

Symbiotic N₂-Fixation in Tropical Legume Species Growing at High Geographic Elevation

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Abstract

Thirteen nodulated suffruticose tropical legume species were collected during the rainy season in full sun-exposed areas of a tropical cloud forest, between 1,400 and 1,650 m above sea level. Annual rain fall averaged 1,080 mm, and the soil and air temperature were 25°C and 22°C, respectively. The soils were clay-loam with poor drainage. According to vegetation inventories carried out in highland and lowland areas the tropical legumes were considered as opportunistic ecological generalist species capable to colonize areas of contrasting geographic elevation. Based on the values of the relative abundance of ureides (RAU), the highland species *Chamaecrista patellaria*, *Aeschynomene brasiliana*, *Calopogonium mucunoides*, *Crotalaria anagiroides*, *Desmodium barbatum*, *Indigofera lepedezioides*, *Stylosanthes leiocarpa*, *Vigna vexillata* and *Mimosa orthocarpa* were categorized as good N₂-fixers (RAU>60%). Whereas, *Calopogonium* sp., *Desmodium intortum* and *Indigofera suffruticosa* behaved as intermediate N₂-fixers (RAU 30–59%). Low rates of N₂-fixation (RAU<30%) were detected only in individuals of *Mimosa albida*. These differences in RAU among the highland species were not correlated with any of the ultrastructural characteristics of nodule infected cells such as the presence of fibre-like structures in the peribacteroid space, number of peribacteroid units per 100 nm, shape of bacteroids, number of bacteroids per peribacteroid unit, or number of poly-β-hydroxybutyrate grains per bacteroid. For all highland legume species the mean RAU value was significantly higher than the mean value reported for all lowland legumes. The enhanced symbiotic efficiency in highland species was

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a consequence of higher leaf non-structural carbohydrate content and a 2.2 fold increase and 1.7 fold reduction in the shoot ureide and nitrate content, respectively, when compared to values of lowland legumes. The higher ureide:nitrate ratio in highland legumes allowed the assumption that symbiotic N₂-fixation prevails above nitrate uptake under the favorable climatic conditions encountered by legumes at high geographic elevation.

Keywords: Symbiotic N₂-fixation, elevation, native legumes

1. Introduction

The profound influence of the environment on the symbiotic N₂-fixation in legumes has been the subject of extensive investigations (Bordeleau and Prévost, 1994). Environmental conditions such as seasonal drought (Sicardi de Mallorca and Izaguirre-Mayoral, 1993; Sicardi de Mallorca and Izaguirre-Mayoral, 1994), adverse soil properties (Izaguirre-Mayoral et al., 1992), high temperatures (Dudeja and Khurana, 1989; Ahmed et al., 1993), and light intensity at which plants are grown (Izaguirre-Mayoral et al., 1995) are considered, at present, as the most important factors hindering the symbiotic efficiency of legume species. However, due to the complexity of the environment at which native leguminous plants are exposed it is possible to assume that other climatic conditions, such as those prevailing at high geographic elevation, might also exert an influence on the symbiotic performance of tropical legumes. Vegetation inventories carried out in highland and lowland areas have shown that the spatial recruitment of legumes is certainly not restricted to lowland-hot weather habitats (De Souza et al., 1992; Belsky et al., 1993; Negi and Pat, 1994; Kelly et al., 1994; Izaguirre-Mayoral and Flores, 1995).

Highland sites typical of tropical cloud forests are characterized by decreased rates of nitrogen mineralization (Medina and Cuevas, 1994), greater water availability and lower soil and air temperatures when compared to lowland areas. The favorable climatic conditions in these highland areas must exert an influence on the physiological performance of tropical legumes (Zachariassen and Power, 1991). Nevertheless, the effect of high geographic elevation on the *Rhizobium*-host symbiotic association have been analyzed only in commercially cultivated legumes (Abaidoo et al., 1990; Rumbaugh and Johnson, 1991) and in few forest legume trees (Izaguirre-Mayoral and Flores, 1995). No data are available on the physiology of highland suffructicose native tropical legumes. Thus, the purpose of this study was to estimate the symbiotic N₂-fixation in native legume species collected in full sun-exposed areas of a highland tropical cloud forest. Outstanding questions included

whether geographic elevation influences the diversity of suffruticose tropical legumes, the nodulation process, and the contribution of the symbiotic N₂-fixation to the nitrogen economy of legume species. The present study of the *Rhizobium* symbiosis in highland species and the comparison of the data with those reported on lowland legumes may contribute to a better understanding of the legume ecology.

