mainly on lateral roots and nodule appearance on the primary root seemed to be a random event, not related to species and site of collection.

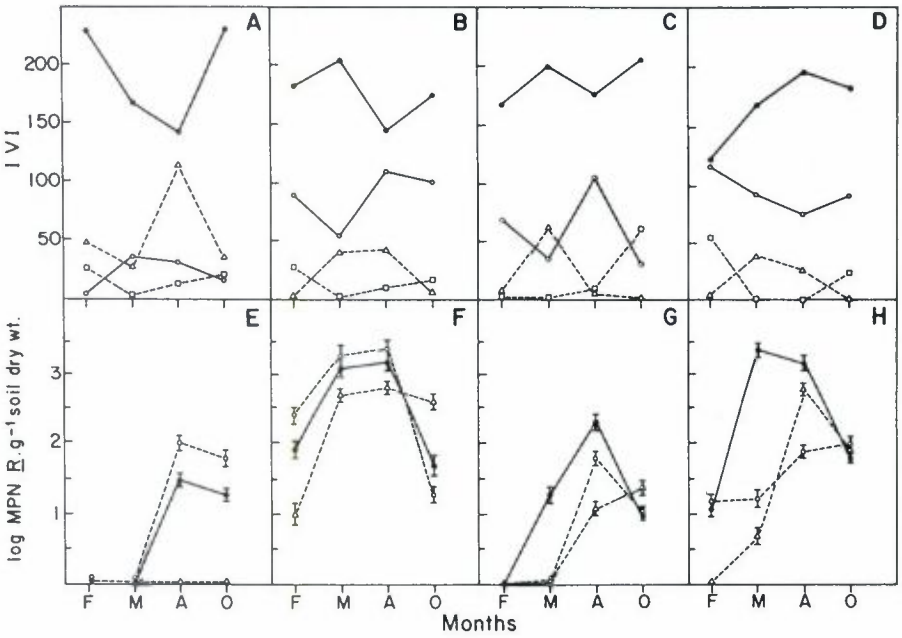

Figure 1. A to D, Importance value index (IVI) of grasses (●), legumes (○), Cyperaceae (△) and other native species (□) in the four contrasting savanna sites as detected in the transect analysis. E to H, Most probable number (MPN) of rhizobia in the 0–20 cm (●), 20–40 cm (○) and 40–60 cm (△) soil horizons of the four contrasting savanna sites. A and E, site 1; B and F, site 2; C and G, site 3; D and H, site 4. F, February; M, May; A, August; O, October.

Analysis of native rhizobial populations

The dynamics of NRP at different soil horizons of the savanna sites is shown in Fig. 1E–H. During the dry season, NRP were not detected in any of the three soil horizons at site 1. With the onset of rains (after May) the size of NRP increased in the two uppermost soil horizons (0–40 cm) at site 1, but remained undetected at greater soil depths. At site 2, the size of NRP at each of the soil horizons increased between February and May, remaining constant up to August, followed by a decrease in October at upper soil horizons. Between February and August the number of rhizobia in this site was higher at upper soil horizons (0–40 cm) than in soils below 40 cm deep. Differences in rhizobial number between the two uppermost horizons were observed only in February and October. At site 3, NRP were not detected in February regardless of soil depths and in May, at soil depths below 20 cm. In the upper soil horizon there was an increase in the size of NRP in May, increasing more in August. At

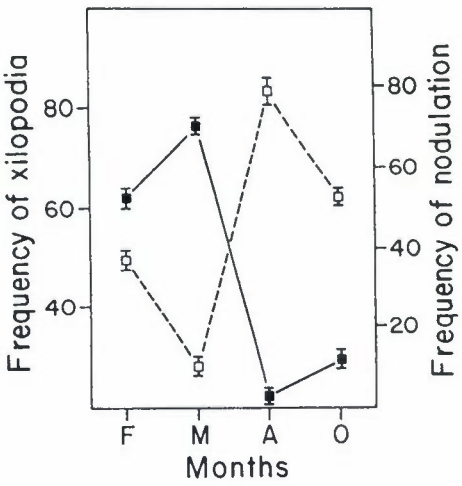

Figure 2. Frequency of native legume individuals with active nodules on the roots (□) and frequency of native legume individuals with xylopodia (■) detected in February (F), May (M), August (A) and October (O) in the savanna under study.

this time of the year an enhancement in the size of NRP was also observed at deeper soils, the number of rhizobia being higher at soil depths of 20–40 cm when compared to that of soils located below 40 cm depth. AT site 4, in February, NRP were confined to a soil depth of 0–40 cm, and a high number of rhizobia were observed at the upper soil horizon during May and August, followed by a decrease in October. With the onset of the rainy season, there was a progressive increase in the number of rhizobia at soil depths below 40 cm and in August the value was higher in the 40–60 cm soil horizon than at a 20–40 cm soil depth. Differences in the size of NRP at different soil depths at site 4 were not detected in October.

