

## **Symbiotic Nitrogen Fixation in *Dioclea guianensis* Benth., a Shade-Tolerant and Aluminium-Accumulator Legume Species Native of Tropical Cloud Forests**

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### **Abstract**

Understorey seedlings and juveniles as well as adult plants of *Dioclea guianensis* were analyzed for the content of ureide,  $\alpha$ -amino-N, nitrate and non-structural carbohydrate in the different plant compartments. Plants were harvested during the rainy season in a tropical cloud forest located at 1,747 m above sea level. At this time of the year the photosynthetically active radiation at the understorey stratum was 92% lower than that recorded at the forest canopy, and the clay loam soils had a pH of 4.1, 91% Al saturation and a 3:1  $\text{NH}_4^+:\text{NO}_3^-$  ratio. In this forest, all individuals of *D. guianensis* with stem height higher than 17 cm were nodulated. Nodules were first observed on seedlings with stem heights of 10 cm and there was a synchronization between the depletion of the cotyledon reserves and the onset of the symbiotic nitrogen fixation. For all individuals, the determinate nodules formed by a *Bradyrhizobium* strain were located mainly at the 0–20 cm uppermost soil horizon. The ultrastructural analysis of nodules revealed bacteroid units with  $4\pm 1$  rod-shaped bacteroids containing a dense fibrillar nuclear material and scarce poly-3-hydroxybutyric acid grains. The nodule mass increased with plant height and nodulated juveniles showed significantly lower shoot nitrate and higher shoot/root ratios than non-nodulated individuals. In

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seedlings and juvenile plants the nodulation process induced a significant increase in the ureide content in leaves, roots and shoots. In addition, a significant decrease was observed in the  $\alpha$ -amino-N and non-structural carbohydrate content in leaves and roots, when compared to values in non-nodulated individuals of similar stem heights. The rates of nitrogen fixation, estimated from measurements of the relative abundance of ureides (RAU) in shoots, ranged from 90% in seedlings and juveniles to 60% in adult plants with leaves exposed to full sunlight. Such ontogenetic changes in RAU appear to be related to a significant progressive increase in the shoot nitrate content rather than to alterations in the plant ureide content. Based on present results *D. guianensis* was categorized as a shade-tolerant, good N<sub>2</sub>-fixer and Al-accumulator species. The tolerance to shade, the accumulation of aluminium in roots and leaves and the partial exclusion of this ion from nodule tissues were considered to be the probable mechanisms underlying the efficiency of the symbiotic nitrogen fixation under the tropical cloud forest conditions.

Keywords: symbiotic nitrogen fixation, tropical cloud forest, *Dioclea guianensis*.

## 1. Introduction

Strong water availability, low soil fertility (Wright, 1982), high percentage of soil aluminium (Cuenca and Herrera, 1988) and a marked reduction in the photosynthetically active radiation with depth in the leaf canopy (Hirose and Werger, 1994) are the main characteristics of tropical cloud forests. Nevertheless, highland tropical forests are regions of an extraordinary floristic diversity which include legume species of the genus *Brownea*, *Dioclea*, *Hymenolobium*, *Dipterix*, *Pithecellobium*, *Swartia*, *Inga* and *Ormosia* (Kelly et al., 1994, Clark and Clark, 1994).

In general, the recruitment and spatial distribution of forest legumes have been associated with the presence of canopy gaps (Milton et al., 1994). However, a number of legume species are capable to grow under the shade conditions encountered in the understorey stratum of tropical forests and have been categorized as shade tolerant (Lovelock et al., 1994, Osunkoya et al., 1994). For these legume species, the shade tolerance is demonstrated by the survival and growth of seedlings and juveniles in the forest shady habitats (Flores, 1992). This capacity of plants to tolerate shade appears to be achieved through the development of adaptive strategies such as production of large seeds (Osunkoya et al., 1994, Leishman and Westoby, 1994) and a positive carbon balance as well as a high shoot/root ratio in the understorey seedlings (Callaway, 1992, Lehto and Grace, 1994, Kitajima, 1994, Kasperbauer and Hunt, 1994). In turn, the onset of the symbiotic process at the earliest stages of

growth may be of great importance for the establishment of legumes in the shady understorey N-deficient soils. As previously shown, soil mineralization rates decrease with geographic altitude (Medina and Cuevas, 1994) and for understorey seedlings low light intensity may not constitute a handicap for nodule development (Malik et al., 1984, Streeter, 1993).