2. Materials and Methods

Characteristics of the study site

The study site was confined to previously disturbed areas of a tropical cloud forest located in the Miranda State, Venezuela (10° 20' N, 66° 55' W), between 1,400 and 1,650 m above sea level. The full sun-exposed areas under study originated from landscape disturbances carried out 35 years ago for construction of buildings and roads, and regeneration of the forest dominant species has not occurred, so far. Annual rainfall average is 1,080 mm, and at the rainy season the air and soil temperature average is 22°C and 25°C, respectively. The soils are clay-loam with poor drainage (L.O. Marulanda, personal communication).

Plant material and plant analyses

All suffruticose legume species growing in the study area were collected during the rainy season (August) in 1994. Plant harvesting consisted of the entire above-ground biomass and underground organs (roots and nodules) contained in a soil volume of approximately 0.027 m³. Plants were analyzed for the ureide (allantoin plus allantoic acid), α -amino-N, nitrate, chlorophyll, total reducing sugars and starch content as described in Sicardi de Mallorca and Izaguirre-Mayoral (1993). The relative abundance of ureides (RAU) in shoots was used as an index of the symbiotic N₂-fixation rate in the individual legume species. Based on RAU values, legume species were classified as good (RAU>60%), intermediate (RAU 30–59%) and low N₂-fixers (RAU<30%) (Sicardi de Mallorca and Izaguirre-Mayoral, 1993). Analysis of nodulation and physiological parameters related to symbiotic N₂-fixation were carried out on a minimum of 6 individuals for each of the legume species. All collected individuals were adults at the vegetative stage of growth.

For ultrastructural studies, nodules harvested from individuals of several legume species were fixed, post-fixed and stained as described in Mayoral et al. (1989). Electron microscopic observations were carried out in nodules having an internal reddish color. The quantitative analysis of ultrastructural

characteristics of infected cells were performed in a minimum of 20 electron micrographs taken from randomly selected areas of equivalent nodule cross sections.

3. Results

Legume inventory

The legume composition of the area comprised one Caesalpiaceae, ten Fabaceae and two Mimosaceae species (Table 1). Nodulation was observed in all individuals, with indeterminate nodules in Caesalpiaceae and Mimosaceae, and either determinate or indeterminate type of nodules in Fabaceae species. A combined population of determinate and indeterminate nodules was detected only in *I. suffruticosa*. Nodules were found mainly on lateral roots and were rarely formed on the underground xylopodia.

Table 1. Inventory of tropical legume species growing in full sun-exposed areas of a tropical cloud forest located between 1,400 and 1,650 m above sea level.

| Species | Type of nodule | Type of underground organ |
|--|----------------|---------------------------|
| Caesalpiaceae | | |
| <i>Chamaecrista patellaria</i> DC. | I | X |
| Fabaceae | | |
| <i>Aeschynomene brasiliana</i> (Poir.) DC. | D | X |
| <i>Calopogonium mucunoides</i> Desv. | D | R |
| <i>Calopogonium</i> sp. | I | R |
| <i>Cratalaria anagiroides</i> Desv. | I | R |
| <i>Desmodium barbatum</i> Benth | D | X |
| <i>Desmodium intortum</i> Mill | D | X |
| <i>Indigofera lespedezioides</i> HBK | I | R |
| <i>Indigofera suffruticosa</i> L. | I+D | X |
| <i>Stylosanthes leiocarpa</i> Vog. | D | R |
| <i>Vigna vexillata</i> (L.) Rich. | D | X |
| Mimosaceae | | |
| <i>Mimosa albida</i> H&B. | I | R |
| <i>Mimosa orthocarpa</i> Spruce | I | X |

I = indeterminate nodules; D = determinate nodules; R = root; X = xylopodium.

Physiological parameters in native legumes

Of all legumes detected in the area, *D. barbatum* presented the highest significant chlorophyll content, *C. patellaria*, *C. mucunoides* and *M. orthocarpa* showed the highest significant leaf total reducing sugar content, whereas the highest significant starch content was detected in leaves of *C. anagiroides* (Table 2). The total reducing sugar content in roots did not significantly differ among species, except for *I. suffruticosa* and *V. vexillata* which showed the highest and lowest significant values, respectively. In turn, the highest significant starch content in roots was detected in *M. orthocarpa*.

