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A Comparative Evaluation of the Symbiotic N_2-Fixation and Physiological Performance of Thirty-Six Native Legume Species Collected in a Tropical Savanna During the Rainy and Dry Seasons

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Abstract

The savanna under study was covered mainly by vegetatively growing native legume species during the rainy season. Seven species of Caesalpiniaceae, 22 species of Fabaceae and 5 species of Mimosaceae were found nodulated. The exceptions were *Centrosema venosum* and *Tephrosia sessiliflora* with nodules absent in the sampled root volume. Determination of the relative abundance of ureides (RAU) at this time of the year allowed the classification of all Caesalpiniaceae, 13 Fabaceae and 2 mimosaceae species as intermediate N_2-fixers (RAU 30–59%), whereas good N_2-fixers (RAU> 60%) were found only in the Fabaceae. Outstanding symbiotic N_2-fixation was detected in *Aeschynomene* sp., *Calopogonium mucunoides*, *Centrosema pascuorum*, *Phaseolus diversifolius* and *Phaseolus gracilis*. Low N_2-fixation (RAU 14–29%) was observed in *Crotalaria stipularis*, *Galactia jussieuana*, *Tephrosia sessiliflora* (Fabaceae), *Mimosa camporum*, *M. orthocarpa* and *M. pudica* (Mimosaceae).

The dry season caused a 25% decrease in the number of native legume species, and reduced to 8 the number of species with effective nodules on the roots.

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Most of the individuals were at flowering and fruiting stages, presented nodule decay, and 69% of the species showed significantly reduced relative ureides content (RUC) in shoots and roots. Determination of RAU during the dry season showed 50% of the species with a 70–100% reduction in N_2-fixation. Such species were categorized as non-tolerants to drought. Seven species were considered as drought-tolerants due to the 50% decrease in RAU. *A. hystrix*, *G. jussieuana*, *Indigofera pascuorum*, *M. pudica*, *C. mucunoides* and *P. gracilis* presented RAU values similar to those observed during the rainy season, and were identified as insensitive to drought. The last two species showed RAU above 60%. The effect of seasonal drought on RUC but not on the α-amino-N content in roots and shoots evidenced the ureide producer status of all sampled native legumes. On the other hand, there were no significant effects of drought on the chlorophyll, protein, total-N (Kjeldahl) and non-structural carbohydrate content of the plants. These results emphasize the greater sensitivity of nodules to water stress when compared to that of the host. The present investigation also revealed a significant increase in nitrate levels of shoots and roots of several native legumes during the dry season, and the general inverse relationship between RUC and the shoot relative nitrate content. Thus, a plant metabolic switch from symbiotic N_2-fixation to nitrate reduction is proposed, as an alternative way for native species to fix soil nitrogen under drought.

Keywords: symbiotic nitrogen fixation, native legume species, seasonal effect, tropical savanna

1. Introduction

Tropical savannas are characterized by a strong seasonality in water availability. Throughout the year, two seasons are easily distinguished according to the amount of rain fall. Drought is known to prevail during the driest months. On the other hand, savannas in Venezuela are covered all year round by a wide diversity of legume species, most of them capable of fixing N_2 at relatively high or intermediate rates during the rainy season (Izaguirre-Mayoral et al., 1992). However, the question arises about the effectiveness of the symbiotic process in native species subjected to seasonal drought.

To date, studies related to the effect of water stress on the symbiotic N_2-fixation have focussed predominantly on cultivated legumes (Osunkoya and Sobogum, 1989; Aguirreolea and Sánchez-Diaz, 1989; Venkateswarlu et al., 1989; Guerin et al., 1990; Irigoyen et al., 1992; Castrillo, 1992; Castonguay and Markhart, 1992; Westgate and Peterson, 1993). Rather less is known about the response of native legume species to temporal low soil water conditions. Therefore, the present investigation was undertaken to examine the physiological and symbiotic performance of native legume species collected at

the rainy and dry seasons. Plants were analysed in terms of nodulation, ureide, α-amino-N, nitrate, proteins, chlorophyll and non-structural carbohydrate content. In order to consider N input from the symbiont to be relevant for the N-economy of the plants, the N-products from N_2-fixation should at least be equal or higher than the nitrate incorporated by the plants. For our purposes, values of relative abundance of ureides of 60% or above are considered as indicator of an effective symbiosis.

2. Materials and Methods

Soil sampling and plant collection were carried out in the Estación Experimental La Iguana, Guarico State, Venezuela (8° 25′N, 65° 24′W) (Universidad Experimental Simón Rodriguez), during the rainy (August) and dry (February-May) seasons of 1991–1992.

Soil characterization

Triplicate soil samples to a depth of 20 cm were collected, at random, in representative areas of the savanna under study. The physicochemical analyses of soils were carried out on dried subsamples as described by Izaguirre-Mayoral et al. (1992).

Vegetation analysis and plant material

Vegetation analyses at the rainy and dry seasons were carried out by harvesting individuals every meter along 10 m long transects. The importance value index (IVI) of individual legume species was calculated as the relative density + relative frequency + relative dominance of each species in the transects (Muller-Dombois and Ellemberg, 1974).

Analysis of nodulation and physiological parameters related to symbiotic N_2-fixation were carried out on a minimum of 6 individuals for each of the native legume species. Plant harvesting during the rainy and dry seasons consisted of the entire above-ground biomass and underground organs (roots and xylopodia) contained in a soil volume of approximately 0.027 m^3.

Determination of N-compounds, non-structural carbohydrates and chlorophyll content in different plant compartments

The first 5 cm of green shoots and roots were extracted twice for 30 min in hot 10 mM buffer phosphate, pH 7.2, containing 50% ethanol. Ureides (allantoin plus allantoic acid), α-amino-N and nitrate content were determined

in aliquots of the plant extracts, as previously described by Izaguirre-Mayoral et al. (1992). Values of relative ureides (RUC), α-amino-N (RAC) and nitrate (RNC) content were calculated as the percentage of each N-compound in the total-N extracted from different plant organs (van Kessel et al., 1988). The relative abundance of ureides in shoots was calculated, taking into account the 4× molar concentration of ureides relative to the molar concentration of nitrate (Herridge et al., 1990). Non-structural carbohydrates were determined in dried subsamples of shoots and roots (McCready et al., 1950). Chlorophyll (Hiscox and Israelstam, 1978) and protein (Lowry et al., 1951) content were measured in mature leaves.

