

New Insights into the Symbiotic Performance of Native Tropical Legumes: I. Analysis of the Response of Thirty-Seven Native Legume Species to Artificial Shade in a Neotropical Savanna

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Abstract

Several physiological parameters related to symbiotic N₂-fixation were analysed in native legume species subjected to an artificial 75% reduction of the incident sunlight in a neotropical savanna. To insure a full growing cycle of legumes under artificial shade, three savanna areas were totally covered with a black greenhouse net from the beginning of the dry season up to the end of the following rainy season. Analyses carried out at the end of the rainy season indicated that shade did not hamper nodulation in any of the species in spite of the smaller size of native rhizobial populations in shaded soils, but reduced by 71%, 62% and 60% the number of Caesalpiniaceae, Fabaceae and Mimosaceae species, respectively. According to vegetation inventories, native legume species absent in shaded areas were categorized as shade-intolerant. By contrast, *Chamaecrista tetraphila*, *C. rotundifolia*, *Aeschynomene brasiliana*, *Desmodium barbatum*,

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Eriosema simplicifolium, *Galactia jussieuana*, *Indigofera lespedezioides*, *Phaseolus diversifolius*, *Stylosanthes capitata*, *S. guianensis*, *Zornia curvata*, *Mimosa camporum* and *M. debilis*, present in shaded as well as in full sun-exposed areas, were classified as facultative shade-tolerant species. *Desmodium mollicullun* was considered, at present, as the only shade-obligate species due to the total absence of individuals in full sun-exposed areas. Based on the values of relative abundance of ureides (RAU), *C. tetraphila*, *A. brasiliana*, *E. simplicifolium*, *G. jussieuana*, *I. lespedezioides*, *S. guianensis*, *M. camporum* and *M. debilis* were further classified as species that benefit from shade, since shade significantly enhanced RAU in all individuals. *C. rotundifolia*, *P. diversifolius*, *S. capitata* and *Z. curvata* were categorized as shade-insensitive due to the similar RAU values in shaded and full sun-exposed individuals. The reduced RAU displayed by shaded individuals of *D. barbatum* indicated the symbiotically shade-intolerance of this species. For all tested native species, shade significantly decreased the chlorophyll, leaf starch and shoot nitrate content. In contrast, shade significantly increased RAU and the shoot ureide:nitrate ratio of the plants. Between shaded and full sun-exposed individuals there were no significant differences in the ureide, α -amino-N, leaf total reducing sugars and either total reducing sugars or starch content in roots. From present data it might be concluded that native species considered to be good N₂-fixers at full sunlight were unable to grow at low light, and only those species with a spare capacity to enhance their symbiotic process were capable to exploit shaded habitats.

Keywords: Symbiotic nitrogen fixation, native legumes, shade-tolerant

1. Introduction

The efficiency of the symbiotic process in legumes is dependent on the host characteristics and rhizobial strains, and may be limited by the environmental conditions at which nodulated plants are grown. Seasonal drought, high temperatures, acid soil complex of high aluminium saturation and low soil fertility have been considered among the most important factors hindering the symbiotic N₂-fixation in either cultivated or native legume species (Izaguirre-Mayoral et al., 1992a; Sicardi de Mallorca and Izaguirre-Mayoral, 1993; Bordeleau and Prévost, 1994). Recent reports have shown that light conditions also influence the symbiotic efficiency of cultivated legumes via an effect on the rate of synthesis and allocation of photoassimilates to nodulating roots (Kasperbauer and Hunt, 1994). Dark-induced changes in the functioning of stem nodules have been described in *Sesbania rostrata* (Parsons et al., 1993), of root nodules in *Vigna unguiculata* (Swaraj et al., 1988), and in *Trifolium repens* as well as in some tropical pasture legumes (Whiteman and Lulham, 1970; Eriksen and Whitney, 1982). The observed impairment of both growth and nodule

activity under low light conditions were probably a consequence of reduced carbohydrate availability in roots of those nodulated legumes (James et al., 1993).

The capacity of plants to tolerate shade has been well recognized in a number of non-legume species native of a wide variety of climates (Medina et al., 1991; Osunkoya et al., 1994; Kelly et al., 1994; Kitajima, 1994). However, a number of tree legume species are capable to grow under the shade conditions encountered in tropical forests and have been categorized as shade tolerant (Lovelock et al., 1994; Osunkoya et al., 1994). Among those legumes, the efficiency of the symbiotic process has been determined solely in the understory forest species *Acacia pulchella*, *Acacia alata* (Hansen and Pate, 1987), *Bossiaea laidlawiana* (Grove and Malajczuk, 1992) and *Dioclea guianensis* (Izaguirre-Mayoral and Flores, 1995). For commercially cultivated legumes *Pueraria lobata* constitutes, so far, the only species categorized as shade-tolerant. Under mild shading, this species showed relatively higher activity of N₂-fixation than *Centrosema pubescens*, *Macroptilium atropurpureum* and *Glycine max* plants subjected to similar shaded conditions (Fujita et al., 1993).