Table 2. Chlorophyll, and the sugars and starch content in leaves and roots of highland tropical legume species.

| Species | Leaves | | | Roots | |
|--------------------------|--|------------------------------|------------------------------|------------------------------|------------------------------|
| | Chlorophyll ($\mu\text{g}/\text{cm}^2$) | Sugars (mmol/g dry wt) | Starch (mmol/g dry wt) | Sugars (mmol/g dry wt) | Starch (mmol/g dry wt) |
| Caesalpinaceae | | | | | |
| <i>C. patellaria</i> | nd | 1.4a | 0.6c | nd | nd |
| Fabaceae | | | | | |
| <i>A. brasiliana</i> | nd | 0.6c | 0.5c | 0.4bc | 0.3d |
| <i>C. mucunoides</i> | 5.4b | 1.0ab | 0.7b | 0.6b | 1.1ab |
| <i>Calopogonium</i> sp. | 3.4b | 0.6c | 0.3d | 0.3bc | 0.3d |
| <i>C. anagiroides</i> | 2.7b | 0.8bc | 1.2a | 0.4bc | 0.4cd |
| <i>D. barbatum</i> | 10.6a | 0.5c | 0.5c | 0.6b | 0.4cd |
| <i>D. intortum</i> | 4.6b | 0.8bc | 0.2d | 0.4bc | 1.1ab |
| <i>I. lespedezioides</i> | 3.2b | 0.6c | 0.2d | 0.4bc | 0.2d |
| <i>I. suffruticosa</i> | 3.0b | 0.5c | 0.2d | 0.9a | 1.1ab |
| <i>S. leiocarpa</i> | nd | 0.5c | 0.7bd | 0.6b | 0.3d |
| <i>V. vexillata</i> | 3.4b | 0.4c | 0.2d | 0.2d | 0.5bc |
| Mimosaceae | | | | | |
| <i>M. albida</i> | 5.3b | 0.6c | 0.2d | 0.3bc | 0.7bc |
| <i>M. orthocarpa</i> | nd | 1.2a | 0.5c | 0.6b | 1.8a |

Numbers in columns followed by the same letter(s) are not statistically different at $p < 0.05$.
nd = not determined.

N-compounds in native legumes

As shown in Table 3, *C. mucunoides* and *V. vexillata* had the highest significant ureide content, and *I. lespedezioides* the highest significant α -amino-N content. Nitrate content was significantly higher in *D. intortum* and *S. leiocarpa*, while the lower significant values were detected in *I. lespedezioides*. *C. mucunoides* and *V. vexillata* showed the highest significant RAU values, with the lowest significant value in *M. albida*.

Table 3. Ureide, α -amino-N and nitrate content, and the relative abundance of ureides (RAU) in shoots of highland tropical legume species.

| Species | Ureides (mmol/g dry wt) | α -amino-N (mmol/g dry wt) | Nitrate (mmol/g dry wt) | RAU (%) |
|--------------------------|----------------------------|--------------------------------------|----------------------------|------------|
| Caesalpinaceae | | | | |
| <i>Cassia patellaria</i> | 4.6d | 56.3be | 8.8c | 62.9c |
| Fabaceae | | | | |
| <i>A. brasiliana</i> | 36.4b | 58.8be | 25.0b | 85.9b |
| <i>C. mucunoides</i> | 163.2a | 62.7be | 9.6b | 98.5a |
| <i>Calopogonium</i> sp. | 2.3e | 40.2e | 22.9b | 28.6e |
| <i>C. anagiroides</i> | 4.8d | 45.6de | 19.9b | 68.0c |
| <i>D. barbatum</i> | 9.2c | 69.8be | 10.3c | 81.0b |
| <i>D. intortum</i> | 4.4d | 111.0b | 34.0a | 35.9e |
| <i>I. lespedezioides</i> | 4.2d | 195.9a | 2.2d | 88.4b |
| <i>I. suffruticosa</i> | 4.0d | 56.4be | 19.9b | 46.9d |
| <i>S. leiocarpa</i> | 10.4c | 47.4ce | 31.0a | 57.6c |
| <i>V. vexillata</i> | 178.7a | 107.0bc | 19.2b | 96.8a |
| Mimosaceae | | | | |
| <i>M. albida</i> | 0.7f | 99.7be | 22.1b | 11.4f |
| <i>M. orthocarpa</i> | 5.4d | 92.4be | 13.3c | 61.2c |

Numbers in columns followed by the same letter(s) are not statistically different at $p < 0.05$.