The symbiotic nitrogen fixation process has been the subject of intensive investigations in cultivated legumes. However, data on forest legumes are scarce and no information is available on the ontogenesis of the symbiotic process and on the physiological performance of native legumes growing in tropical cloud forests. Therefore, the present research was undertaken to analyze the nodulation, physiology and the symbiotic nitrogen fixation in the understorey *Dioclea guianensis* at different stages of plant development. In the cloud forest under study, *D. guianensis* is considered an obligate seed reproducing native legume species. Seedlings and juveniles of this species are capable to grow and nodulate in the understorey stratum, and only adults have leaves exposed to full sunlight. For the present investigation, understorey seedlings, juveniles as well as adult individuals were analyzed for the concentration of ureides,  $\alpha$ -amino-N, nitrate and non-structural carbohydrate in the different plant compartments. The efficiency of symbiotic nitrogen fixation was estimated according to values of the relative abundance of ureides in shoots. The mortality rates of seedlings and juveniles, the relative growth rates as well as the aluminium (Al) concentration in the different plant compartments were also determined.

## 2. Materials and Methods

This study was carried out during the rainy season of 1994 in an undisturbed tropical cloud forest located in the Miranda State, Venezuela (10° 20' N, 66° 55' W), at an altitude of 1,747 m above sea level. The photosynthetically active solar radiation (PAR) in the understorey stratum was measured along a diurnal cycle with a Quantum/Radiometer/Photometer Model LI-185B Licor Inc. The physicochemical properties of the soil were determined in triplicate samples to a depth of 20 cm collected at random in representative areas of the forest (Izaguirre-Mayoral et al., 1992a).

*Dioclea guianensis* Benth. plants at the seedling and juvenile stages of growth were collected in the understorey of the forest. According to the stem height, seedlings and juveniles were categorized in the following size intervals (SI): SI-6 (5.5 to 6.5 cm), SI-8 (7 to 8.5 cm), SI-10 (9 to 10.5 cm), SI-12 (11 to 13 cm), SI-15 (14 to 15.5 cm), SI-17 (16.5 to 17.5 cm), SI-20 (18 to 22 cm), SI-26 (24 to 28 cm), and SI-50 (40 to 63 cm). The seedling stage included plants with

cotyledons attached (SI-6 to SI-12), whereas the abscission of the cotyledons marked the beginning of the juvenile stage (SI-15 to SI-50). Plant collection also included adults with stem heights higher than 5 m and a diameter of  $3.8 \pm 0.2$  cm at breast height, with upper leaves exposed to full sunlight. For seedlings and juveniles the harvest consisted on the entire above and below-ground biomass. For adult plants, the sampling comprised sections of the main stem and of the root and nodules biomass as well as a set of mature leaves developed in the understory of the forest.

The analysis of nodulation and physiological parameters were carried out in a minimum of 6 individuals for each SI. Ureides (allantoin plus allantoic acid),  $\alpha$ -amino-N, total reducing sugars and starch concentration were determined in dried subsamples of cotyledons, shoots, leaves and roots. The nitrate concentration and the relative abundance of ureides (RAU) were determined in shoots. Similar analyses were also carried out in nodules harvested from juveniles at SI-15 and SI>15, since a minimum nodule dry weight of 20 mg for the analyses was not attained at SI-10 to SI-12. The colorimetric measurements of N-compounds and non-structural carbohydrates and the calculation of RAU were performed as described in Sicardi de Mallorca and Izaguirre-Mayoral (1993). The Al concentration in shoots, leaves, roots and nodules from juvenile and adult plants was measured by atomic absorption spectrometry with nitrous oxide-acetylene flame. For ultrastructural studies, nodules harvested from juveniles and adults were fixed, post-fixed and stained as described in Izaguirre-Mayoral et al. (1992b). For individual plant parameters, statistical differences among SI were analyzed using one way analysis of variance. Comparison of means between nodulated and non-nodulated individuals at SI-10 to SI-15 was carried out using the students' t test.

The abundance of *D. guianensis* was determined in four quadrates of  $10 \times 10$  m demarcated in randomly-selected locations in the cloud forest. The percent of mortality at the relative growth rate (Hunt 1982) during the seedling and juvenile stages of growth was assessed monthly. For this purpose about 100 seedlings at SI-6 were labeled with a yellow ribbon in June of 1994 and measurements were carried out up to January 1995.