On the other hand, the possible effect of contrasting light intensities on the symbiotic performance of legumes native of tropical savannas has not been reported. Also, the possibility of a shade-tolerance or the flexibility of the symbiotic N₂-fixation process under different light environments have not been defined in annual native legume species. Therefore, the present study was conducted to analyze the symbiotic activity and the physiology of native legume species growing in a neotropical savanna at either high or at artificial low light conditions. The description and the comparative analysis of native legumes growing under full sunlight and in artificially shaded areas offered an opportunity to answer the following questions: 1) Is the magnitude of the symbiotic process in native legumes modulated by light?, 2) Can native legume species be categorized as shade-tolerant?, and 3) Can native legume species benefit from low light habitats?

2. Material and Methods

Experimental design and soil characteristics

The present study was carried out in a *Trachypogon* savanna located at the Estación Experimental La Iguana, Universidad Experimental Simón Rodríguez, Guárico State, Venezuela (8° 25'N, 65° 24'W). The limits of the experimental areas were confined within the previously denoted soil site 2 (Sicardi de

Mallorca and Izaguirre-Mayoral, 1994), characterized by the highest importance value index for native legumes when compared to other savanna sites. In this soil site, three areas of 20 m² each were randomly delimited and covered with a black greenhouse net at the beginning of the dry season (January) in 1994. The nets were placed at 1.5 m above ground with closed lateral sides to avoid the penetration of reflected sunlight. Nets were removed toward the end of the rainy season (September) of 1994. The allocation of black nets at the beginning of the dry season insured that all legume individuals sampled during the subsequent rainy season grew under constant shade from the time of seed germination or from the beginning of the regrowth process induced by rains. At the time of net removal, the maximum light intensity at noon time in the full sun-exposed areas and under the nets were 1900 and 480 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. Thus, nets caused a 75% reduction in the incident sunlight. The light intensity was measured with a Quantum/Radiometer/Photometer Model LI-185B Licor Inc. Concomitantly, triplicate soil samples to a depth of 20 cm were collected, at random, in representative areas of the shaded and full sun-exposed areas of the savanna. The physicochemical analyses of soils were performed on dried subsamples as described in Izaguirre-Mayoral et al. (1992a).

Analysis of soil native rhizobial populations

The size of native rhizobial populations was determined in triplicate soil samples collected in shaded and full sun-exposed areas of the neotropical savanna. 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 *Macropitium atropurpureum* (Sicardi de Mallorca and Izaguirre-Mayoral, 1994). Results are expressed as the log₁₀ of the most probable number of native rhizobia (MPNR) g⁻¹ soil dry wt.

Plant material and determination of plant parameters

For the present work only those species concomitantly collected on the shaded and full sun-exposed areas of the neotropical savanna were the subject of analyses. For individual legumes, the entire above-ground biomass and underground organs (roots and nodules) contained in a soil volume of approximately 0.027 m³ were harvested at both full sun-exposed and artificially shaded areas. Plants were analyzed in terms of ureide (allantoin plus allantoic acid), α -amino-N, nitrate, chlorophyll, total reducing sugars and starch content as described in Izaguirre-Mayoral et al. (1992a). The

relative abundance of ureides (RAU) in shoots was used as an index of the symbiotic N_2 -fixation rate in the individual legume species. RAU was demonstrated to constitute a significant indicator of effective symbiosis in native legumes (Sicardi de Mallorca and Izaguirre-Mayoral, 1993, Sicardi de Mallorca and Izaguirre-Mayoral, 1995). Analysis of physiological parameters related to symbiotic N_2 -fixation were carried out on a minimum of 6 individuals for each of the native legume species. All collected individuals were adults in the vegetative stage of growth.

Statistical analyses

Data in percentage were transformed to degrees by angular transformation. Statistical differences for individual plant parameters, between species collected in artificially shaded or in full sun-exposed areas, were analyzed 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 experimental areas was carried out using the student' *t* test.

3. Results

Soil conditions and vegetation inventories

The texture of the savanna soil was sandy (90% sand), with a pH of 5.3. Chemical composition of soils was: available P, $0.33 \mu\text{g g}^{-1}$ soil; Mg, $0.46 \text{ cmol kg}^{-1}$ soil; K, $0.03 \text{ cmol kg}^{-1}$ soil; Ca, $1.12 \text{ cmol kg}^{-1}$ soil and 0.53 mg N g^{-1} soil. Effective soil cation exchange capacity was $1.61 \text{ cmol kg}^{-1}$ soil. Percent aluminium saturation was 2.4. Soil temperatures in the shaded and full sun-exposed areas at the end of the rainy season were 27°C and 28.6°C , respectively. The soil water content was 9.2%, regardless of the location.

Shade decreased the size of native rhizobial populations from 4 in soils collected in full sun-exposed areas to $1.9 \log_{10}$ MPNR g^{-1} dry soil. Shade also caused a 39% reduction in the number of native legume species detected in full sun-exposed areas (Table 1) and inhibited the growth of all grasses (not shown). Only two Caesalpinaceae, nine Fabaceae and two Mimosaceae species were simultaneously present in shaded and full sun-exposed areas. *D. mollicullum* was detected exclusively in the shaded areas. All individuals collected at both experimental areas were nodulated.

Table 1. Inventory of native legume species growing under artificial shade and in full sun-exposed areas of a neotropical savanna.