Ultrastructural analyses

Electron micrographs of cross sections of nodules harvested from several legume species are shown in Fig. 1. Ovoid bacteroids with seldom pleomorphic

(Y and T) shapes occurred in nodules of *C. anagiroides*. The shape of bacteroids in nodules from *C. patellaria* and *I. lespedezioides* were also ovoid, and elongated bacteroids were observed in nodules from *C. mucunoides*, *D. intortum*, *M. albida*, *M. orthocarpa*, and *V. vexillata*. Fibrous nucleoid material were easily detected in bacteroids of nodules from *C. anagiroides*, *D. intortum*, *I. lespedezioides* and *I. suffruticosa*. Nodules from the latter species also showed electron dense fibre-like structures within the peribacteroid space. No infection threads were noted in any of the nodule cross sections, nor were there any interstitial cells in the central infected zone. The quantitative analysis of the ultrastructure of infected cells in nodules from several legume species is summarized in Table 4.

Table 4. Ultrastructural characteristics of *Rhizobium*-infected cells in nodules harvested from highland tropical legume species.

| Species | Number of bacteroids/ PBU (1) | Bacteroid volume (μm^3) | Number of poly- β -hydroxy butyrate grains/ bacteroid | Number of bacteroids/ 100 μm | Number of PBU/ 100 μm |
|--------------------------|----------------------------------|---|--|--|-------------------------------------|
| <i>C. anagiroides</i> | 1 | 19.6 \pm 0.9 | 0-10 | 38 \pm 2 | 63 \pm 4 |
| <i>C. patellaria</i> | 1-7 | 1.1 \pm 0.2 | 1-5 | 59 \pm 2 | 23 \pm 3 |
| <i>C. mucunoides</i> | 2-7 | 0.6 \pm 0.1 | >15 | 95 \pm 3 | 35 \pm 3 |
| <i>D. intortum</i> | 1-2 | 0.9 \pm 0.1 | 1-2 | 82 \pm 5 | 73 \pm 6 |
| <i>I. lespedezioides</i> | 1-2 | 4.7 \pm 1.9 | 1-2 | 56 \pm 3 | 55 \pm 3 |
| <i>I. suffruticosa</i> | 10-12 | 7.1 \pm 0.9 | 1-2 | 76 \pm 10 | 38 \pm 4 |
| <i>M. albida</i> | 1-3 | 0.7 \pm 0.1 | >15 | 53 \pm 4 | 50 \pm 6 |
| <i>M. orthocarpa</i> | 1-7 | 1.0 \pm 0.1 | 5-10 | 67 \pm 5 | 34 \pm 2 |
| <i>V. vexillata</i> | 1-5 | 0.8 \pm 0.1 | >15 | 119 \pm 2 | 69 \pm 7 |

(1) = PBU, peribacteroid unit.

4. Discussion

The study area had a reduced legume diversity when compared with neotropical lowland savannas for which we have carried out a detailed study (Sicardi de Mallorca and Izaguirre-Mayoral, 1993). The poor drainage of montane clay-loam soils has been postulated to be a determining factor for the establishment of suffruticose legume species at high geographic elevation