A highly significant negative correlation between the size of NRP and the soil temperature was obtained for both seasons ($r=-0.84$, $p < 0.01$). Significant positive correlations at $p < 0.01$ were detected between the size of NRP and the soil water content at site 1 (r=-0.921), site 2 (4=0.712) and site 3 (r=0.767). However, the correlation value was not significant at site 4 (r=0.526). Results of the correlation analysis between the size of NRP and soil chemical properties of the uppermost soil horizon are summarized in Table 4. The correlation between the size of NRP and the percentage of native legume individuals is shown in Fig. 3.

During the dry season the highest size of NRP was detected in the rhizosphere of several native legumes randomly collected at site 1 to 4 (Table 5),

Table 4. Correlation analysis between the most probable number (MPN) of rhizobia and several soil chemical characteristics during the rainy or dry seasons. Correlation tests were carried out with the data obtained at the 0–20 cm soil horizon of the four savanna sites.

	Soil	Rainy season	Dry season
Log MPN of rhizobia g^{-1} soil dry wt	pH (H_2O)	0.64 S	0.73 S
	Int. ac[1]	−0.62 S	−0.82 S
	Al	−0.64 S	−0.39 NS
	$\sum$ bases	−0.24 NS	0.94 S
	Ca	0.10 NS	0.56 S
	CEC[2]	0.72 S	0.99 S
	K	0.23 NS	0.73 S
	Mg	−0.59 NS	0.68 S
	OM[3]	−0.88 S	−0.50 NS
	N	−0.46 NS	−0.26 NS
	P	0.36 NS	−0.11 NS
	% Al sat[4]	−0.45 NS	−0.79 S

[1], interchangeable acidity; [2], cation exchange capacity; [3], organic matter; [4], aluminium saturation; S, correlation between variables significant at $p < 0.01$; NS, correlation between variables not significant at $p < 0.01$.

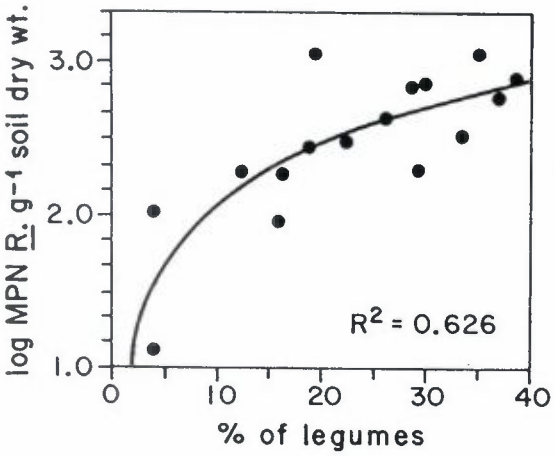

Figure 3. Interaction between the percent of native legume individuals and the most probable number (MPN) of rhizobia in the savanna.

Table 5. The most probable number (MPN) of rhizobia detected in the rhizosphere of several native legume species collected in February (dry season) in four savanna sites

Species	Log MPN rhizobia g^{-1} soil dry wt.
Soil site 1	
Chamaecrista calycioides	1.7
Clitoria guianensis	2.2
Crotalaria stipularis	3.3
Desmodium barbatum	3.7
Galactia jussieuana	3.5
Mimosa camporum	3.3
Phaseolus gracilis	3.8
Stylosanthes guianensis	3.2
Zornia curvata	3.7
Soil site 2	
Chamaecrista tetraphila	3.6
C. rotundifolia	2.4
Eriosema crinitum	2.9
Galactia jussiaena	2.8
Indigophera lespedezioides	3.8
Phaseolus sp.	2.9
Stylosanthes capitata	2.6
S. guianensis	3.2
Zornia curvata	3.3
Soil site 3	
Stylosanthes sp.	4.1
Soil site 4	
Chamaecrista tetraphila	2.8
Mimosa debilis	2.0

the maximum number of rhizobia being detected in the soil surrounding *Stylosanthes* sp. (site 3). In contrast, rhizobial counts in the rhizosphere of a similar set of native legumes collected during the rainy season revealed values below 10 cells g^{-1} soil regardless of the species and site of collection (data not shown).