Species reference number*		Full sun	Shade
Caesalpinaceae			
1	<i>Chamaecrista bauhiniifolia</i> Benth.	+	-
2	<i>Chamaecrista calycioides</i> (DC.) Er.	+	-
3	<i>Chamaecrista flexuosa</i> (Moench) DC.	+	-
4	<i>Chamaecrista hispidula</i> (L.) Vahl.	+	-
5	<i>Chamaecrista rotundifolia</i> Benth.	+	+
6	<i>Chamaecrista serpens</i> L.	+	-
7	<i>Chamaecrista tetraphila</i> Mart.	+	+
Fabaceae			
8	<i>Aeschynomene brasiliiana</i> (Poir.) DC.	+	+
9	<i>Aeschynomene evenia</i> Wright	+	-
10	<i>Aeschynomene hystrix</i> Poir.	+	-
11	<i>Aeschynomene paniculata</i> Willd.	+	-
12	<i>Calopogonium mucunoides</i> Desv.	+	-
13	<i>Centrosema pascuorum</i> Mart.	+	-
14	<i>Centrosema venosum</i> Mart.	+	-
15	<i>Clitoria guianensis</i> (Aubl.) Benth.	+	-
16	<i>Crotalaria stipularis</i> Desv.	+	-
17	<i>Desmodium barbatum</i> (L.) Benth.	+	+
18	<i>Desmodium mollicullum</i> (H.B. & K.) DC.	-	+
19	<i>Eriosema crinitum</i> (H.B. & K.) Benth.	+	-
20	<i>Eriosema rufum</i> (H.B. & K.)	+	-
21	<i>Eriosema simplicifolium</i> (H.B. & K.) G. Don.	+	+
22	<i>Galactia jussieuana</i> Kunth.	+	+
23	<i>Indigofera lespedezioides</i> Benth.	+	+
24	<i>Indigofera pascuorum</i> Benth.	+	-
25	<i>Phaseolus diversifolius</i> Pittier	+	+
26	<i>Phaseolus gracilis</i> Poepp.	+	-
27	<i>Phaseolus</i> sp.	+	-
28	<i>Stylosanthes capitata</i> Vog.	+	+
29	<i>Stylosanthes guianensis</i> (Aubl.) Sw.	+	+
30	<i>Stylosanthes</i> sp.	+	-
31	<i>Tephrosia sessiliflora</i> (Poir.) Hassl.	+	-
32	<i>Zornia curvata</i> Mohl.	+	+
Mimosaceae			
33	<i>Mimosa camporum</i> Benth.	+	+
34	<i>Minosa debilis</i> (H. & B.)	+	+
35	<i>Mimosa martensis</i> Br. & Rose	+	-
36	<i>Mimosa orthocarpa</i> Spruce	+	-
37	<i>Mimosa pudica</i> L.	+	-

+ = Species present in the sampled area; - = Species absent in the sampled area.

*Species reference numbers as in Fig. 1.

Chlorophyll content in native species collected in shaded and full sun-exposed areas

Except for *M. debilis*, the chlorophyll content did not significantly differ among native species harvested in shaded areas (Table 2). In the full sun-exposed areas not all species were tested for chlorophyll content due to the reduced leaf area of many individuals. Nevertheless, statistical significant differences in chlorophyll content were detected between species collected in full sun-exposed areas. Within species with individuals concomitantly analysed in shaded and full sun-exposed areas, shade induced a significant reduction in the chlorophyll content in *C. tetraphila*, *Z. curvata* and *M. debilis*.

Table 2. Chlorophyll content in native legume species collected in artificially shaded and full sun-exposed areas of a neotropical savanna.

	Chlorophyll ($\mu\text{g cm}^{-2}$)	
	Shade	Full sun
Caesalpinaceae		
<i>C. rotundifolia</i>	7.4 \pm 1.4 ab	7.3 \pm 1.0 c (NS)
<i>C. tetraphila</i>	7.9 \pm 1.0 ab	16.7 \pm 1.2 a (S)
Fabaceae		
<i>A. brasiliana</i>	5.2 \pm 0.2 b	nd
<i>D. barbatum</i>	6.3 \pm 1.7 ab	nd
<i>D. mollicullum</i>	6.3 \pm 0.9 ab	nc
<i>E. simplicifolium</i>	9.1 \pm 1.4 a	11.9 \pm 1.9 b (NS)
<i>G. jussieuana</i>	9.9 \pm 1.2 a	12.4 \pm 1.8 b (NS)
<i>I. lespedezioides</i>	5.0 \pm 3.6 b	5.8 \pm 0.7 c (NS)
<i>P. diversifolius</i>	9.4 \pm 1.4 a	nd
<i>S. capitata</i>	6.5 \pm 2.7 ab	nd
<i>S. guianensis</i>	9.1 \pm 2.1 a	nd
<i>Z. curvata</i>	9.4 \pm 0.2 a	18.5 \pm 0.7 a (S)
Mimosaceae		
<i>M. debilis</i>	2.2 \pm 0.5c	7.7 \pm 0.3 c (S)

Numbers in columns followed by the same letter(s) are not statistically different at $p < 0.05$. Statistical significance at $p < 0.05$ between means is indicated by (S) = significant; (NS) = not significant; nd = not determined; nc = species not collected.