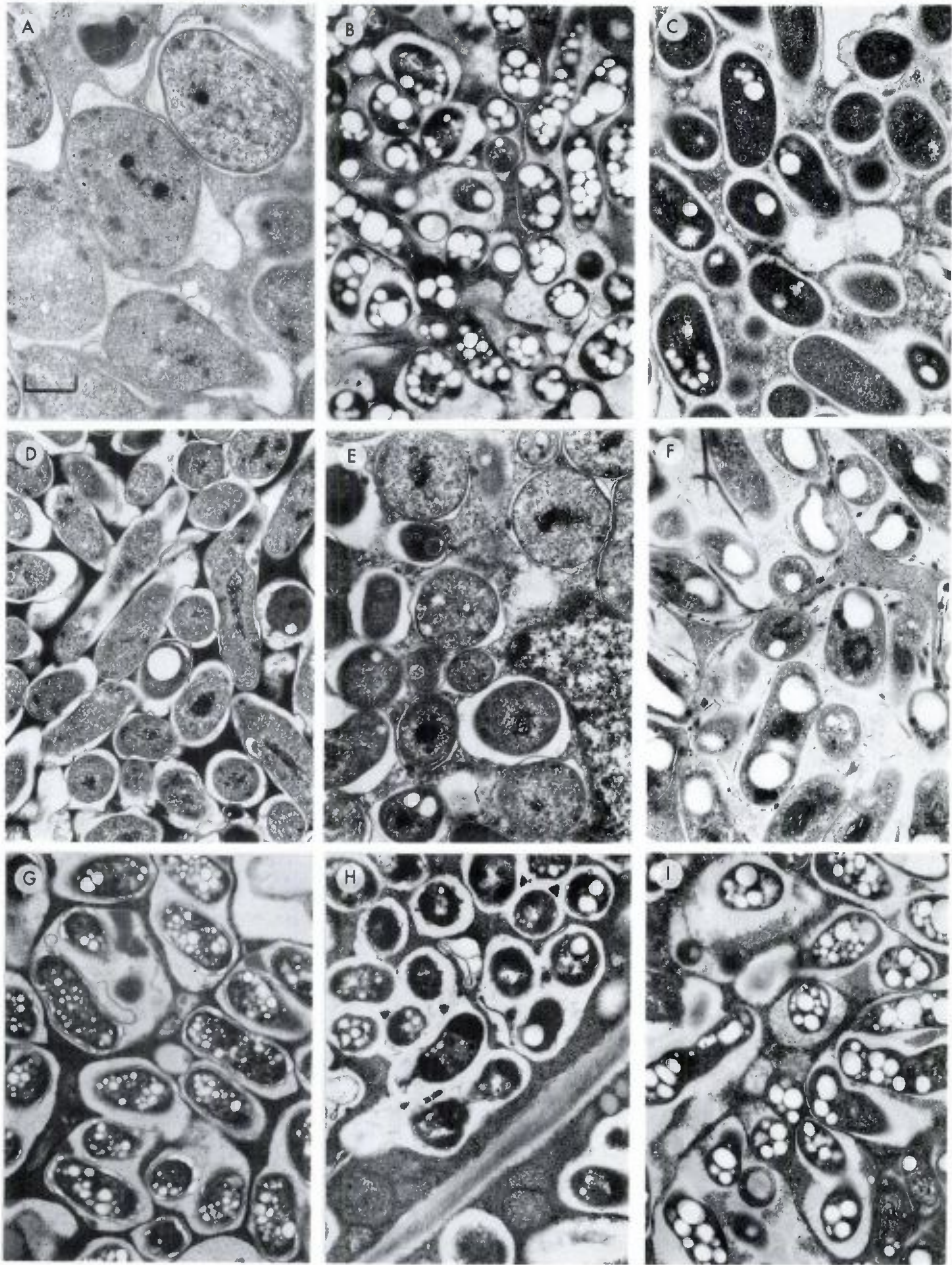


Figure 1. Electron micrographs of infected cells in nodules from: A) *Crotalaria anagiroides*; B) *Calopogonium mucunoides*; C) *Chamaecrista patellaria*; D) *Desmodium intortum*; E) *Indigofera lespedezioides*; F) *Indigofera suffruticosa*; G) *Mimosa albida*; H) *Mimosa orthocarpa*; and I) *Vigna vexillata*. For all electron micrographs the bar represents 0.8 μm .

(Cocks, 1994). Nevertheless, the observation that highland and lowland areas share a high percentage of legume species (Barrios and Gonzalez, 1971; Ishizaki et al., 1991; Izaguirre-Mayoral et al., 1992; Sicardi de Mallorca and Izaguirre-Mayoral, 1993; Osonkuya and Oyesiku, 1994; Izaguirre-Mayoral et al., 1995) indicate that suffructicose tropical legumes present a low degree of endemism and can be categorized as "preponderantly widely distributed opportunistic ecological generalist species" (Kelly et al., 1994).