Statistical analyses

Data in percentage were transformed to degrees by angular transformation. Statistical differences for individual plant parameters, between species collected during the rainy or dry seasons, were analysed using one way analysis of variance. Statistical differences between data of each variable were determined using LSD test (Least Significant Difference). Comparison of means between seasons was carried out using the students' t test.

3. Results

Climatic and soil characteristic of the savanna

During the rainy season the savanna under study presented an average of 25±2° C in the air and 28±1° C in superficial soil horizons (0–20 cm), and a mean rain fall of 1175 mm. During the dry season, temperatures were of 36±2° C and of 37±2° C air and soil, respectively, and a mean rain fall of 81 mm.

Soil texture was predominantly sandy with 80–90% of sand. Soil pH ranged between 5.2 to 5.7. Chemical composition of soil was of 2.1±0.2 cmol Kg^{-1} dry mass cation exchange capacity, 0.18±0.92 μg g^{-1} soil available P, and 19.5±1.1% aluminium saturation. Organic matter content was below 1.7% with 0.7 mg of N/g of soil.

Physiognomic observations

Native legumes mainly in vegetative stages of growth were observed during the rainy season (Table 1). With the onset of drought the savanna was covered by legumes in flowering or fruiting stages, increasing the number of individuals presenting underground xylopodia.

Table 1. Percentage of total native legume individuals in vegetative, flowering and fruiting stages, and with underground xylopodium during the rainy and dry seasons

	Rainy	Dry
Number of individuals collected	278	154
% of individuals with xylopodium	17	30
% of individuals in vegetative stage	70	38
% of individuals in flowering and fruiting stages	30	62

Of all the legumes detected in the transect analysis, *D. barbatum*, *G. jussieuana* and *M. debilis* presented the highest importance value index (IVI) in the rainy season (Table 2). *C. flexuosa*, *C. rotundifolia*, *M. camporum* and *M. pudica* showed high IVI during the dry season. The presence of *C. tetraphila*, *G. jussieuana*, *I. pascuorum*, *S. capitata*, *Z. curvata*, *M. camporum* and *M. debilis* throughout the year was ascertained by the IVI values.

Table 2. Importance Value Index (IVI = relative density + relative frequency + relative dominance) for native legume species during the rainy and dry seasons of the savanna sampled

	IVI	
Species	Rainy	Dry
Caesalpiniaceae		
Chamaecrista bauhiniaefolia	15.1	
Chamaecrista calycioides	21.1	
Chamaecrista flexuosa		52.1
Chamaecrista rotundifolia		78.3
Chamaecrista serpens		7.3
Chamaecrista tetraphila	10.7	24.8
Fabaceae		
Aeschynomene brasiliana	15.6	
Aeschynomene evenia	13.7	
Aeschynomene hystrix	10.7	
Desmodium barbatum	49.3	
Eriosema crinitum		23.6
Eriosema rufum		5.8
Galactia jussieuana	49.2	26.1
Indigofera pascuorum	13.2	5.1
Stylosanthes capitata	12.7	5.1
Stylosanthes sp.		18.9
Zornia curvata	19.7	5.2
Mimosaceae		
Mimosa camporum	15.6	38.5
Mimosa debilis	40.1	4.4
Mimosa pudica		31.9

Nodulation in native legumes and vegetation analysis

Seven species of Caesalpiniaceae, 24 species of Fabaceae and 5 species of Mimosaceae were collected during the rainy season (Table 3). Nodulation was observed in 34 species, with coralloid, finger-like (indeterminate) or spherical (determinate) nodules found mainly along lateral roots. Nodules were rarely formed on the underground xylopodia. A combined population of coralloid and spherical nodules was observed in *C. bauhiniaefolia, C. calycioides, I. pascuorum, M. camporum, M. debilis* and *M. pudica.* Otherwise, each individual species had a predominant nodule shape. The best nodulation, as indicated by nodule distribution along the roots, nodule size and internal red color was detected in *C. calycioides, C. flexuosa, C. mucunoides, C. pascuorum, D. barbatum, P. diversifolius, P. gracilis, M. martensis* and *M. orthocarpa* plants collected during the rainy season.

With the onset of drought, there was a significant reduction in the number of species (Table 3). Out of the 26 species collected in the driest months only 8 were found nodulated. Most of the nodules lacked the internal red color and showed symptoms of senescence. Visual observations of species reported as non-nodulated during the dry season denoted the presence of the outer cortex of nodules from the previous rainy season. In *C. venosum* and *T. sessiliflora*, nodules were never observed in the analysed root volume.

N-compounds in native legumes

Data of relative ureide (RUC), α-amino-N (RAC) and nitrate content (RNC) in shoots of plants collected during the rainy and dry seasons are listed in Table 4. During the rainy months, RUC values were not significantly different between Caesalpiniaceae species, except for *C. rotundifolia.* Among Fabaceae *Aeschynomene* sp., *C. mucunoides*, *C. pascuorum*, *P. diversifolius*, *P. gracilis* and *Phaseolus* sp. showed the highest RUC. Whereas, RUC below 12% were detected in all Mimosaceae, with no statistical differences between species. RAC was statistically similar among Caesalpiniaceae, except for *C. flexuosa* which showed the lowest value. Within Fabaceae and Mimosaceae there were no statistical differences between species with regard to RAC. Significant differences in RNC were found among Caesalpinaceae or Fabaceae species collected during the rainy season. The highest significant RNC was detected in *C. bauhiniaefolia, C. hispidula, C. serpens, A. evenia, C. pascuorum, C. venosum*, and *E. crinitum.* RNC did not significantly differ among Mimosaceae.

Decreased RUC was detected in *C. flexuosa, C. tetraphila, D. barbatum, E. rufum, E. simplicifolium, Stylosanthes* sp., *M. debilis, M. martensis* and

Table 3. Description of underground organs, nodule type and nodulation in native legume species collected during the rainy and dry seasons