Non-structural carbohydrate content in leaves and roots of native species collected in shaded and full sun-exposed areas

There were significant differences in the content of total reducing sugars and starch in leaves (Table 3) and roots (Table 4) according to species and light intensity. *C. tetraphila* and *G. jussieuana* were the only species to show significant reduction in the total reducing sugar content in leaves induced by shade. Shade significantly decreased the starch content in leaves of *C. rotundifolia*, *A. brasiliensis*, *D. barbatum*, *S. guianensis*, *Z. curvata* and *M. debilis*. The comparative analyses of mean values showed a significant reduction in the total reducing sugar contents in roots of *P. diversifolius* and *M. camporum* subjected to artificial shade. Shade also induced a significant reduction in the root starch content in *G. jussieuana*, *S. guianensis*, *Z. curvata* and *M. camporum*. In contrast, shade significantly increased the starch content in roots of *D. barbatum* above values detected in full sun-exposed individuals.

Table 3. Total reducing sugar and starch content in leaves of native legume species collected in artificially shaded and full sun-exposed areas of a neotropical savanna.

	Total reducing sugars (mmol g ⁻¹ dry wt)		Starch (mmol g ⁻¹ dry wt)	
	Shade	Full sun	Shade	Full sun
Caesalpinaceae				
<i>C. rotundifolia</i>	0.5±0.1 b	0.3±0.09 de (NS)	0.2±0.02 c	0.4±0.1 b (S)
<i>C. tetraphila</i>	1.6±0.03 a	2.1±0.03 a (S)	0.5±0.02 a	0.7±0.2 a (NS)
Fabaceae				
<i>A. brasiliensis</i>	0.3±0.06 cd	0.5±0.1 cd (NS)	0.1±0.05 c	0.8±0.05 a (S)
<i>D. barbatum</i>	0.3±0.05 cd	0.3±0.03 e (NS)	0.1±0.03 c	0.3±0.02 b (S)
<i>D. mollicillum</i>	0.1±0.005 d	nc	0.1±0.01 c	nc
<i>E. simplicifolium</i>	0.2±0.02 cd	0.3±0.01 e (NS)	0.1±0.05 c	0.2±0.07 bc (NS)
<i>G. jussieuana</i>	0.2±0.01 d	0.7±0.06 b (S)	0.1±0.05 c	0.1±0.03 c (NS)
<i>I. lespedezioides</i>	0.5±0.04 b	0.5±0.05 c (NS)	0.1±0.04 c	0.3±0.02 b (NS)
<i>S. capitata</i>	0.4±0.03 bc	nc	0.2±0.04 bc	nc
<i>S. guianensis</i>	0.4±0.02 bc	0.4±0.02 cde (NS)	0.4±0.02 ab	0.6±0.02 b (S)
<i>Z. curvata</i>	0.3±0.02 cd	0.4±0.04 cde (NS)	0.1±0.01 c	0.3±0.04 b (S)
Mimosaceae				
<i>M. camporum</i>	0.2±0.03 d	0.2±0.05 e (NS)	0.1±0.05 c	0.1±0.02 bc (NS)
<i>M. debilis</i>	0.5±0.03 b	0.5±0.02 cd (NS)	0.2±0.06 bc	0.5±0.02 b (S)

Numbers in columns followed by the same letter(s) are not statistically different at $p < 0.05$. Statistical significance at $p < 0.05$ between means of individual plant parameters is indicated by (S) = significant; (NS) = not significant; nc = species not collected.

Table 4. Total reducing sugar and starch content in roots of native legume species collected in artificially shaded and full sun-exposed areas of a neotropical savanna.

	Total reducing sugars (mmol g ⁻¹ dry wt)		Starch (mmol g ⁻¹ dry wt)	
	Shade	Full sun	Shade	Full sun
Caesalpinaceae				
<i>C. rotundifolia</i>	0.1±0.01 d	0.2±0.03 d (NS)	0.1±0.03 c	0.1±0.02 d (NS)
<i>C. tetraphila</i>	0.4±0.01 a	0.4±0.01 bc (NS)	2.3±0.06 a	1.2±0.3 b (NS)
Fabaceae				
<i>A. brasiliana</i>	0.1±0.03 d	0.2±0.02 d (NS)	0.3±0.02 c	0.2±0.03 cd (NS)
<i>D. barbatum</i>	0.3±0.01 bc	0.2±0.04 d (NS)	0.2±0.04 c	0.08±0.02 d (S)
<i>D. mollicullum</i>	0.4±0.02 a	nc	nd	nc
<i>E. simplicifolium</i>	0.3±0.01 ab	0.4±0.05 b (NS)	1.2±0.07 ab	1.3±0.5 b (NS)
<i>G. jussieuana</i>	0.4±0.05 a	0.4±0.04 b (NS)	0.3±0.08 c	0.8±0.2 b (S)
<i>I. lespedezioides</i>	0.2±0.01 cd	0.3±0.03 bc (NS)	0.2±0.01 c	0.3±0.05 c (NS)
<i>P. diversifolius</i>	0.2±0.01 cd	0.4±0.02 b (S)	0.3±0.01 c	0.5±0.05 bc (NS)
<i>S. capitata</i>	0.3±0.03 bc	nd	0.2±0.04 c	nd
<i>S. guianensis</i>	0.1±0.01 d	0.1±0.01 d (NS)	0.2±0.04 c	0.8±0.06 b (S)
<i>Z. curvata</i>	0.2±0.01 cd	0.3±0.07 bc (NS)	0.3±0.02 c	2.1±0.2 a (S)
Mimosaceae				
<i>M. camporum</i>	0.4±0.02 a	0.7±0.01 a (S)	0.1±0.02 c	1.1±0.2 b (S)
<i>M. debilis</i>	0.2±0.02 cd	0.3±0.06 bc (NS)	0.1±0.07 c	0.2±0.04 cd (NS)