### 3. Results

#### *Site and soil description*

In the forest under study, a total rainfall of 1,063 mm was registered during the rainy season (May to October) and day/night air temperatures averaged

22±2°C/15±2°C, respectively. The clay loam soils had a pH of 4.1 in water and 3.5 in KCl, and a chemical composition of: available P, 0.89 µg/g dry soil; Ca, 0.31 cmol<sub>c</sub>/kg; K, 0.29 cmol<sub>c</sub>/kg; Mg, 0.07 cmol<sub>c</sub>/kg; effective cation exchange capacity, 6.7 cmol<sub>c</sub>/kg, and 91% Al saturation. Organic matter concentration was below 7%, with 0.29% total nitrogen and a C/N ratio of 14.5±0.6 g/g dry soil. The average NH<sub>4</sub><sup>+</sup> concentration in the soils was 34.7 µg/g dry soil, whereas the NO<sub>3</sub><sup>-</sup> concentration was 3 fold lower. PAR at the understorey stratum oscillated between 10 mmol/m<sup>2</sup>/s (9:00 h) and 20 mmol/m<sup>2</sup>/s (13:00 to 14:00 h). Thereafter, PAR showed a progressive decrease with day time. At noon time, PAR at the understorey stratum was 92% lower than the values recorded outside of the forest canopy.

### *Plant phenology*

In the cloud forest, *D. guianensis* is characterized by its lianoid-climber type of growth. Plants showed an abundance of three adults per 100 m<sup>2</sup> and the root biomass was located mainly in the upper soil layers. Adult individuals flowered during the dry season and the dispersal of the large seeds took place at the beginning of the following rainy season. The maximum rate of seed germination occurred in May and June, and either seeds or seedlings were found scattered on the forest floor without any detectable pattern of distribution with respect to parent plants. Seeds exhibited hypogeous germination, the cotyledons never loose the seed coat and were always found buried in the forest litter. Two opposite trifoliolate leaves appear upon germination and the growth rate of seedlings was 0.0415±0.001 cm/month.

### *Nodulation, mortality and plant shoot/root ratio*

Seedlings at SI-6 and SI-8 were not nodulated. Nodules appearance was recorded in seedlings at SI-10. At this SI, nodules had a mean diameter of 2.5±0.3 mm and were present in number of 1 to 3 per plant. The occurrence of non-nodulated individuals was detected up to SI-15. Thereafter, all individuals including adults were nodulated. Nodules were observed in either primary or lateral roots regardless of the SI. In adults the massive cluster of nodules were formed mainly on lateral roots located in the upper 20 cm of the soil. The absence of nodules at soil depths below 20 cm was ascertained by digging into deeper soil horizons. In this species, almost all mortality was recorded in seedlings (Fig. 1), and none of the dead seedlings and juveniles were nodulated.

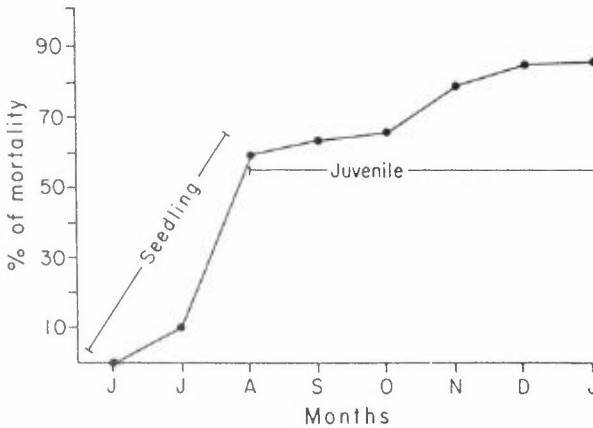


Figure 1. Percentage of mortality in seedlings and juveniles of *Dioclea guianensis* in the understorey stratum of the tropical cloud forest. Data were collected monthly between June (1994) and January (1995).

In nodulated seedlings and juveniles between SI-10 and SI-17 the nodule mass per plant did not show a significant increase (Table 1), although results indicated a direct relationship between nodule mass and height of the plants. A significant increase in nodule mass was detected at SI>17. Nodule formation induced a significant increase in the shoot/root ratio of seedlings and juveniles between SI-10 and SI-15 when compared to that of non-nodulated individuals at the same SI (Table 1). At SI>17, nodules showed a determinate type of growth with a diameter of  $0.8 \pm 0.09$  cm (Fig. 2A) and the *Rhizobium*-infected area represented about 85% of the nodule transversal sections. The rod-shaped bacteroids contained a dense fibrillar nuclear material, scarce poly-3-hydroxybutyric acid grains and were present in a number of  $4 \pm 1$  per bacteroid unit (Fig. 2B). The peribacteroid cytoplasm was dense and a number of small vesicles were observed. The morphology and ultrastructure of nodules was similar regardless of the SI. Preliminary experiments indicated that nodules in *D. guianensis* were constituted by *Bradyrhizobium* of the "cowpea miscellany".