The ability of tropical legumes to grow in a fairly broad range of habitats seems to be associated with the physiological adaptability of the plants to contrasting climatic conditions, rather than to the efficiency of the symbiotic process under different environments (Izaguirre-Mayoral et al., 1995). This hypothesis is supported by the following observations: a) highland legumes showed increased leaf non-structural carbohydrate content above values reported in lowland species. This can be a result of higher rates of CO₂ fixation brought about by the greater water availability and the lower air and soil temperatures in the highland area. As previously shown, air temperatures between 15–25°C favor the optimal operation of the Calvin photosynthetic cycle for all legumes so far analyzed (Bordeleau and Prévost, 1994). In turn, an adequate carbon supply to nodules will insure high rates of N₂-fixation and, therefore, enhanced shoot ureide content and RAU values. For all tested highland species the mean RAU value was 1.7 fold higher than the mean value reported in lowland species (Sicardi de Mallorca and Izaguirre-Mayoral, 1993); b) the higher RAU in highland legumes was a consequence of a 2.2 fold increase and 1.7 fold reduction in the shoot ureide and nitrate content, respectively, when compared to that in lowland species. This finding implies that the process of nitrate uptake and N₂-fixation are closely synchronized, and that nitrate uptake seems to dominate in lowland species subjected to higher air temperature. At present we cannot discard increased nitrate content during the dry season because our sampling was carried out at the rainy season when nitrate levels in soils are lower (Giambiagi et al., 1993); c) the response of highland legumes to high geographic elevation was species-specific. The species *C. patellaria*, *A. brasiliiana*, *C. anagiroides*, *D. barbatum*, *I. lespedezioides*, *S. leiocarpa* and *M. orthocarpa* were considered as good N₂-fixers, whereas *Calopogonium* sp., *D. intortum* and *I. suffructicosa* were classified as intermediate N₂-fixers. Low rates of N₂-fixation were detected only in individuals of *M. albida*, and outstanding rates of symbiotic N₂-fixation were displayed by *C. mucunoides* and *V. vexillata* (RAU>96%); and d) the efficiency of the symbiotic process in the highland legume species was not related with any of the ultrastructural characteristics quantified in nodule cross sections. High number of peribacteroid units per 100 mm of nodule tissue and a high number of bacteroid per bacteroid unit as well as of poly-β-

hydroxybutirate grains were detected in either good or low N₂-fixers. Fibre-like structures in the peribacteroid space, probably constituted by structural polysaccharides (Izaguirre-Mayoral et al., 1994), were exclusively detected in nodules from *I. suffruticosa*. The high RAU values in most highland legumes suggest that soil rhizobial populations native in this montane region are fully competent symbiotically.

Finally, from the results presented, two main conclusions might be drawn:

1) The physiological performance of the plants and the genetic characteristics of the species determine the symbiotic efficiency of the plants in areas of contrasting geographic elevation. This suggestion is based on the comparative analysis of data obtained in highland and lowland legumes which indicate that: a) Mimosaceae, Caesalpiniaceae and Fabaceae species can be considered as low, intermediate and good N₂-fixers, respectively, regardless of the geographic elevation at which the plants were collected (Sicardi de Mallorca and Izaguirre-Mayoral, 1993), and b) only legume species with a spare capacity to enhance their symbiotic efficiency seem to be capable to colonize areas with different climatic conditions (Izaguirre-Mayoral et al., 1995).

2) High geographic elevation, per se, should not be included among the environmental factors known to control the symbiotic process in native tropical legumes. Rather, the positive effect of the favorable climatic conditions encountered in full sun-exposed highland areas on the physiology of the plants seem to be the main factors responsible for the enhanced symbiotic efficiency of tropical legumes at high geographic elevation.

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REFERENCES

- Abaidoo, R.C., George, T., Bohlool, B.B., and Singleton, P.W. 1990. Influence of elevation and applied nitrogen on rhizosphere colonization and competition for nodule occupancy by different rhizobial strains on field-grown soybean and common bean. *Canadian Journal of Microbiology* **36**: 92-96.
- Ahmed, F.E., Hall, A.E., and Madore, M.A. 1993. Interactive effects of high temperature and elevated carbon dioxide concentration on cowpea (*Vigna unguiculata* L.). *Plant, Cell and Environment* **16**: 835-842.