Numbers in columns followed by the same letter(s) are not statistically different at $p < 0.05$. Statistical significance at $p < 0.05$ between means of individual plant parameters is indicated by (S) = significant; (NS) = not significant; nd = not determined; nc = species not collected.

Ureide, α -amino-N and nitrate content in shoots of native species collected in shaded and full sun-exposed areas

Ureide and α -amino-N content significantly differed among native species growing either under shade or in full sun-exposed areas (Table 5). When compared to full sun-exposed individuals, shade induced a significant decreases in the ureide content in *D. barbatum* and *G. jussieuana*. Whereas, shade significantly increased the ureide content in *I. lespedezioides*. In contrast, the α -amino-N content was not significantly altered by shade in any of the native species. The nitrate content did not differ between shaded and full sun-exposed individuals of *D. barbatum*, *P. diversifolius* and *Z. curvata* (Fig. 1). For the rest of the species there was a marked reduction in the nitrate content in all individuals subjected to shade.

Table 5. Ureide and α -amino-N content in shoots of native legume species collected in artificially shaded and full sun-exposed areas of a neotropical savanna.

	Ureides ($\mu\text{mol g}^{-1}$ dry wt)		α -amino-N (mmol g^{-1} dry wt)	
	Shade	Full sun	Shade	Full sun
Caesalpinaceae				
<i>C. rotundifolia</i>	2.6 \pm 1.0 d	4.2 \pm 0.3 d (NS)	81.8 \pm 4.0 b	72.7 \pm 7.6 b (NS)
<i>C. tetraphila</i>	6.7 \pm 0.5 bc	5.0 \pm 0.6 d (NS)	125.8 \pm 15.1 ab	97.0 \pm 7.3 b (NS)
Fabaceae				
<i>A. brasiliana</i>	19.6 \pm 3.2 a	14.6 \pm 3.9 c (NS)	66.9 \pm 19.8 b	45.9 \pm 8.4 c (NS)
<i>D. barbatum</i>	4.1 \pm 0.3 c	16.1 \pm 0.4 c (S)	69.0 \pm 17.2 b	63.4 \pm 6.8 b (NS)
<i>D. mollicullum</i>	9.3 \pm 2.3 b	nc	266.1 \pm 26.2 a	nc
<i>E. simplicifolium</i>	7.2 \pm 1.2 bc	6.3 \pm 0.2 d (NS)	102.5 \pm 9.9 b	109.0 \pm 9.2 b (NS)
<i>G. jussieuana</i>	23.0 \pm 2.1 a	52.7 \pm 17.0 b (S)	104.2 \pm 36.2 b	113.6 \pm 21.9 b (NS)
<i>I. lespedezioides</i>	16.3 \pm 8.3 a	4.3 \pm 0.8 d (S)	140.4 \pm 30.0 b	113.6 \pm 9.8 b (NS)
<i>P. diversifolius</i>	17.9 \pm 1.7 a	17.8 \pm 3.6 a (NS)	204.1 \pm 20.4 a	216.0 \pm 24.6 a (NS)
<i>S. capitata</i>	4.6 \pm 0.1 c	5.0 \pm 0.5 d (NS)	57.1 \pm 8.9 b	108.0 \pm 14.6 b (NS)
<i>S. guianensis</i>	6.0 \pm 0.2 bc	6.6 \pm 0.3 d (NS)	58.6 \pm 10.2 b	41.6 \pm 4.9 c (NS)
<i>Z. curvata</i>	7.7 \pm 0.7 bc	7.3 \pm 0.5 cd (NS)	84.0 \pm 8.4 b	126 \pm 26.0 b (NS)
Mimosaceae				
<i>M. camporum</i>	7.5 \pm 0.2 bc	7.6 \pm 0.6 d (NS)	94.8 \pm 7.8 b	72.5 \pm 7.5 b (NS)
<i>M. debilis</i>	6.4 \pm 0.5 bc	9.7 \pm 0.9 d (NS)	53.4 \pm 6.2 b	57.6 \pm 5.0 b (NS)

Numbers in columns followed by the same letter(s) are not statistically different at $p < 0.05$; Statistical significance at $p < 0.05$ between means of individual plant parameters is indicated by (S) = significant; (NS) = not significant; nc = species not collected.