#### *Non-structural carbohydrates*

In seedlings the total reducing sugars concentration in the cotyledons decreased with plant height, the lowest value detected at SI-12 (Fig. 3). A decrease in the total reducing sugars was also observed in shoots, leaves and roots of seedlings between SI-6 and SI-10. Nodule appearance at SI-10 markedly decreased the total reducing sugars concentration in roots and leaves

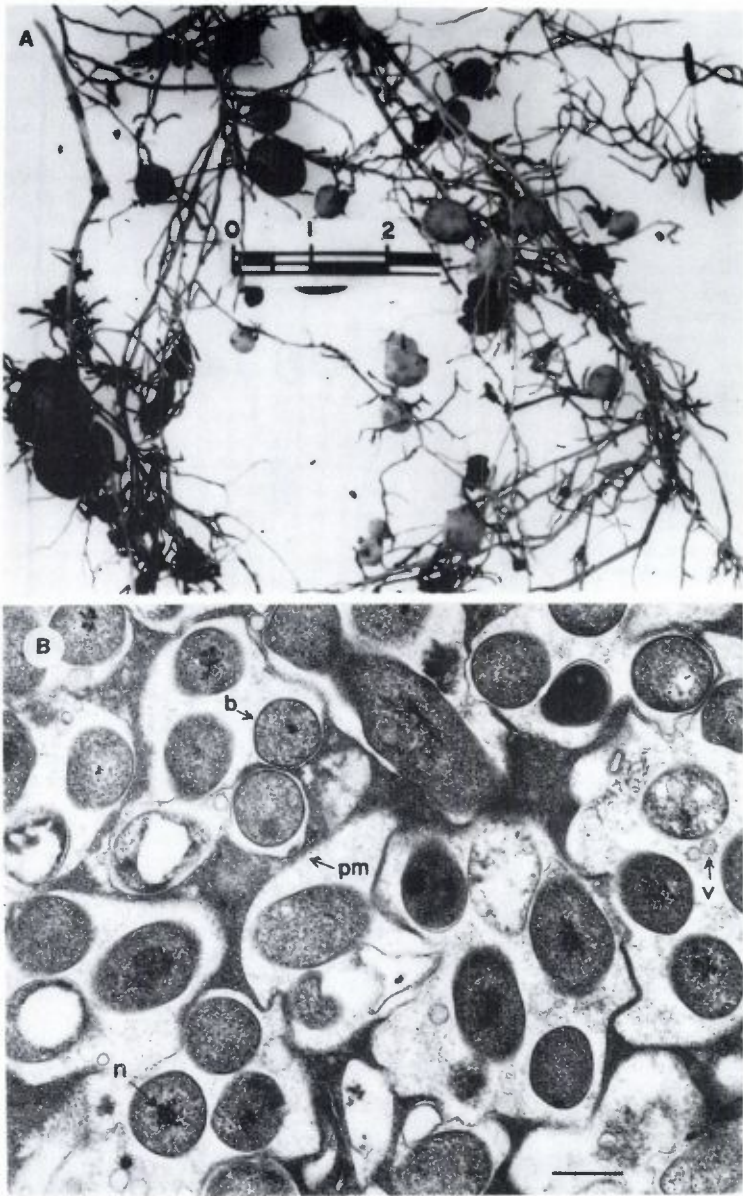


Figure 2. (A), determinate nodules on lateral roots of *Dioclea guianensis* collected in the 0–20 cm depth uppermost soil horizon of the tropical cloud forest. Scale in cm. (B), ultrastructure of the central portion of nodules from *D. guianensis*: b, bacteroids; n, nuclear fibrillar material; pm, peribacteroid membrane; v, vacuoles within the cytoplasm of the bacteroid unit. Bar represent 0.6  $\mu\text{m}$ .