	Species	Type of underground organ	nodule	Nodules Rainy	Dry
Caesalpiniaceae					
1*	*Chamaecrista bauhiniaefolia* Berth	root	I+D	+	+
2	*Chamaecrista calycioides* (DC) Er.	root	I+D	+	nc
3	*Chamaecrista flexuosa* (Moench) DC.	xylopodium	I	+	–
4	*Chamaecrista hispidula* L.	xylopodium	D	+	–
5	*Chamaecrista rotundifolia* Berth	root	I	+	–
6	*Chamaecrista serpens* L.	xylopodium	I	+	+
7	*Chamaecrista tetraphila* (Martyn)	xylopodium	I	+	–
Fabaceae					
8	*Aeschynomene brasiliana* (Poir.) DC.	xylopodium	D	+	nc
9	*Aeschynomene evenia* Wright	root	D	+	nc
10	*Aeschynomene hystrix* Poir.	xylopodium	D	+	+
11	*Aeschynomene paniculata* Willd	root	D	+	–
12	*Aeschynomene* sp.	root	D	+	nc
13	*Calopogonium mucunoides* Desv.	root	D	+	+
14	*Centrosema pascuorum*	xylopodium	D	+	nc
15	*Centrosema venosum* Mart	xylopodium		–	nc
16	*Clitoria guianensis* (Aubl) Berth	xylopodium	D	+	–
17	*Crotalaria stipularis* Desv.	xylopodium	D	+	–
18	*Desmodium barbatum* Berth.	root	D	+	+
19	*Eriosema crinitum* (HBK) G. Don.	xylopodium	D	+	–
20	*Eriosema rufum* (HBK)	xylopodium	D	+	–
21	*Eriosema simplicifolium* (HBK)	xylopodium	D	+	–
22	*Galactia jussieuana* K	xylopodium	D	+	+
23	*Indigofera pascuorum* Berth.	root	I+D	+	–
24	*Phaseolus diversifolius* Pittier	xylopodium	D	+	nc
25	*Phaseolus gracilis* Poepp	xylopodium	D	+	+
26	*Phaseolus* sp.	xylopodium	D	+	nc
27	*Stylosanthes capitata* Vogels	root	D	nc	+
28	*Stylosanthes guianensis* (Aubl.) Sw.	root	D	+	–
29	*Stylosanthes* sp.	xylopodium	D	+	+
30	*Tephrosia sessiliflora*	root		–	nc
31	*Zornia curvata* Mohl	xylopodium	D	+	–
Mimosaceae					
32	*Mimosa camporum* Berth	xylopodium	I+D	+	–
33	*Mimosa debilis* Humb. et Bompl.	root	I+D	+	–
34	*Mimosa martensis* Br & Rose	xylopodium	I	+	–
35	*Mimosa orthocarpa* Spruce	root	I	+	nc
36	*Mimosa pudica* L.	xylopodium	I+D	+	–

I, indeterminate nodules; D, determinate nodules; +, nodules present; – nodules absent; nc, species not collected. *, number identifying legume species in Fig. 1.

Table 4. Percentage of ureide (RUC), α-amino-N (RAC) and nitrate (RNC) content in shoots of native legumes collected during the rainy and dry seasons

Species		RUC	RAC	RNC	Total N** mmol g^{-1} dry wt.
Caesalpiniaceae					
C. bauhiniaefolia	R	10.0ab	76.2a	13.8b	0.19ab
	D	1.1b	63.1ac	35.8a	0.08c
C. calycioides	R	11.1ab	58.8ac	30.0a	0.13bc
C. flexuosa	R	21.9a	48.5c	29.5a	0.11c
	D	3.4b	60.2ac	34.3a	0.18ac
C. hispidula	R	10.5ab	66.8ab	22.7b	0.21ab
	D	1.9b	56.4ac	41.7a	0.16bc
C. rotundifolia	R	4.7*	91.8*	3.5*	0.21*
	D	0.0b	83.2a	16.8b	0.11bc
C. serpens	R	15.4a	68.5ab	16.1b	0.26a
	D	6.1ab	68.1ab	26.3ab	0.18bc
C. tetraphila	R	20.2a	55.5ac	24.2ab	0.10c
	D	2.0b	57.2ac	40.8a	0.17bc
Fabaceae					
A. brasiliana	R	20.2bd	51.9bc	27.8b	0.17ae
A. evenia	R	9.9fg	79.5ab	10.6c	0.19ae
A. hystrix	R	14.1be	68.5ac	17.4bc	0.25ac
	D	14.4be	50.9bc	34.6ab	0.09df
A. paniculata	R	12.2cf	69.8ac	18.0bc	0.10df
	D	5.4fh	73.4ab	21.2bc	0.10df
Aeschynomene sp.	R	35.7a	44.1bc	20.2bc	0.28ab
C. mucunoides	R	28.2ac	54.5ac	17.3bc	0.22ad
	D	29.8ac	82.3a	17.9bc	0.34a
C. pascuorum	R	37.4a	54.3ac	8.3c	0.35a
C. venosum	R	11.2dg	80.2a	8.6c	0.27ac
C. guianensis	R	11.0dg	69.2ac	19.8bc	0.15cf
	D	3.9fi	66.1ac	30.0b	0.12df
C. stipularis	R	9.4fg	60.6ac	30.0b	0.21ad
	D	4.3fh	45.8bc	49.9a	0.14cf
D. barbatum	R	9.7fg	69.6ac	20.7bc	0.15cf
	D	1.8hi	64.2ac	34.0ab	0.17af
E. crinitum	R	4.7fh	91.8a	3.5c	0.25ac
	D	1.6hi	66.3ac	32.0b	0.29a
E. rufum	R	13.5*	58.7*	27.8*	0.12*
	D	2.7gi	76.7ab	20.6bc	0.17ae
E. simplicifolium	R	6.3fh	72.7ab	20.9bc	0.15bf
	D	0.0i	58.4ac	41.6a	0.14df
G. jussieuana	R	3.9fi	75.1ab	21.0bc	0.15cf
	D	3.0gi	77.7ab	14.5bc	0.15cf
I. pascuorum	R	5.0fh	74.7ab	20.3bc	0.17ae
	D	3.6fi	83.3a	13.1bc	0.07ef
P. diversifolius	R	28.2ac	51.7bc	18.8bc	0.19ae
P. gracilis	R	34.2ab	48.0bc	17.8bc	0.22ad
	D	34.7ab	56.0ac	9.3c	0.19ae

Table 4. (Continued)

Species		RUC	RAC	RNC	Total N** mmol g^{-1} dry wt.
Phaseolus sp.	R	37.3a	37.9c	24.8bc	0.14cf
S. capitata	D	3.0gi	75.1ab	21.9bc	0.17af
S. guianensis	R	11.3dg	68.8ac	19.9bc	0.06ef
	D	3.9fi	36.7c	59.4a	0.06ef
Stylosanthes sp.	R	11.7dg	73.4ab	14.9bc	0.10df
	D	0.4hi	86.5a	13.1bd	0.11df
T. sessiliflora	R	12.8cf	51.9bc	35.2ab	0.11df
Z. curvata	R	10.0fg	70.2ab	19.8bc	0.18ae
	D	6.4fh	75.9ab	17.5bc	0.21ad
Mimosaceae					
M. camporum	R	6.6ab	60.2b	35.9b	0.12cd
	D	5.2bc	64.9ab	23.2b	0.14cd
M. debilis	R	10.9a	67.1ab	21.9bc	0.13cd
	D	2.3bc	37.1c	61.1a	0.10d
M. martensis	R	10.5a	72.1ab	17.4bc	0.19bc
	D	2.5bc	85.2a	12.3c	0.51a
M. orthocarpa	R	3.4bc	77.2ab	19.5bc	0.06d
M. pudica	R	11.9a	60.2b	27.9b	0.08d
	D	3.1bc	78.6ab	18.4bc	0.17bd

For individual plant subfamilies, numbers in columns followed by the same letters are not statistically different at $P< 0.05$. R, rainy season, D, dry season; nd, not determined, *, value not included in ANOVA; **, Total-N ethanol extracted.