Relative abundance of ureides (RAU)

The calculated RAU values in shaded and full sun exposed native species are summarized in Table 6. Among the species subjected to artificial shade *C. tetraphila*, *A. brasiliana*, *D. mollicullum*, *G. jussieuana*, *I. lespedezioides*, *M. camporum* and *M. debilis* showed the highest RAU values. In the full sun-exposed areas the highest RAU value was detected in *P. diversifolius*. The shade conditions significantly increased RAU in *C. tetraphila*, *A. brasiliana*, *E. simplicifolium*, *G. jussieuana*, *I. lespedezioides*, *S. guianensis*, *M. camporum*

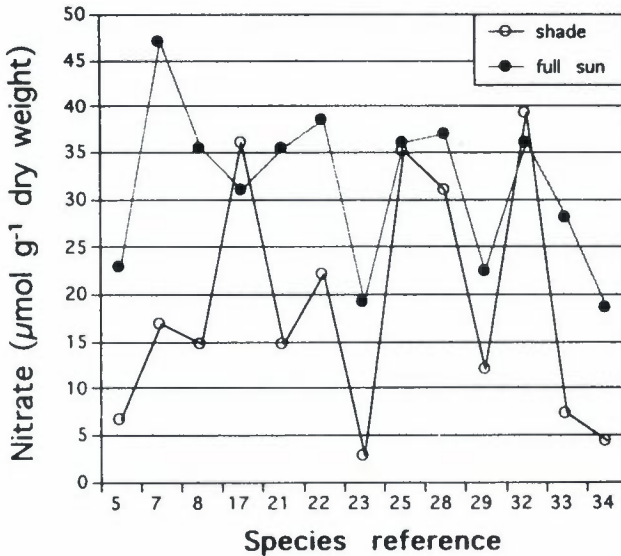


Figure 1. Nitrate content in shoots of native legume species collected in shaded and full sun-exposed areas of neotropical savanna. Species reference numbers correspond to Table 1.

and *M. debilis* above values detected in individuals of the same species collected in full sun-exposed areas. In contrast, RAU values in *C. rotundifolia*, *P. diversifolius*, *S. capitata* and *Z. curvata* were not affected by the light conditions. A significant decrease was observed in *D. barbatum* subjected to shade.

Statistical comparison of means of different plant variables

The statistical comparison of mean for individual plant parameters showed that for all native legume species the chlorophyll, leaf starch and shoot nitrate content were significantly higher in full sun-exposed individuals (Table 7). In contrast, RAU and the ureide:nitrate ratio were significantly higher in shoots of individuals collected under shade. The ureide, α -amino-N, total reducing sugars in leaves and roots as well as the starch content in roots did not significantly differ between shaded and full sun-exposed individuals.

Table 6. Relative abundance of ureides (RAU) in native legume species collected in artificially shaded and full sun-exposed areas of a neotropical savanna.

	RAU (%)	
	Shade	Full sun
Caesalpinaceae		
<i>C. rotundifolia</i>	43.0±3.5 c	40.8±3.3 b (NS)
<i>C. tetraphila</i>	81.1±0.5 a	50.9±2.8 ab (S)
Fabaceae		
<i>A. brasiliana</i>	87.8±5.6 a	48.5±1.3 ab (S)
<i>D. barbatum</i>	28.7±4.2 d	44.4±4.4 ab (S)
<i>D. mollicullum</i>	77.2±7.7 b	nc
<i>E. simplicifolium</i>	65.5±3.5 b	41.7±0.9 b (S)
<i>G. jussieuana</i>	82.3±3.5 a	13.8±1.3 c (S)
<i>I. lepedezioides</i>	92.8±3.3 a	46.5±4.2 ab (S)
<i>P. diversifolius</i>	52.3±5.2 b	58.3±5.3 a (NS)
<i>S. capitata</i>	37.4±0.3 c	30.8±3.8 b (NS)
<i>S. guianensis</i>	51.7±5.1 b	35.2±3.6 b (S)
<i>Z. curvata</i>	39.3±3.9 c	43.9±4.4 b (NS)
Mimosaceae		
<i>M. camporum</i>	80.3±0.4 a	15.9±2.3 c (S)
<i>M. debilis</i>	85.3±0.9 a	36.4±3.6 b (S)

Numbers in columns followed by the same letter(s) are not statistically different at $p < 0.05$. Statistical significance at $p < 0.05$ between means is indicated by (S) = significant; (NS) = not significant; nc = species not collected.

4. Discussion

The marked reduction in the number of native legume species and the disappearance of all C_4 -species constituted the first evidences of the effect of shade on the savanna ecosystem. When compared to the legume physiognomy of full sun-exposed areas, shade caused a 71%, 62% and 60% reduction in the number of Caesalpinaceae, Fabaceae and Mimosaceae species, respectively, suggesting a greater effect of shade on Caesalpinaceae than on Fabaceae and Mimosaceae species. On the other hand, the phenotype of legumes growing under shade did not undergo major alterations (not shown), and chlorosis was not observed in any of the individuals in spite of the significant decreased chlorophyll content in *C. tetraphila*, *Z. curvata* and *M. debilis*.

Table 7. Statistical comparison of means of different plant variables for all individuals of native legume species collected under artificial shade and in full sun-exposed areas of the neotropical savanna.