Host range and symbiotic efficiency of native rhizobial populations

NRP present in the upper soil horizon of the four savanna sites induced nodule formation in *M. atropurpureum*, *P. vulgaris* and *V. unguiculata* regardless of the month of soil collection. In contrast, nodulation in *C. pubescens*,

L. leucocephala and *P. tetragonolobus* took place mainly in plants inoculated with soils from the four sites collected in May. Results also indicate nodule formation in *S. capitata* inoculated with soils from sites 1, 2 and 4 collected in October. Nodules were absent in all inoculated *G. max* and *P. sativum*.

The symbiotic efficiency of NRP located in the upper horizon of the four sites in whole-soil-inoculated (WSI) host plants is summarized in Table 6. The aerial biomass of *V. unguiculata* plants inoculated with soils collected in February did not differ from that of uninoculated (−N). When compared to plants inoculated with the *Bradyrhizobium* strain I-700, WSI plants presented a reduced nodule number and fixed about 60% less N_2, according to RAU values. Enhanced symbiotic N_2 fixation, nodule number and aerial biomass were observed in *V. unguiculata* plants inoculated with all soils collected in May and with soils from sites 1 and 2 collected in October. Nevertheless, the best nodulation recorded in plants inoculated with soils from sites 3 and 4 (May) and from site 2 (October) represented 25% of the nodule mass, 75% RAU and 46% of the aerial biomass of plants inoculated with the *Bradyrhizobium* strain I-700. For WSI *P. vulgaris* the average RAU was 28.7 ± 2.7, 3.2-fold lower than RAU values in plants inoculated with the *Rhizobium phaseoli* strain I-113. The aerial biomass of the plants was 82% smaller than that of I-113 inoculated ones and in most cases was similar or even smaller than that of uninoculated (−N) plants. In WSI *P. tratagonolubus* a growth promotion was exclusively observed in plants inoculated with soil from site 1 collected in May. Whole soil inoculation in *C. pubescens* and *L. leucocephala* induced the formation of 5 ± 1 nodules pl^{-1} with a mean weight of 3.1 ± 0.6 mg dry wt pl^{-1} and the average aerial biomass did not differ from that of uninoculated (−N) plants (data not shown).

Nodulation in WSI *V. unguiculata* was characterized by the formation of indeterminate and determinate nodules with external black or pink color with or without dark strips as well as by the occurrence of collars of inefficient white nodules. Predominance of determinate black nodules without strips was observed in plants inoculated with soil site 2 collected in October. In WSI *P. vulgaris* there was a predominance of determinate nodules externally white with an internal greenish pigmentation. In turn, pink determinate nodules with an internal red color were exclusively observed in *P. tetragonolobus* inoculated with soil from site 1 collected in May.

Cultural characteristics and symbiotic efficiency of native rhizobial isolates

Of the 52 rhizobial isolates, 69% were obtained at pH 5.5 and 31% at pH 7.0. Forty-eight percent were alkali producers, 46% were acid producers and 6%

Table 6. Nodulation, relative abundance of ureides (RAU) and aerial biomass in legume species inoculated with the upper soil horizon collected at four different savanna sites during the dry (February and May) and rainy (October) seasons. Values represent the mean of four replicates ± standard error of the mean.