M. pudica collected during the dry season (Table 4). Ureides were not detected during the dry season in *C. rotundifolia* and *E. simplicifolium*. On the other hand, RAC in shoots was not affected by the dry season. The apparent reduction in RAC observed only in *M. debilis* was attributed to a concomitantly and significant increase in RNC. There was also a significant increase in RNC in shoots of *C. bauhiniaefolia, C. hispidula, C. stipularis, E. crinitum, E. simplicifolium, S. guianensis* and *M. debilis* plants collected in the dry season.

High RUC was detected in roots of *C. calycioides, C. flexuosa, C. serpens, A. brasiliana, C. mucunoides, D. barbatum* and *P. gracilis* during the rainy season (Table 5). The highest RAC in roots were observed in *C. rotundifolia, E. simplicifolium, G. jussieuana* and *Phaseolus* sp. Whereas, *C. tetraphila* and *Stylosanthes* sp. were the species to show the highest RNC in roots. Mimosaceae species did not differ statistically with regards to root RUC, RAC and RNC.

As shown in Table 5, all Caesalpiniaceae and Mimosaceae as well as most of the Fabaceae species collected during the dry season showed significantly

Table 5. Percentage of ureide (RUC) α-amino-N (RAC) and nitrate (RNC) content in roots of native legume species collected during the rainy and dry seasons

Species		RUC	RAC	RNC	Total-N** mmol g^{-1} dry wt.
Caesalpiniaceae					
C. bauhiniaefolia	R	22.4ab	57.4b	20.2c	0.13bc
	D	0.0*	73.6*	26.5*	0.18*
C. calycioides	R	35.9a	35.3c	31.7bc	0.15bc
C. flexuosa	R	30.5a	51.2b	18.3c	0.18bc
	D	1.7c	55.6b	45.3b	0.17bc
C. hispidula	R	15.2b	62.4b	22.3bc	0.20bc
	D	0.0c	57.1b	42.9bc	0.20bc
C. rotundifolia	R	10.6b	78.3a	11.1c	0.09c
	D	2.7c	78.6a	18.7c	0.30b
C. serpens	R	24.3a	55.5b	20.2c	0.41a
	D	6.1bc	58.9b	35.2bc	0.23b
C. tetraphila	R	6.5b	31.9c	61.6a	0.15bc
	D	2.1c	54.2b	43.7bc	0.11c
Fabaceae					
A. brasiliana	R	23.4ab	42.2c	34.5ab	0.18ce
A. evenia	R	11.4ae	57.9ac	30.6ac	0.24bd
A. hystrix	D	11.5ae	65.3ac	27.2ac	0.15ce
A. paniculata	R	42.5*	51.3*	6.2*	0.25*
	D	11.4ae	54.8bc	33.8ab	0.15de
Aeschynomene sp.	R	66.1*	24.4*	9.5*	0.31*
C. mucunoides	R	33.9a	51.9bc	16.8bc	0.18ce
	D	2.8eg	73.7ab	23.5bc	0.16ce
C. venosum	R	16.0ad	52.0bc	31.9ac	0.38ab
C. guyanensis	R	1.2eg	71.9ab	26.9bc	0.12de
	D	1.3eg	78.9ab	19.8bc	0.18ce
C. stipularis	R	7.9be	64.7ac	27.3ac	0.17ce
	D	7.0ce	56.1ac	36.9ab	0.10e
D. barbatum	R	23.0ab	47.1bc	29.9ac	0.10e
	D	1.0eg	73.9ab	25.1bc	0.17ce
E. crinitum	R	0.5fg	63.6ac	35.5ab	0.39a
	D	0.5fg	63.6ac	35.5ab	0.16ce
E. rufum	R	11.8ad	67.7ab	20.5bc	0.28ad
	D	2.7eg	76.7ab	20.6bc	0.19be
E. simplicifolium	R	5.9cf	81.3a	12.8cd	0.22be
	D	2.4eg	72.9ab	24.8bc	0.16ce
G. jussieuana	R	4.1dg	81.3a	14.7cd	0.16ce
	D	4.1dg	78.5ab	15.8cd	0.19ce
I. pascuorum	R	8.1be	68.5ab	23.4bc	0.17ce
	D	3.6eg	83.3a	13.2cd	0.14de
P. diversifolius	R	22.0ac	55.4ac	22.5bc	0.39a
P. gracilis	R	25.2ab	52.7bc	22.1bc	0.29ad
	D	33.8a	55.5ac	10.8cd	0.26ad
Phaseolus sp.	R	1.8eg	81.6a	16.8bc	0.08e
S. capitata	D	3.0eg	75.2ab	21.9bc	0.19be
S. guianensis	R	13.2ad	57.8ac	29.0ac	0.24bd

Table 5. (Continued)

Species		RUC	RAC	RNC	Total-N** mmol g^{-1} dry wt.
	D	4.7cg	38.2c	57.1a	0.07e
Stylosanthes sp	R	10.9be	48.8bc*	40.3a	0.09*
	D	0.2fg	62.2ac	37.6ab	0.10e
T. sessiliflora	R	16.9ac	66.7ab	16.4bc	0.06e
Z. curvata	R	22.2ac	57.5ac	20.3bc	0.14de
	D	6.8cf	67.6ab	25.7bc	0.15de
Mimosaceae					
M. camporum	R	9.1a	60.6a	30.3a	0.15b
	D	2.7b	51.8a	31.1a	0.17ab
M. debilis	R	13.5a	63.6a	22.9ab	0.15b
	D	5.7b	63.8a	30.5a	0.19ab
M. martensis	R	10.4a	65.7a	23.9ab	0.18ab
	D	0.0b	76.1a	23.9ab	0.28a
M. pudica	R	12.1a	53.5a	34.4a	0.09c
	D	4.9b	60.7a	34.4a	0.17ab

For individual plant subfamilies, numbers in columns followed by the same letters are not statistically different at $P < 0.05$. R, rainy season; D, dry season; nd, not determined; *, data not included in ANOVA, **, Total-N in ethanol extracts.

reduced RUC in roots. Dry season had no effect on RAC in roots of Caesalpiniaceae, Fabaceae and Mimosaceae, except for *C. tetraphila.* Increased RNC was measured in roots of *C. flexuosa* when compared to values obtained in the rainy months. *C. tetraphila* was the only species to show significantly reduced nitrate content in roots during the dry season.