Table 1. Shoot/root ratio and nodule mass in *Dioclea guianensis* plants collected at different size intervals in the tropical cloud forest

Size intervals (cm)	Shoot/root ratio		Nodule mass (mg dry wt/pl)
	Nod <sup>-</sup>	Nod <sup>+</sup>	
SI-6	6.4 a		
SI-8	3.9 b		
SI-10	1.4 c	3.6 a (s)	0.7 a
SI-12	1.4 c	3.6 a (s)	1.9 a
SI-15	1.0 c	2.4 b (s)	2.5 ab
SI-17		1.5 cd	4.2 ab
SI-20		1.9 bc	7.1 b
SI-26		1.2 d	31.0 c
SI-50		1.1 d	52.1 d

Nod<sup>-</sup>, non-nodulated individuals; Nod<sup>+</sup>, nodulated individuals; Numbers in columns followed by the same letter are not statistically different at  $p < 0.01$ . (s), difference between means of Nod<sup>-</sup> and Nod<sup>+</sup> statistically significant at  $p < 0.05$ .

when compared to values in non-nodulated plants at SI-10 to SI-12. In contrast, the formation of nodules did not affect the total reducing sugars concentration in the shoot. At SI > 15 the total reducing sugars concentration in each compartment of nodulated plants remained constant regardless of the SI. In nodules, the highest total reducing sugars concentration was detected at SI-15 followed by a decrease.

The starch concentration in cotyledons also underwent a reduction with increasing plant height (Fig. 4). In leaves and roots of non-nodulated seedlings (SI-6 to SI-10) the starch concentration showed a decrease, followed by a transitory increase between SI-10 and SI-15. A similar situation was detected in nodulated seedlings, although at SI-10 and up to SI-15 the starch concentration was lower than in non-nodulated individuals. From SI-50 up to adults, the starch concentration remained relatively constant. In nodules, the starch concentration decreased with plant height and the lowest value was first detected at SI-26.

#### *Ureides and $\alpha$ -amino-N concentration*

The ureide concentration in cotyledons decreased with the increasing height of seedlings (Fig. 5). In shoots and leaves of seedlings between SI-6 and SI-8 the



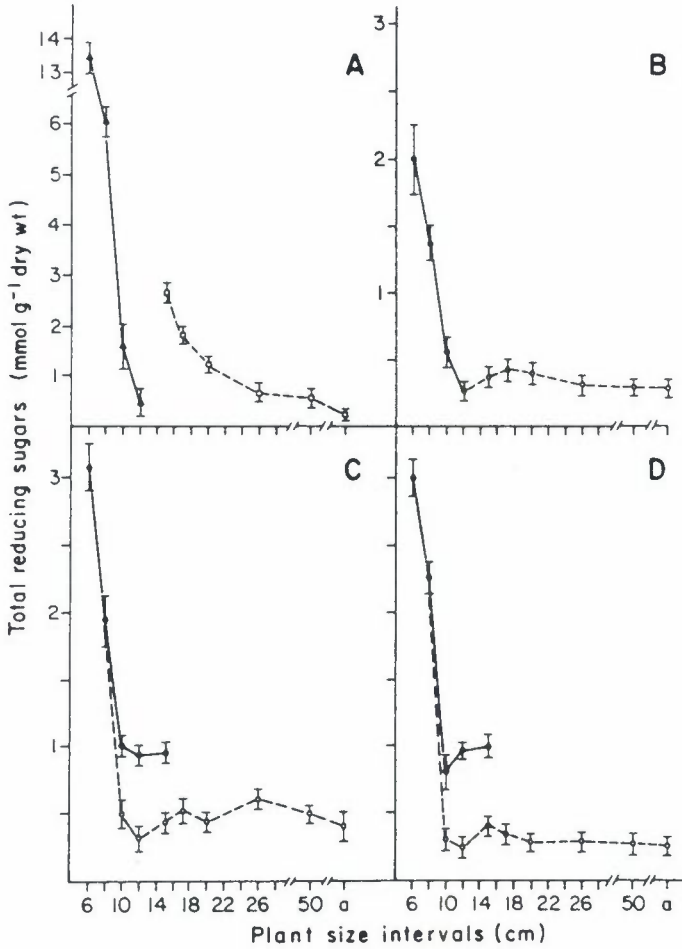


Figure 3. Total reducing sugars content in: (A), cotyledons (line) and nodules (dotted line); (B), shoots; (C), leaves; and (D), roots of *Dioclea guianensis* collected at different size intervals in the understorey of the tropical cloud forest. Size interval a: adults with stem height higher than 5 m. In (B), (C) and (D), dark symbols represent data from non-nodulated individuals and open symbols represent data from nodulated individuals. Results are the mean of at least 6 replicates for each size interval and the bars indicate the standard error of the mean.