Inoculation treatments Soil sites	Month	Nodule number pl^{-1}	Nodule mass (mg dry wt pl^{-1})	Aerial biomass (g dry wt pl^{-1})	RAU (%)
		Vigna unguiculata			
1	February	8±1	30.2±0.5	0.37±0.05	34.4±1.6
2	February	29±1	14.8±0.8	0.24±0.04	27.2±2.4
3	February	8±1	9.6.0.4	0.25±0.01	41.6±2.2
4	February	12±1	31.5±0.4	0.34±0.03	46.2±7.3
1	May	41±1	89.9±2.4	0.70±0.05	69.0±6.2
2	May	27±1	98.2±4.6	0.70±0.09	59.6±4.7
3	May	20±2	52.4±3.1	0.90±0.09	78.4±6.9
4	May	55±2	137.4±3.2	0.90±0.02	70.4±8.7
1	October	58±1	52.3±1.9	0.73±0.08	68.1±7.0
2	October	65±2	65.0±1.5	1.10±0.04	70.2±4.1
3	October	8±1	8.1±0.5	0.43±0.07	33.2±3.2
4	October	50±3	81.7±2.3	0.40±0.03	43.6±1.0
uninoculated (−N)[1]		–	–	0.32±0.05	< 0
uninoculated (+N)[1]		–	–	1.31±0.05	3.3±0.1
inoculated (I-700)[1]		46±3	259.9±9.8	2.40±0.1	93.2±1.0
		Phaseolus vulgaris			
1	February	8±1	4.3±0.9	0.24±0.01	21.6±1.0
2	February	4±1	3.5±0.2	0.67±0.03	31.4±8.2
3	February	11±1	5.3±0.6	0.44±0.07	25.4±3.0
4	February	13±1	6.4±0.2	0.30±0.05	33.4±1.5
1	May	8±1	6.3±0.5	0.46±0.02	24.4±1.8
2	May	41±2	33.1±0.8	0.50±0.07	34.9±1.5
3	May	18±2	35.0±1.5	0.44±0.02	20.6±1.6
4	May	18±2	28.0±1.3	0.60±0.09	26.2±1.7
1	October	17±1	26.2±1.4	0.62±0.03	25.9±0.9
2	October	68±3	62.5±5.2	0.55±0.08	20.6±1.3
4	October	35±1	28.4±0.9	0.44±0.03	51.6±2.4
uninoculated (−N)		–	–	0.22±0.02	< 0
uninoculated (+N)		–	–	1.22±0.08	3.7±1.0
inoculated-(I-113)[1]		nd	24.2±0.01	2.70±0.2	91.0±2.5
		Psophocarpus tetragonolobus			
1	February	8±1	4.2±0.2	0.31±0.04	32.9±3.3
4	February	5±1	3.0±0.2	0.30±0.07	38.3±3.8
1	May	35±2	42.0±1.4	1.20±0.08	65.2±5.9
2	May	31±1	34.1±0.5	0.42±0.01	36.0±4.5
3	May	4±1	4.5±0.03	0.53±0.05	39.3±2.2
4	May	8±1	2.7±0.3	0.10±0.008	28.3±2.2
1	October	22±1	48.5±3.0	0.38±0.05	33.4±1.1
2	October	15±2	33.6±2.1	0.34±0.06	36.3±4.4
4	October	28±1	46.7±2.7	0.49±0.05	38.6±0.4
uninoculated (−N)		–	–	0.13±0.01	< 0
uninoculated (+N)		–	–	0.81±0.04	13.9±0.9

[1], see Materials and Methods; –, nodules absent.

did not induce pH changes in the growth media. The bulk of rhizobial isolates were slow growers and there was no relationship between pH changes and the generation time in the growth media.

Eleven slow growing bradyrhizobial isolates capable of nodulating *V. unguiculata* were selected for further analyses. The *Bradyrhizobium* isolates I-709, I-714, I-715 and I-718 showed good growth at pH 7.2, whereas the rest grew poorly at this pH. Good growth at pH 4.5 was displayed only by isolates I-707 and I-711. All bradyrhizobial isolates formed nodules in *M. atropurpureum*. The isolates I-703, I-706, I-711, I-714, I-715, I-717, I-718 and I-719 nodulated *C. pubescens*.

When inoculated in *V. unguiculata* the 11 bradyrhizobial isolates formed a reduced nodule number and there was a lower chlorophyll concentration in mature leaves when compared to that of plants inoculated with the efficient *Bradyrhizobium* strains I-125, I-700 or I-38 (Table 7). With regard to aerial biomass and ureide concentration, the isolates I-703, I-706, I-711, I-715, I-717 and I-719 induced a similar response to that of plants inoculated with the *Bradyrhizobium* strain I-125. In contrast, isolates I-701, I-707, I-709, I-714 and I-718 formed inefficient nodules and the aerial biomass of the plants was similar

Table 7. Nodule hydrogenase, nodule mass, aerial biomass, ureide concentration in the petiole of the first trifoliolate leaf and chlorophyll concentration in 45-d-old *Vigna unguiculata* var. Tuy plants inoculated with *Bradyrhizobium* sp. isolates. Values represent the mean of 4 replicates ± standard error of the mean.