Mean values of relative abundance of ureides (RAU) in shoots of native species collected during the rainy and dry season are listed in Table 6. RAU was significantly reduced in 69% of the species collected during the dry season. Seventy-eight per cent of the species affected by drought showed RAU below 11%. At this time of the year, *C. mucunoides* and *P. gracilis* were the only species to present RAU values above 60%.

Physiological parameters in native legumes

Values of chlorophyll, proteins, total-N (Kjeldahl) and non-structural carbohydrates in different plant compartments are summarized in Table 7. Within individual subfamilies the highest chlorophyll content for both seasons were detected in *C. hispidula, C. tetraphila, E. simplicifolium, Stylosanthes* sp., *M. camporum* and *M. martensis.* Among all species, *C. flexuosa* and *M. debilis* showed low chlorophyll content regardless of the season. Chlorophyll content in most species was not significantly affected by drought. Significantly reduced

Table 6. Relative abundance of ureides (RAU) in shoots of native legume species collected during the rainy and dry seasons

Species	RAU (%)	
	Rainy	Dry
Caesalpiniaceae		
C. bauhiniaefolia	37.2a	10.4ab (s)
C. calycioides	32.6a	nd
C. flexuosa	44.7a	6.5b (s)
C. hispidula	33.8a	9.9ab (s)
C. rotundifolia	32.3a	0.0b (s)
C. serpens	41.8a	19.5a (s)
C. tetraphila	36.3a	7.1b (s)
Fabaceae		
A. brasiliana	53.8bd	nd
A. evenia	49.1ce	nd
A. hystrix	44.9cf	30.4c (ns)
A. paniculata	46.9cf	20.4cd (s)
Aeschynomene sp.	62.8ab	nd
C. mucunoides	67.2ab	60.2b (ns)
C. pascuorum	83.5a	nd
C. venosum	47.9ce	nd
C. guianensis	40.6dg	27.9c (s)
C. stipularis	26.1gh	7.9de (s)
D. barbatum	44.4cf	6.9de (s)
E. crinitum	57.1bd	6.1de (s)
E. rufum	51.1be	27.9c (s)
E. simplicifolium	32.9fg	0.0e (s)
G. jussieuana	13.8h	9.6de (ns)
I. pascuorum	27.8gh	19.6cd (ns)
P. diversifolius	58.3bc	nd
P. gracilis	69.6ab	78.6a (ns)
Phaseolus sp.	34.8eg	nd
S. capitata	nd	20.8cd
S. guianensis	35.2eg	6.9de (s)
Stylosanthes sp.	40.4dg	3.8e (s)
T. sessiliflora	18.4gh	nd
Z. curvata	43.9cf	28.7c (s)
Mimosaceae		
M. camporum	17.1c	7.4b (s)
M. debilis	36.4ab	7.5b (s)
M. martensis	48.2a	19.9a (s)
M. orthocarpa	17.2c	nd
M. pudica	26.1bc	16.4ab (s)

For individual plant subfamilies, numbers in columns followed by the same letters are not statistically different at $P < 0.05$. nd, not determined. (ns), difference between means not statistically significant at $P < 0.05$; (s), difference between means statistically significant at $P < 0.05$.

values were detected in *Stylosanthes* sp. and *M. camporum* collected during the dry season. A significant increase was observed in *D. barbatum*. An increase in leaf protein content took place in *M. camporum* and *M. debilis* during the dry season with a decrease in *C. flexuosa*. Otherwise, there were no statistical differences in leaf protein content between the same species collected at both seasons.

The dry season did not alter the total reducing sugars content in shoots of native legumes, except for a significant decrease in *I. pascuorum, S. guianensis* and *M. camporum*. Significantly higher total reducing sugars were detected in roots of *M. debilis*, *M. martensis* and *M. pudica* during the dry season, while decreased levels were measured in roots of *P. gracilis* and *M. camporum*. The starch content in roots was statistically higher in *C. tetraphila*, *C. stipularis*, *E. simplicifolium*, and *M. martensis* collected during the dry season. Significantly decreased values were only detected in *P. gracilis* and *Z. curvata*.

Statistically higher total-N content (N-Kjeldahl) in shoots was detected in *C. venosum* and *Phaseolus* sp. at the rainy season. There were significant increases in *C. tetraphila*, *M. martensis* and *M. pudica* collected during the dry season when compared to nitrogen values obtained in the rainy months.

Statistical comparison of mean values of different plant variables

For all native legume species, the statistical comparison of mean values for different plant variables measured during the rainy or dry seasons are summarized in Table 8. There were non-statistical differences between RAU in shoots and roots for both seasons.

Within subfamilies and during the rainy season a significantly higher protein content was found in Caesalpiniaceae, with no statistical differences detected between Fabaceae and Mimosaceae (Table 9). The chlorophyll content was significantly higher in Mimosaceae followed by Fabaceae and Caesalpiniaceae. Significantly lower total-N content was detected in Mimosaceae. Differences in protein, chlorophyll and total-N content among subfamilies were not found during the dry season. Between rainy and dry seasons there were significant differences in the leaf protein content in Fabaceae. There was no seasonal effect on the chlorophyll and total-N content in Caesalpiniaceae, Fabaceae and Mimosaceae. RAU in shoots was significantly higher in Fabaceae when compared to Caesalpiniaceae and Mimosaceae regardless of the season, with significant differences between rainy and dry seasons means for individual subfamilies (Table 9).