ureide concentration showed a slight increase, whereas the concentration decreased progressively in roots. In non-nodulated seedlings and juveniles at SI>10 cm the ureide concentration decreased with plant height. In contrast, a transitory increase in the ureide concentration was observed in shoots, leaves

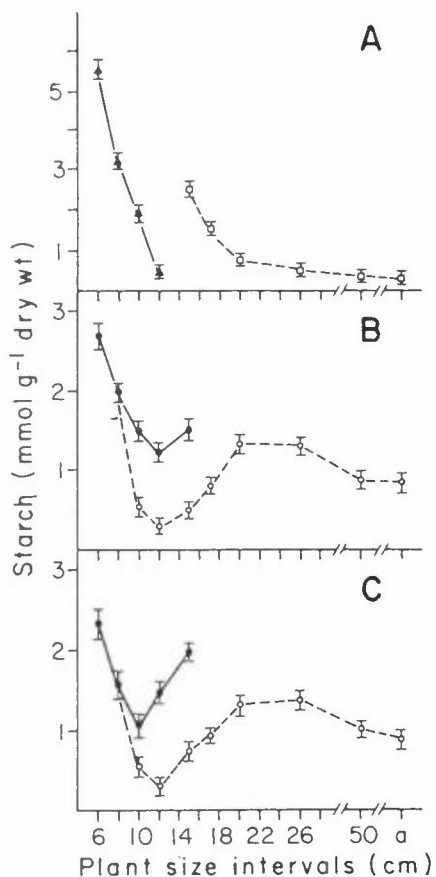


Figure 4. Starch content in: (A), cotyledons (line) and nodules (dotted line); (B), leaves; and (C), roots of *Dioclea guianensis* collected at different size intervals in the understorey of the tropical cloud forest. Size interval a: adults with stem height higher than 5 m. In (B) and (C), dark symbols represent data from non-nodulated individuals and open symbols represent data from nodulated individuals. Results are the mean of at least 6 replicates for each size interval and the bars indicate the standard error of the mean.

and roots of nodulating seedlings at SI-10 and SI-12, followed by a decrease with increasing height of the plants. The lowest ureide concentration in the different plant compartments was recorded in nodulated juveniles at SI-20. During the following SI, the ureide concentration remained constant in leaves and roots. An increase, albeit weak, was detected in shoots of adult individuals. A progressive increase in the ureide concentration was also observed in nodules harvested from juveniles between SI-15 and SI-20. Thereafter, the ureide concentration in nodules decreased up to a constant value.