- Barrios, S. and Gonzalez, V. 1971. Rhizobial symbiosis on Venezuelan savannas. *Plant and Soil* **34**: 707-719.
- Belsky, A.J., Mwonga, S.M., Amundson, R.G., Duxbury, J.M., and Ali, A.R. 1993. Comparative effects of isolated trees on their undercanopy environments in high- and low-rainfall savannas. *Journal of Applied Ecology* **30**: 143-155.
- Bordeleau, L.M. and Prévost, D. 1994. Nodulation and nitrogen fixation in extreme environments. *Plant and Soil* **161**: 115-125.
- Cocks, P.S. 1994. Colonization of a South Australian grassland by invading Mediterranean annual and perennial pasture species. *Australian Journal of Agricultural Research* **45**: 1063-1076.
- De Souza Moreira, F.M., Da Silva, M.F., and De Faria, S.M. 1992. Occurrence of nodulation in legume species in the Amazon region of Brazil. *New Phytologist* **121**: 563-570.
- Dudeja, S.S. and Khurana, A.L. 1989. The pigeonpea-*Rhizobium* symbiosis as affected by high root temperature: effect on nodule formation. *Journal of Experimental Botany* **40**: 469-472.
- Giambiagi, N., Rimolo, M., and Pirolo, T. 1993. Influence of drought on the production of mineral nitrogen in a typical argiudol of the pampas. *Soil Biology and Biochemistry* **25**: 101-108.
- Ishizaki, S.M., Silva, J.A., and Toma, W.Y. 1991. Soil-plant relationships in pangola grass (*Digitaria decumbes* Stent.) and intortum (*Desmodium intortum* (Mill.) Urb.) fertilized with molybdenum and copper. *Journal of Plant Nutrition* **14**: 295-313.
- Izaguirre-Mayoral, M.L., Carballo, O., Flores, S., Sicardi de Mallorca, M., and Oropeza, T. 1992. Quantitative analysis of the symbiotic N₂-fixation, non-structural carbohydrates and chlorophyll content in sixteen native legume species collected in different savanna sites. *Symbiosis* **12**: 293-312.
- Izaguirre-Mayoral, M.L., Carballo, O., Sicardi de Mallorca, M., Marys, E., and Gil, F. 1994. Symbiotic nitrogen fixation and physiological performance of bean (*Phaseolus vulgaris* L.) plants as affected by *Rhizobium inoculum* position and bean rugose mosaic virus infection. *Journal of Experimental Botany* **45**: 373-383.
- Izaguirre-Mayoral, M.L. and Flores, S. 1995. Symbiotic nitrogen fixation in *Dioclea guianensis*, a shade-tolerant and aluminium-accumulator legume species native of a tropical cloud forest. *Symbiosis* **19**: 51-69.
- Izaguirre-Mayoral, M.L., Vivas, A.I., and Oropeza, T. 1995. 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. *Symbiosis* **19**: 111-129.
- Kelly, D.L., Tanner, E.V.J., NicLughadha, E.M., and Kapos, V. 1994. Floristics and biogeography of a rain forest in the Venezuelan Andes. *Journal of Biogeography* **21**: 421-440.
- Mayoral, M.L., Sicardi de Mallorca, M., and Uzcátegui, R.C. 1989. Comparative response of nodulated and nitrogen-supplied cowpea (*Vigna unguiculata* (L.) Walp. var. Tuy) plants to infection by cowpea mosaic virus. *Journal of Experimental Botany* **40**: 159-165.

- Medina, E. and Cuevas, E. 1994. Mineral nutrition: Humid tropical forest. In: *Progress in Botany*, Vol. 55, Springer-Verlag, Berlin and Heidelberg, pp. 115–129.
- Negi, K.S. and Pant, K.C. 1994. Genetic wealth of agri-horticultural crops, their wild relatives, indigenous medicinal and aromatic plants at U.P. Himalaya. *Journal of Economic and Taxonomic Botany* **18**: 17–41.
- Okusanya, O.T. and Oyesiku, O. 1994. Comparative salinity tolerance of two legumes, *Vigna luteola* and *Vigna vexillata* from the coast of Trinidad. *Canadian Journal of Botany* **72**: 1216–1221.
- Rumbaugh, M.D. and Johnson, D.A. 1991. Field acetylene reduction rates of *Lupinus argenteus* along an elevational gradient. *Great Basin Naturalist* **51**: 192–197.
- Sicardi de Mallorca, M. and Izaguirre-Mayoral, M.L. 1993. A comparative evaluation of the symbiotic N₂-fixation and physiological performance of thirty-six native legume species collected in a tropical savanna during the rainy and dry seasons. *Symbiosis* **16**: 225–247.
- Sicardi de Mallorca, M. and Izaguirre-Mayoral, M.L. 1994. Seasonal dynamic, host range and symbiotic efficiency of native rhizobial populations in three soil horizons of four contrasting savanna sites. *Symbiosis* **17**: 43–63.
- Zachariassen, J.A. and Power, J.F. 1991. Growth rate and water use by legume species at three soil temperatures. *Agronomy Journal* **83**: 408–413.