For all native legumes collected during the rainy season, the nitrate content of shoots and roots ranked around 38 and 34 μmol g^{-1} dry wt.,

Table 7. Non-structural carbohydrates, total-N, chlorophyll and protein content in different plant compartments in native legumes collected during the rainy and dry seasons

Species		Shoots		Roots		Leaves	
		Sugars mmol g^{-1} dry wt.	Total-N mg g^{-1} dry wt.	Sugars mmol g^{-1} dry wt.	Starch mmol g^{-1} dry wt.	Chlorophyll mg g^{-1} dry wt.	Protein mg g^{-1} fresh wt.
Caesalpiniaceae							
C. bauhiniaefolia	R	0.26b	7.0df	0.17a	0.16b	2.9de	48.9c
	D	0.30ab	6.1ef	0.38a	0.17b	nd	nd
C. calycioides	R	0.29ab	6.5ef	0.17a	0.28b	1.9de	109.2b
C. flexuosa	R	0.24bc	9.1be	0.17a	0.37b	1.5e	231.7a
	D	0.15c	8.1bf	0.32a	0.32b	1.5e	138.9b
C. hispidula	R	0.14c	6.5ef	nd	nd	10.7a	nd
	D	0.21bc	7.5cf	0.34a	0.45ab	9.5b	67.9c
C. rotundifolia	D	0.19bc	8.5bf	0.33a	0.32b	7.7b	nd
C. serpens	R	0.40a	8.1bf	nd	nd	3.3ce	23.9c
	D	0.29ab	7.4df	0.27a	0.20b	6.3bc	43.9c
C. tetraphila	R	0.16bc	5.3f	0.24a	0.42b	6.1bc	36.9c
	D	0.29ab	11.8a	0.30a	0.80a	7.5b	65.3c
Fabaceae							
A. brasiliana	R	0.19cd	8.7bf	0.08e	0.57dg	5.8ac	124.6bc
A. evenia	R	0.31ab	10.9ab	nd	nd	nd	nd
A. hystrix	D	0.13d	8.2bf	0.19ce	0.41eg	7.3ab	114.5bd
A. paniculata	R	0.13d	8.0bf	0.09e	0.31eg	3.1bd	42.4de
Aeschynomene sp.	R	0.22cd	10.1ac	0.14de	0.32eg	4.3bd	41.7de
C. mucunoides	R	0.23cd	10.9ab	0.30be	0.42eg	5.0bd	33.7e
	D	0.16cd	13.8a	0.40ae	0.59dg	2.0cd	63.8de
C. venosum	R	0.47a	12.4a	0.92a	1.5ab	nd	nd
C. guianensis	R	0.38ab	7.5cf	0.57ab	1.7a	6.6ac	35.4e
	D	0.33ab	7.3df	0.55ac	1.2ac	nd	nd
C. stipularis	R	0.29bc	8.8bf	0.50ac	0.89cf	4.1bd	31.1e
	D	0.38ab	4.9f	0.49ae	1.4ab	nd	nd
D. barbatum	R	0.19cd	7.0df	0.17de	0.55dg	1.5d	139.5bc
	D	0.17cd	7.8cf	0.23be	0.57dg	6.0ac	143.2ab
E. crinitum	D	0.19cd	6.7ef	0.36be	0.47dg	4.4bd	218.9*
E. rufum	R	0.19cd	5.3f	0.25be	0.25eg	nd	nd
	D	0.28bc	8.1bf	0.48ad	0.25eg	nd	nd
E. simplicifolium	R	0.28bc	8.2bf	0.28be	0.23fg	10.1a	40.2de
	D	0.19cd	6.8df	0.22ce	1.8a	nd	nd
G. jussieuana	R	0.15cd	7.8cf	0.19ce	0.83cg	4.8bd	36.7e
	D	0.18cd	9.4be	0.33be	0.90ce	4.6bd	85.5ce
I. pascuorum	R	0.30ab	6.3ef	0.18ce	0.73cg	nd	nd
	D	0.13d	4.3f	0.22ce	0.50dg	nd	nd
P. diversifolius	R	0.28bc	7.1df	0.39be	0.50dg	7.0ac	27.1e
P. gracilis	R	0.21cd	6.9df	0.79a	1.1bd	2.9bd	86.2be
	D	0.27bc	9.9bd	0.37be	0.42eg	7.2ab	92.9bd
Phaseolus sp.	R	0.45a	11.6a	0.10e	0.88cf	nd	33.1e
S. capitata	D	0.41a	9.6bd	0.55ac	1.2ac	nd	nd

Table 7. (Continued)

Species		Shoots Sugars mmol g^{-1} dry wt.	Shoots Total-N mg g^{-1} dry wt.	Roots Sugars mmol g^{-1} dry wt.	Roots Starch mmol g^{-1} dry wt.	Leaves Chlorophyll mg g^{-1} dry wt.	Leaves Protein mg g^{-1} fresh wt.
S. guianensis	R	0.49a	nd	nd	nd	7.1ab	41.7de
	D	0.21cd	6.2ef	0.49ad	0.24eg	5.6bd	69.4de
Stylosanthes sp.	R	0.29bc	10.7ac	0.38be	0.77cg	11.0a	81.7ce
	D	0.24cd	9.9bd	0.40ae	0.87cg	4.1bd	75.4de
T. sessiliflora	R	0.22cd	4.3f	0.28be	0.12fg	nd	nd
Z. curvata	R	0.18cd	10.1ac	0.30be	1.7a	3.9bd	56.9de
	D	0.21cd	12.7a	0.36be	0.56dg	4.6bd	51.1de
Mimosaceae							
M. camporum	R	0.27a	6.9df	0.28c	0.32ab	12.9a	112.8bd
	D	0.15b	6.9df	0.18de	0.57a	7.0bc	232.2a
M. debilis	R	0.16b	8.4bf	0.09f	0.14b	3.9c	71.2de
	D	0.20ab	7.7cf	0.24cd	0.30b	4.7c	155.1b
M. martensis	R	0.19b	8.0bf	0.13ef	0.24b	9.4ab	75.3ce
	D	0.23ab	11.4a	0.75a	0.48a	5.0bc	135.1bc
M. pudica	R	0.22ab	6.5ef	0.07f	0.37a	nd	nd
	D	0.20ab	13.7a	0.25cd	0.72a	4.8bc	44.6e

For individual plant subfamilies, numbers in columns followed by the same letters are not statistically different at $P< 0.05$. R, rainy season; D, dry season, nd not determined, *, number not included in ANOVA.