Isolates[1]	Hup	Nodule mass (mg pl^{-1})	Aerial biomass (g pl^{-1})	Ureides (mmol g^{-1}dry wt)	Chlorophyll (μg cm^{-2})
I-701	–	28.5±3.1	0.33±0.02	13.2±0.7	2.8±0.1
I-703	–	39.7±1.4	0.52±0.05	25.4±2.4	42.2±3.8
I-706	–	70.6±8.3	1.20±0.09	63.6±5.1	46.7±3.7
I-707	–	5.5±0.5	0.19±0.01	8.6±0.8	2.0±0.2
I-709	–	11.9±1.1	0.13±0.01	22.0±0.2	16.0±1.9
I-711	–	80.0±8.8	0.81±0.06	34.1±2.8	43.8±2.6
I-714	–	3.9±0.2	0.13±0.01	13.7±1.7	7.9±.2
I-715	–	78.7±7.8	0.89±0.20	58.8±5.7	55.6±2.9
I-717	–	88.1±8.8	1.11±0.06	20.8±2.3	42.5±2.0
I-718	–	3.2±0.2	0.26±0.03	10.1±1.2	9.4±0.8
I-719	–	67.7±0.6	1.20±0.01	37.0±3.3	37.8±2.7
I-125[2]	+	410.7±12.1	0.85±0.03	35.4±2.1	72.8±3.9
I-700[2]	–	259.2±9.8	2.14±0.13	97.4±3.4	97.7±8.6
I-38[2]	–	298.2±10.1	1.84±0.10	25.3±1.6	52.2±2.0

[1], see Table 1; [2], commercially recommended effective *Bradyrhizobium* strains; Hup^-, hydrogenase negative; Hup^+, hydrogenase positive.

to that of uninoculated (−N). For all plant-isolate combinations there was a positive correlation between nodule mass and ureide concentration ($r=0.438$, $p< 0.01$) and between nodule mass and aerial biomass ($r=0.784$, $p< 0.01$). The qualitative determination of the hydrogenase in nodules formed by the individual native isolates indicated their Hup^- phenotype.

4. Discussion

The onset of rains markedly enhanced the size of NRP at all soil horizons of the savanna sites 2, 3 and 4 and in the two uppermost soil horizons of site 1. Thus, interseasonal changes in the size of NRP within individual soil units were ascribed mainly to the improved soil temperature and water content brought about by the rains (Mahler and Wollum, 1981; Rupela et al., 1987), rather than to interseasonal alterations in the soil physicochemical properties. On the other hand, the nondetection of NRP in the 40–60 cm deep soil horizon of site 1, characterized by a high % Al saturation at both seasons and the marked differences in the size of NRP among sites and horizons indicate the deleterious effect excerted by the soil physico-chemical traits upon native rhizobia. Thus, it might be postulated that during the rainy season the size of the NRP at different sites and horizons was the result of a counteraction between adverse soil chemical conditions and the beneficial effect of rains (Woomer et al., 1990). The influence of soil constituents on the size of NRP was emphasized by the results of the correlation analysis between the number of rhizobia and soil properties.

Present data also revealed a positive correlation between the size of NRP and legume density in the four savanna sites. Throughout the year the smaller NRP were found at sites 1 and 3, characterized by a low legume IVI, whereas sites 2 and 4, with high legume IVI at the onset of the dry season harbored the largest NRP at the rainy season. The increased size of NRP along soil horizons during the rainy season might constitute an advantage for the seasonal nodulation of perennial legumes with deep roots, since nodules formed at deeper root segments are known to have an active participation on the N-economy of legumes (Davey et al., 1989, Izaguirre-Mayoral et al., 1994).

The important role played by the hosts was also ascertained by the high number of rhizobia in the rhizosphere of native legumes collected during the dry season regardless of soil sites, and by the increased size of NRP observed in May at sites 2, 3 and 4. Such high rhizobial numbers in the rhizosphere and bulk soils in May (dry season) has been associated with the release or rhizobia from shedding nodules of water-stressed individuals (Bushby, 1984; Richardson and Simpson, 1988). This assumption is further supported by the reduction

in the frequency of nodulated individuals and the nondetection of rhizobia at site 1 in May, time at which IVI values indicated the lowest legume density among sites. The more favourable conditions of the rhizosphere during the dryest months might also help to sustain NRP for the next nodulating cycle (Bushby, 1993), and the high nitrogen content in the rhizosphere (Wardle and Greenfield, 1991) could contribute to the N-economy of droughted plants. From the native species analysed, the rhizosphere of *C. tetraphila*, *D. barbatum*, *G. jussieuana*, *L. leucocephala*, *P. gracilis*, *Stylosanthes* sp. and *Z. curvata*, widely distributed among savanna sites, proved to be important reservoirs of NRP during the dry season.