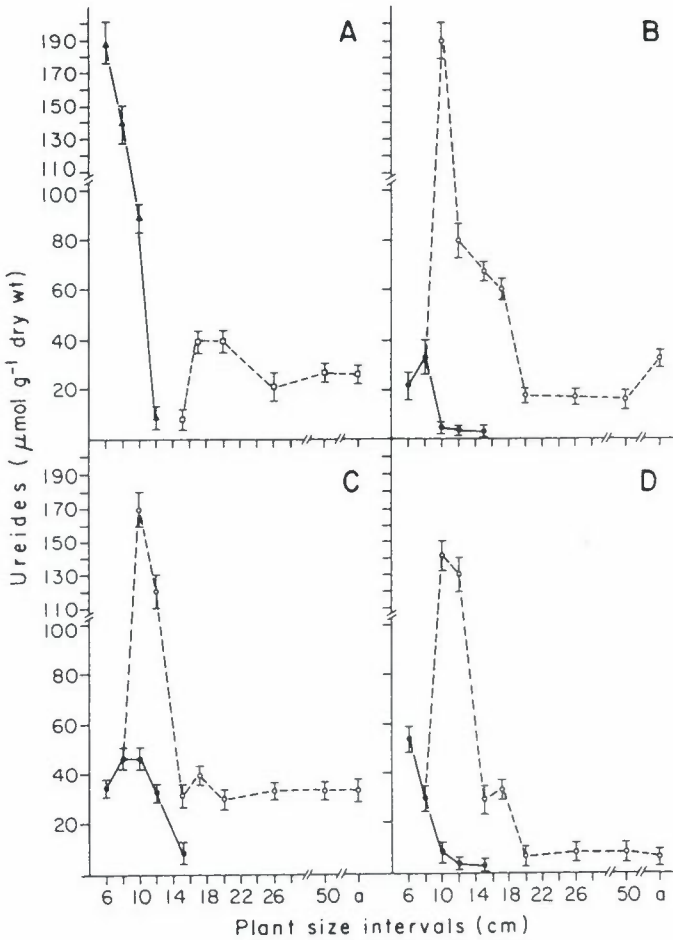


Figure 5. Ureide content in: (A), cotyledons (line) and nodules (dotted line); (B), shoots; (C), leaves; and (D), roots of *Dioclea guianensis* collected at different size intervals in the understorey of the tropical cloud forest. Size interval a: adults with stem height higher than 5 m. In (B), (C) and (D), dark symbols represent data from non-nodulated individuals and open symbols represent data from nodulated individuals. Results are the mean of at least 6 replicates for each size interval and the bars indicate the standard error of the mean.

In cotyledons the  $\alpha$ -amino-N concentration also decreased with the increasing height of the seedlings (Fig. 6). In contrast, the  $\alpha$ -amino-N concentration underwent a temporary increase in shoots, leaves and roots of seedlings at SI-6 and SI-8, followed by a decrease with increasing plant

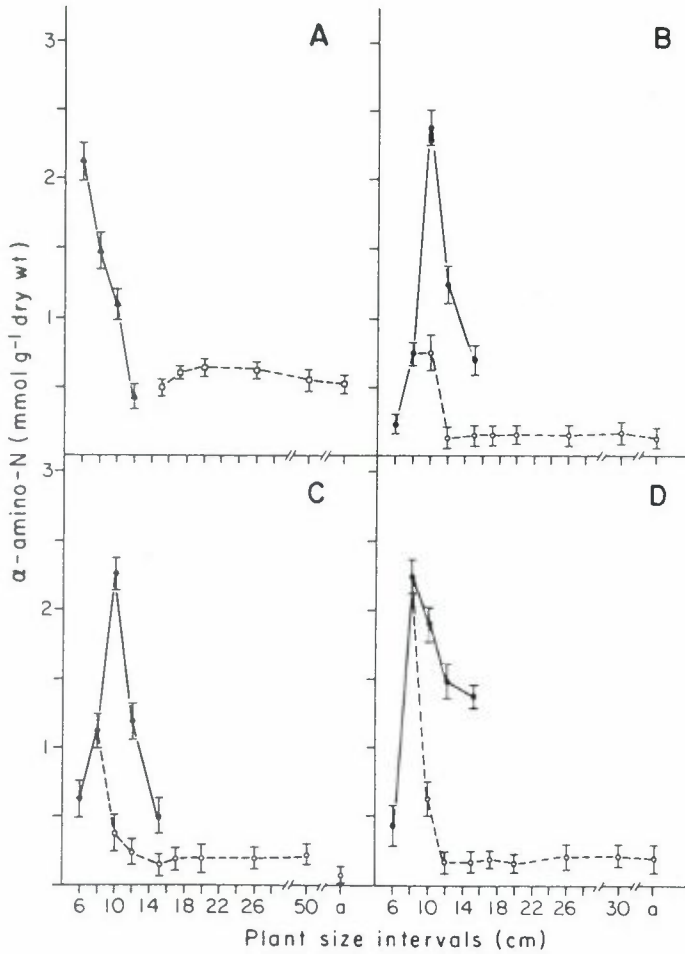


Figure 6.  $\alpha$ -amino-N content in: (A), cotyledons (line) and nodules (dotted line); (B), shoots; (C), leaves; and (D), roots of *Dioclea guianensis* collected at different size intervals in the understorey of the tropical cloud forest. Size interval a: adults with stem height higher than 5 m. In (B), (C) and (D), dark symbols represent data from non-nodulated individuals and open symbols represent data from nodulated individuals. Results are the mean of at least 6 replicates for each size interval and the bars indicate the standard error of the mean.

height. The magnitude of the observed decrease in the  $\alpha$ -amino-N concentration was more marked in nodulated than in non-nodulated seedlings and juveniles between SI-10 and SI-15. In nodulated juveniles at SI>15 the  $\alpha$ -amino-N remained constant in the different plant compartments. Whereas, in nodules there were not major changes in the  $\alpha$ -amino-N concentration with increasing SI.