Table 8. Statistical comparison of means of different plant variables for all native legume species measured during the rainy and dry seasons

Variable	Variable	Means	
	Rainy season		
RAU[1] in shoots	RAU in roots	39.5	42.5 (ns)
RAU in shoots	RAU in xylopodia	39.5	28.5 (ns)
RAU in xylopodia	RAU in roots	28.5	42.5 (s)
Sugars[2] in shoots	Sugars in roots	0.2	0.2 (ns)
Starch[2] in roots	Starch in xylopodia	0.5	1.0 (s)
Sugars in roots	Sugars in xylopodia	0.2	0.5 (s)
	Dry season		
RAU in shoots	RAU in roots	16.5	16.3 (ns)
RAU in shoots	RAU in xylopodia	16.5	12.2 (ns)
RAU in xylopdia	RAU in roots	12.2	16.3 (s)
Sugars in shoots	Sugars in roots	0.3	0.3 (ns)
Starch in roots	Starch in xylopodia	0.5	0.9 (s)
Sugars in roots	Sugars in xylopodia	0.3	0.4 (ns)

[1]Relative abundance of ureides in %; [2]mmol of glucose g^{-1} dry wt.; (ns) means not statistically different at $P< 0.05$; (s) means statistically different at $P< 0.05$.

Table 9. Comparative analysis of the protein chlorophyll, relative abundance of ureides (RAU) and total-N content between subfamilies of native legume and between the rainy and dry season

	Rainy	Dry	Rainy	Dry
	Protein		Chlorophyll	
Subfamily	μg^{-1} fresh wt.		μg^{-1} dry wt.	
Caesalpiniaceae	90.1a*	81.0a (ns)	4.4b	5.9a (ns)
Fabaceae	56.8b	101.6a (s)	5.5b	5.5a (ns)
Mimosaceae	86.1b	128.0a (ns)	8.7a	6.4a (ns)
	RAU %		Total-N mg g^{-1} dry wt.	
Caesalpiniaceae	37.7b	15.3b (s)	9.1s	9.2a (ns)
Fabaceae	43.6a	24.1a (s)	9.0a	9.6a (ns)
Mimosaceae	34.4b	17.4b (s)	7.9b	8.7a (ns)

* Numbers in columns for individual plant variables followed by the same letter are not statistically different at $P< 0.05$.
(ns) means not statistically different at $P< 0.05$.
(s) means statistically different at $P< 0.05$.

respectively. Nitrate content in either shoots or roots significantly increased up to 48 μmol g^{-1} dry wt. during the dry season. Statistical analysis of data did not indicate significant differences between shoots and roots with regard to nitrate content. There was a general tendency of native species with low RUC to present higher RNC (Fig. 1). The inverse relationship between RUC and RNC was more evident at the driest months of the year.

4. Discussion

The native legumes collected during the rainy season were mainly at the vegetative stages of growth. At this time of the year the statistical comparison of RAU among native legumes allowed the classification of all *Chamaecrista* species as intermediate N_2-fixers (RAU 30–59%), while good N_2-fixers (RAU>60%) were found only in the Fabaceae subfamily. Outstanding symbiotic N_2-fixation rates were recorded in *Aeschynomene* sp., *C. mucunoides*, *P. diversifolius* and *P. gracilis*, the highest RAU being detected in *C. pascuorum*. This species has been considered to be one of the most promising forage legume in Nigeria (Tarawali, 1991). Within Fabaceae and Mimosaceae, *A. brasiliana, A. evenia, A. hystrix, A. paniculata, C. venosum, C. guianensis, D. barbatum, E. crinitum, E. rufum, E. simplicifolium, Phaseolus* sp., *S. guianensis, Stylosanthes* sp., *Z. curvata, M. debilis* and *M. martensis* showed intermediate rates of N_2-fixation. *C. stipularis,*

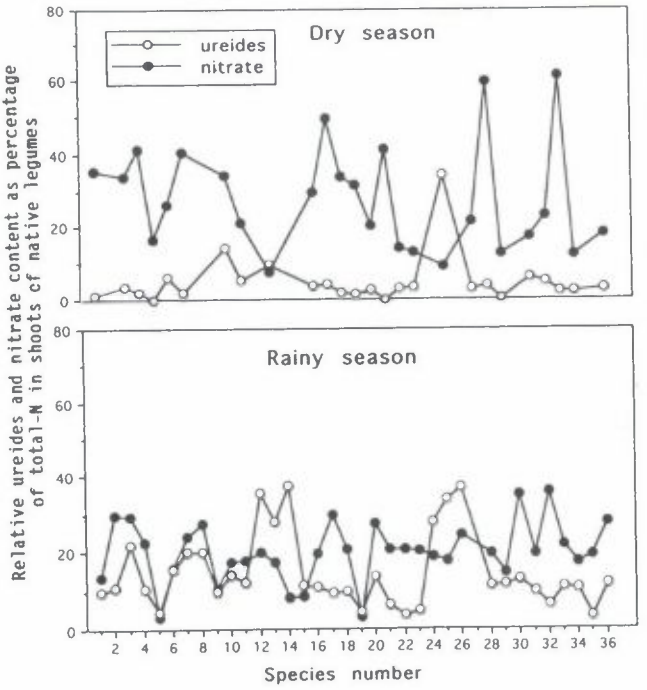

Figure 1. Relative ureides and nitrate content as percentage of total-N in shoots of native legumes collected in a tropical savanna during the dry and rainy seasons. Species number correspond to Table 3.

G. jussieuana, *I. pascuorum*, *T. sessiliflora*, *M. camporum*, *M. orthocarpa* and *M. pudica* were classified as low N_2-fixers (RAU<30%).

The dry season affected the species composition and the physiognomy of the savanna as evidenced by the 25% reduction in the number of native legume species, with 62% of the total individuals in flowering or fruiting stages. Most of the individuals were characterized by having an underground xylopodium. The occurrence of xylopodia constitute an advantage for plants subjected to water stress due to the enlarged capacity for storage of non-structural carbohydrates, as previously suggested (Medina and Bilbao, 1991). However, the present investigation did not reveal clear relationships between the plant's ability to withstand drought and the type of underground organs presented by individual species. Rather, a production of small diameter lateral roots capable of being nodulated and of penetrating finer water-containing soil pores, appears

to be the most efficient approach for plants subjected to long-term water deficit (Smucker and Aiken, 1992).