According to the host range analysis, the rhizobial species composition in the savanna seems to include: *Bradyrhizobium* sp. (*Vigna*) and *Bradyrhizobium* spp. specific for *C. pubescens* (Nurhayati et al., 1988), *L. leucocephala* (Turk and Keyser, 1992), *P. tetragonolobus* and *Stylosanthes capitata*, whereas the presence of *Rhizobium tropici* and *R. etli* in all savanna sites was inferred from the nodule formation in *P. vulgaris* and *L. leucocephala* (Martinez-Romero et al., 1991, Segovia et al., 1993). In contrast, *Rhizobium leguminosarum* bv *viceae* and *Bradyrhizobium japonicum* were apparently absent in the savanna soils due to the nonappearance of nodules in *P. sativum* and *G. max*. Nevertheless, the bulk of data tend to indicate the composition of NRP mainly by *Bradyrhizobium* of the "cowpea miscellany". This suggestion is based on the slow growth displayed by a high number of rhizobial isolates in the growth media and by the occurrence of nodules in *V. unguiculata* plants inoculated with most of the rhizobial isolates obtained either from nodules of *M. atropurpureum* inoculated with soil water suspensions or from nodules of *C. calycioides, C. rotundifolia, D. barbatum, M. camporum* and *S. guianensis* collected in different savanna sites. The presence in the savanna soils of different native *Bradyrhizobium* spp. strains (Thies et al., 1991) was also inferred from the simultaneous appearance of indeterminate and determinate dark, pink and piebald nodules in WSI *V. unguiculata* (Eaglesham et al., 1982).

The host range analysis also allowed us to infer the greatest susceptibility of native *Bradyrhizobium* spp. (*Centrosema*, *Leucaena* and *Psophocarpus*) to drought. This was ascertained by the absence of nodules in *C. pubescens*, *L. leucocephala* and *P. tetragonolobus* plants inoculated with soils collected in February and October, attributed mainly to the low rhizobial number in soil sites at this time of the year. In contrast, nodulation took place in *P. vulgaris* and *V. unguiculata* regardless of the site and month of soil collection. On the other hand, NRP present in the four soil sites did not elicit a response in WSI *C. pubescens*, *L. leucocephala*, *P. vulgaris* and *P. tetragonolobus* in spite of the

good nutritional and pH conditions within the Leonard jars. These results indicate that the intrinsic inefficiency of NRP was not overcome by nutrient supply and pH amendment. A growth promotion was obtained only in *V. unguiculata* plants inoculated with soils collected in May and October or with the effective native *Bradyrhizobium* isolates I-706, I-711, I-715, I-717 and I-719. Nevertheless, the inoculation of *V. unguiculata* with the efficient *Bradyrhizobium* isolates brought about an increased response of plants above values obtained in WSI plants. Therefore, it might be assumed that the concomitant presence of efficient and inefficient rhizobia in soils may be the reason for the poor performance of WSI *V. unguiculata* plants. It has been shown that inoculation of *V. unguiculata* plants with two *Bradyrhizobium* strains elicit an antagonistic behaviour on plant growth (Sicardi de Mallorca and Izaguirre-Mayoral, 1993).

A detailed analysis of the six efficient *Bradyrhizobium* sp. (*Vigna*) isolates showed five of them to be alkali producers and capable of growing at moderate or good rates at pH 4.5. However, the relative effectiveness, good growth at pH 7.2 and acid production of the isolate I-715 as well as the inefficiency of the I-707 with good growth at pH 4.5 indicate the apparent non-relationship between the isolate cultural characteristics and their symbiotic efficiency. Based on previous reports (Padmanabhan et al., 1990), the acid producer status of several isolates should not affect their inclusion among the group of *Bradyrhizobium* sp. (*Vigna*). Finally, the aerial biomass and RAU values of *V. unguiculata* plants inoculated with efficient Hup^- native *Bradyrhizobium* isolates were similar to those of plants inoculated with the *Bradyrhizobium* strain I-125 (Hup^+), but ranked below that of plants inoculated with the *Bradyrhizobium* strains I-700 or I-38, both Hup^-. Thus, present results support the exclusion of the Hup^+ phenotype as an indicator of the symbiotic efficiency of rhizobial strains (Fuhrmann, 1990).

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