Variables	Shade	Full sun
RAU ¹ , %	68.6	39.0 (S)
Ureide, $\mu\text{mol g}^{-1}$ dry wt	9.9	14.6 (NS)
α -amino-N, mmol g^{-1} dry wt	102.9	91.8 (NS)
Nitrate, $\mu\text{mol g}^{-1}$ dry wt	13.9	30.5 (S)
Ureide:nitrate ratio, $\mu\text{mol g}^{-1}$ dry wt	1.16	0.32 (S)
Chlorophyll, $\mu\text{g cm}^{-2}$	7.2	10.2 (S)
Total reducing sugars in leaves, mmol g^{-1} dry wt	419.1	591.7 (NS)
Total reducing sugars in roots, mmol g^{-1} dry wt	284.4	330.7 (NS)
Starch in leaves, mmol g^{-1} dry wt	199.7	448.2 (S)
Starch in roots, mmol g^{-1} dry wt	567.9	522.4 (NS)

¹Relative abundance of ureides. Statistical significance at $p < 0.05$ between means of individual plant parameters is indicated by (S) = significant or (NS) = not significant.

The comparative analysis of the vegetation inventories carried out at both experimental areas allowed the classification of *C. bauhiniæfolia*, *C. calycioides*, *C. flexuosa*, *C. hispidula*, *C. serpens*, *A. evenia*, *A. hystrix*, *A. paniculata*, *C. mucunoides*, *C. pascuorum*, *C. venosum*, *C. guianensis*, *C. stipularis*, *E. crinitum*, *E. rufum*, *I. pascuorum*, *P. gracilis*, *Phaseolus sp.*, *Stylosanthes sp.*, *T. sessiliflora*, *M. martensis*, *M. orthocarpa* and *M. pudica* as shade-intolerant species due to their absence in all shaded areas. In contrast, *C. tetraphila*, *C. rotundifolia*, *A. brasiliana*, *D. barbatum*, *E. simplicifolium*, *G. jussieuana*, *I. lespedezioides*, *P. diversifolius*, *S. capitata*, *S. guianensis*, *Z. curvata*, *M. camporum* and *M. debilis* were categorized as facultative shade-tolerant species capable to exploit habitats with low light intensity. From the present survey *D. mollicullun* could be considered as the only shade-obligate species due to the total absence of individuals in all full sun-exposed areas of the neotropical savanna under study. The classification of this species is at present dubious since *D. mollicullun* was previously reported in a *Trachypogon* savanna of Venezuela (Barrios and Gonzalez, 1971). Nevertheless, these authors did not indicate the location of the savanna and there are no specification of the spatial recruitment of this species. No explanation could be given for the apparent sensitivity to high light displayed by this species.

The artificial 75% reduction of the incident sunlight did not hamper the nodulation process of any of the facultative shade-tolerant species. As previously observed in full sun-exposed areas (Sicardi de Mallorca and Izaguirre-Mayoral, 1993), determinate nodules were detected in all Fabaceae species and indeterminate in all Caesalpiniaceae as well as in Mimosaceae species subjected to shade. Shade also did not hinder the symbiotic performance of native legumes growing under the nets since the facultative shade-tolerant *C. tetraphila*, *A. brasiliana*, *E. simplicifolium*, *G. jussieuana*, *I. lespedezioides*, *M. camporum* and *M. debilis* as well as the shade-obligate *D. mollicullum* behaved as good N₂-fixers (RAU>60%), and the facultative shade-tolerant *C. rotundifolia*, *D. barbatum*, *P. diversifolius*, *S. capitata*, *S. guianensis* and *Z. curvata* were considered intermediate N₂-fixers (RAU 30–59%) under the shaded conditions. Low N₂-fixers (RAU<30%), however, were not detected in the artificially shaded areas in contrast to observations made in full sun-exposed individuals.

The comparison of RAU values for shaded and full sun-exposed individuals permitted the further categorization of facultative shade-tolerant native species as shade-tolerant that benefit from shade, shade-insensitive and shade-intolerant in terms of symbiotic N₂-fixation. Shade-tolerant that benefit from shade were those species in which shade induced a significant enhancement in the RAU values above that detected in full sun-exposed individuals of the same species. This category was represented by *C. tetraphila*, *A. brasiliana*, *E. simplicifolium*, *G. jussieuana*, *I. lespedezioides*, *S. guianensis*, *M. camporum* and *M. debilis*. On the other hand, the benefit of shade was not evident in the facultative shade-tolerant *C. rotundifolia*, *P. diversifolius*, *S. capitata* and *Z. curvata*. These species showed similar RAU values in the shaded and full sun-exposed areas and were, therefore, considered as shade-insensitive. Finally, the significant decrease in the symbiotic N₂-fixation process in *D. barbatum* caused by the artificial 75% reduction in the incident sunlight allowed the classification of this species as symbiotically shade-intolerant.