Reduction in the number of nodulated species and the scarce nodulation on the roots were the most striking feature of legumes collected during the dry season. The proportion of decaying nodules far exceeded those with the characteristic internal red color. This observation agrees with previous reports indicating that the amount of effective nodular tissues is the main constraint for symbiotic N_2-fixation in water-stressed plants (Kirda et al., 1989). With regard to nodulation, Mimosaceae species were the most affected by the dry season, since none of the collected individuals presented active nodules on the analysed roots. Only the outer cortex of nodules was observed. Nevertheless, by digging into deeper soil horizons it was possible to observe on *M. pudica* and *M. martensis*, nodules on root regions located below 20 cm from the sand surface. This area of the root system was not included in the standardized volume of soil sampled. Therefore, the possibility exists that a similar situation occurs in other native species. An increase in nodule mass at soil depth below 10 cm has been described in clover plants growing in drying soils (Davey et al., 1989). In cowpea, nodules at greater soil depths are as efficient as those located near the crown (Sicardi de Mallorca and Izaguirre-Mayoral, 1993).

The comparison of RAU values for the rainy and dry seasons permitted the categorization of native legume species as non-tolerants, tolerants or insensitive to drought in terms of symbiotic N_2-fixation. Non-tolerant species were those in which drought induced a 70–100% reduction in RAU such as *C. bauhiniaefolia, C. flexuosa, C. hispidula, C. rotundifolia, C. tetraphila, C. stipularis, D. barbatum, E. crinitum, E. simplicifolium, S. guianensis, Stylosanthes* sp., and *M. debilis*. On the other hand, *C. serpens, A. paniculata, C. guianensis, E. rufum, Z. curvata, M. camporum, M. martensis* and *M. pudica*, with an average 50% reduction in RAU, were considered as drought-tolerant. Poor drought tolerance has been recognized in several native forage legumes (Tarawali, 1991; Grof, 1991). Finally, *A. hystrix, C. mucunoides, G. jussieuana, I. pascuorum*, and *P. gracilis* sustained rates of symbiotic N_2-fixation similar to that measured during the rainy season. Therefore, these species were classified as drought-insensitive. At present, no relationships could be detected between the ability of certain species to withstand drought and the N_2-fixation rates displayed by those species during the rainy season. Species such as *C. tetraphila, G. jussieuana, I. pascuorum, S. capitata* and *Z. curvata*, always found in the savanna, according to IVI values, presented intermediate or even low RAU. On the other hand, individuals of *A. brasiliana, A. evenia, Aeschynomene* sp., *C. pascuorum, C. venosum* and *P. diversifolius* with relatively high or intermediate RAU

could not be perceived during the dry season, in spite of the exhaustive survey of the savanna sites.

The insensitivity to water stress has been associated with delayed leaf senescence (Gwathmey and Hall, 1992), decreased leaf area (Rambal, 1993), and the plant capability to maintain stomata open and higher leaf relative water content at low soil water potential (Shimshi et al., 1982; Robin et al., 1989; Minguez and Sau, 1989; Tardieu and Davies, 1993). Such plant adaptative attributes might have contributed to the subsistence of nodule functions under drought via preservation of the leghaemoglobin, a requisite for nitrogenase activity. On the other hand, the negative influence of drought on nodule activity did not seem to be the result of decreased leaf photosynthetic rates (Durand et al., 1987), or photosynthate production. This assumption is strengthened by the non-significant differences between seasons with regard to plant non-structural carbohydrate and chlorophyll content. Recently, a feedback inhibition of N_2-fixation by the end fixation products has been proposed as the mechanisms underlying water stressed nodules (Streeter, 1993).

The complexity of the response of native legumes to drought was further evidenced in the general tendency of native legumes to present higher nitrate levels during the dry season, with no differences in the nitrate partitioning between roots and shoots. The higher nitrate content detected in water-stressed plants could be a consequence of the increased nitrate levels reported in drying soils (Giambiagi et al., 1993). Thus, based on these observations it is tempting to speculate that native species with low or nil rates of N_2-fixation could activate the inducible nitrate reductase (Hoff et al., 1992), as an alternative way to fix soil nitrogen during the dry season. This proposed metabolic switch from symbiotic N_2-fixation to nitrate reduction in water-stressed native species is supported by: (a) the significant negative correlation, albeit weak, detected between RUC and RNC ($r = 0.30$, $P < 0.01$) in native species collected during the dry months; (b) the non-seasonal effect of drought on plant total-N content: (c) the higher nitrate levels measured in non-nodulated when compared to nodulated plants (Goto et al., 1987; Peoples et al., 1989; Thomas et al., 1980); (d) the inverse relationship displayed by nitrate reductase and nitrogenase activities in some cultivated legumes (Harper and Hageman, 1972; Franco et al., 1979); (e) the positive and negative $\delta^{15}N$ values reported for different nodulated legume species (Medina and Bilbao, 1991; Pate et al., 1993); and (f) the knowledge that tropical legumes carry out a substantial proportion of their nitrate assimilation in roots and shoots (Oaks, 1992). According to data obtained on soybean (Raper et al., 1991), nitrogen uptake by roots of native legumes should not be affected by soil

pH within a range of 4.5 to 6.0, or by water stress (Kirda et al., 1989), although there is a report on inhibition of nitrate reductase by water stress in alfalfa (Aparicio-Tejo and Sánchez-Diaz, 1982). On the other hand, the significant positive correlation between RUC and RNC ($r = 0.34$, $P < 0.01$) in native legume species collected during the rainy season might suggest nitrate reductase activity also in well-watered plants with intermediate or low rates of N_2-fixation. This positive cooperation of symbiotic N_2-fixation and nitrate reduction processes has been proposed for legumes growing under limited soil nitrogen supply (Franco et al., 1979; Harper and Hageman, 1972). Certainly, the contribution of the nitrate reductase to the nitrogen economy of native legumes requires further investigation.

From the present data, three major conclusions can be drawn: (1) the apparent non-relationship between RUC reduction in species non-tolerant or tolerant to drought and the plant non-structural carbohydrates, chlorophyll or protein content suggest a greater sensitivity of nodules to water stress, when compared to that of the host. This finding corresponds with previous observations (Guerin et al., 1990; Irigoyen et al., 1992). Decreased N_2-fixation rates was attributed mainly to a combination of nodules senescence and shedding; (2) the effect of drought on RUC but not on RAC could be considered, at present, as an indicator of the ureide producer status of all sampled native legumes. The observed significant differences in RAU values between seasons further justify the use of the ureide technique for detecting and quantifying the symbiotic N_2-fixation in native legumes (Izaguirre-Mayoral et al., 1992). Green shoots can be used for symbiotic N_2-fixation assessment in native legumes, since RUC and RNC did not statistically differ between roots and shoots; and (3) this study identified several Fabaceae species as well-adapted to seasonal drought, with good rates of N_2-fixation during the dry season. *C. mucunoides* and *P. gracilis* can be considered as promising sources of germplasms in tropical savannas.

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