The beneficial effect of shade on the RAU values encountered in several facultative shade-tolerant species could not be attributed to an overall significant increase in the shoot ureide content induced by shade. Rather, the increased RAU in the shaded species that benefit from shade was ascribed to a concomitant significant 2.2 fold decrease in the average shoot nitrate content and a significant 3.6 fold increase in the shoot ureide:nitrate ratio when compared to values detected in full sun-exposed individuals. These observations suggest a synchronization between the symbiotic N₂-fixation process, as the main nitrogen source for the shade-tolerant species at low light, and the utilization of the soil nitrate throughout the nitrate reductase activity

in those facultative shade-tolerant species with intermediate or low N_2 -fixation rates at high light. This hypothesis was further supported by a) the positive effect of light on nitrate reductase activity (Mohr et al., 1992), b) the low nitrate reductase activity recorded in forest plants growing under shade (Medina and Cuevas, 1994) and c) the almost complete absence of available NH_4^+ in savanna soils with high percentage of sand and the reported predominance of NO_3^- above NH_4^+ in soils formed by small aggregates and with low water content (Giambiagi et al., 1993). The cooperation between both nitrogen assimilation pathways in the nitrogen economy of legumes was previously outlined for native species with seasonal hindered N_2 -fixation in a neotropical savanna (Sicardi de Mallorca and Izaguirre-Mayoral, 1993). On the other hand, the maintenance of high nitrate reductase activity at full sunlight and at shade seemed to be the reason for the absence of a response of the symbiotic process in *P. diversifolius* and *Z. curvata* to contrasting light environments. The non-effect of light on the nitrate reductase activity in both species was ascertained by the similar nitrate content found in shaded and full sun-exposed individuals. Therefore, for native legumes the dynamics of the nitrate reductase under contrasting environments seem to be species-specific as previously reported (Herrera et al., 1987; Ohlson and Högbom, 1993).

The most striking observation from the present study was the inability of native legume species considered to be the best N_2 -fixer in full sun-exposed areas to colonize low light habitats. *Aeschynomene* sp., *Calopogonium mucunoides*, *Centrosema pascuorum* and *Phaseolus gracilis* with RAU values above 60% under full sunlight (Sicardi de Mallorca and Izaguirre-Mayoral, 1993) were categorized as shade-intolerant species. These results might indicate that native legumes with a maximum dependence on the symbiotic N_2 -fixation for nitrogen supply at high light were the most prone to be affected by contrasting light environments, and that only those species with a spare capacity to enhance their symbiotic N_2 -fixation were capable to explore low light habitats. On the other hand, it might be postulated that the intermediate or low N_2 -fixation rates in several native legumes under full sunlight were a consequence of the feedback inhibition of photosynthesis mediated by an accumulation of leaf non-structural carbohydrates at high light (Plaut et al., 1987). This hypothesis is strengthened by the significant 2.2 fold greater starch content in leaves of individuals growing under full sunlight when compared to those collected under the artificial shade. The massive accumulation of starch in chloroplasts is known to cause a decrease in the rates of CO_2 -fixation, and can be considered as an indicator of reduction in the rate of sucrose translocation from source to sink organs in plants subjected to full sunlight (Mayoral et al., 1985). Nodules constitute by far the strongest sinks in the legume plants (Izaguirre-Mayoral et al., 1992b; Izaguirre-Mayoral

et al., 1994; Thomas et al., 1991; Izaguirre-Mayoral and Flores, 1995). Thus, the functionality of the symbiotic process in shade-tolerant native species that benefit from shade could have been limited by a low availability of photoassimilates to nodules in individuals exposed to full sunlight. Adequate carbon supply to nodules is required to maintain nitrogenase-linked respiration and hence nitrogen fixation. The possibility for a certain degree of photoinhibition in legumes with intermediate or low rates of N_2 -fixation at full sunlight was ruled out based on the significantly higher chlorophyll contents displayed by all full sun-exposed individuals.

From the present study three main conclusions might be drawn:

1) The establishment of physiological rather than morphological adaptive strategies seemed to be the mechanism underlying the tolerance to shade in all facultative shade-tolerant native species, in agreement with previous observations made in the shade-tolerant forest legume *Castanospermum australe* (Osunkoya et al., 1994). The ability of the facultative shade-tolerant native legumes to grow under shade was associated mainly with their capacity to nodulate and fix N_2 symbiotically at high rates in low light environments.

2) The high RAU values in several shade-intolerant species under full sunlight and the enhanced N_2 -fixation displayed by facultative shade-tolerant species that benefit from shade indicate that native soil rhizobial populations are fully competent symbiotically under both light conditions. The effective nodulation in all shaded individuals and the enhanced symbiotic N_2 -fixation rates in most of the shaded native species occurred in spite of the 2 fold decrease in size of the native rhizobial populations in shaded soils. Such decreases were attributed to the low number of species and of legume individuals (Sicardi de Mallorca and Izaguirre-Mayoral, 1994), rather than to alterations in the soil climatic conditions induced by the artificial shade.

3) The significant differences in RAU values between shaded and full sun-exposed individuals strongly support the inclusion of light intensity among the environmental factors known to control the symbiotic process. The observed benefits of shade on the symbiotic process in shade-tolerant legumes could have been mediated through a direct effect on the leaf temperatures and rate of evapotranspiration in the shade plants (Belsky et al., 1993) and/or indirectly, by increasing the availability of soil nutrients for the growing legumes due to the lack of competition by the otherwise dominating grasses. The type of response elicited by low light habitats on the legume plants was species-related considering that the capability of nodulated legumes to benefit from shade was determined by the host rather than by the rhizobial strains harbored by the plants. An improvement of efficiency in the symbiotic process in legumes might be, therefore, achieved throughout genetic modifications of

the host. The already identified shade-tolerant species constitute an important source of germplasm for future breeding programs.

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