*Nitrate concentration and relative abundance of ureides (RAU) in shoots*

The nitrate concentration in shoots showed a progressive decrease with increasing plant height up to SI-15 (Table 2). In nodulated seedlings and juveniles between SI-10 and SI-15 the nitrate concentration was significantly lower than in non-nodulated individuals of similar SI. In nodulated juveniles the reduction in the nitrate concentration continued up to SI-26, followed by a significantly progressive increase with increasing plant height. The higher significant value was detected in adults. On the other hand, in nodulated seedlings and juveniles at SI-10 to SI-17 the RAU values averaged 90% (Table 2). Whereas, a significant decrease was detected at SI>20, with no significant differences within the following SI. In adults, RAU values showed a non-significant 12% increase with respect to values at SI-50.

Table 2. Nitrate content and relative abundance of ureides (RAU) in shoots of *Dioclea guianensis* plants collected at different size intervals in the tropical cloud forest

Size intervals (cm)	Nitrate ( $\mu\text{mol/g}$ dry wt)		RAU (%)
	Nod <sup>-</sup>	Nod <sup>+</sup>	
SI-6	223.2 a		
SI-8	150.3 b		
SI-10	92.4 c	66.6 a (s)	91.6 a
SI-12	58.1 d	35.7 c (s)	90.7 a
SI-15	40.2 e	21.8 de (s)	90.1 a
SI-17		18.7 e	89.7 a
SI-20		12.3 e	66.2 b
SI-26		16.4 e	57.5 bc
SI-50		32.0 cd	51.8 bc
Adults		54.8 b	63.5 b

Nod<sup>-</sup>, non-nodulated individuals; Nod<sup>+</sup>, nodulated individuals. Numbers in columns followed by the same letter(s) are not statistically different at  $p < 0.01$ . (s), difference between means of Nod<sup>-</sup> and Nod<sup>+</sup> statistically significant at  $p < 0.05$ .

*Aluminium concentration*

Within the plant compartments Al concentration was detected in roots>>leaves>nodules>shoots (Fig. 7). This pattern of Al distribution was observed regardless of the SI of nodulated individuals.

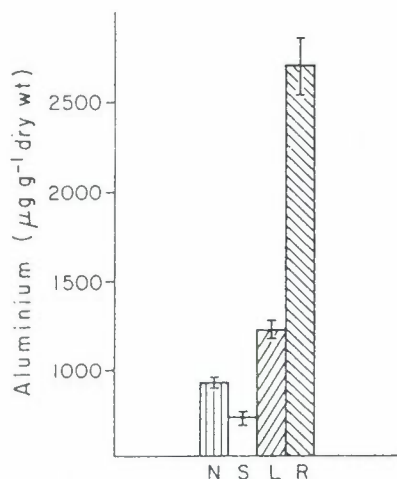


Figure 7. Aluminium content within the different compartments of *Dioclea guianensis* plants collected in a tropical cloud forest. N, nodules; S, shoots; L, leaves; R, roots. Results are the mean of 4 replicates and the bars indicate the standard error of the mean.

#### 4. Discussion

In seedlings between SI-6 and SI-12, the non-photosynthetic cotyledons constituted the main source of ureides, carbohydrates and  $\alpha$ -amino-N for the growing plants. The export of nitrogen and carbon compounds from cotyledons to sink organs occurred throughout the growth of the seedlings, and the early events of nodule formation did not enhance the rate of translocation to sink organs. Previous results have shown that under low light conditions the sink manipulations in cucumber seedlings did not bring about alterations in the non-structural carbohydrate concentration of cotyledons (Mayoral et al., 1985). In seedlings of *D. guianensis* the transfer of substrates from the cotyledons to other organs was largely completed at SI-12, and there was a coordination between the depletion of cotyledon reserves and the beginning of the symbiotic nitrogen fixation process at SI-10. Due to this synchronization, nodulated seedlings and juveniles did not experience a period of nitrogen stress. The establishment of the symbiotic process also appears to be related to the survival of seedlings, since all dead seedlings and juveniles were not nodulated. At present, the possibility that the absence of nodules in all dead individuals was a consequence of the damage to seedlings (Herdina and Silsbury, 1992) by insects

or herbivory (Kitajima, 1994; Christensen et al., 1995) rather than the cause of the seedling mortality could not be ruled out.

In seedlings with cotyledons nearest to abscission and in juveniles nodule formation constituted by far the stronger sink within the growing plant, and leaves were the main source of assimilates for the increasing nodule mass. This assumption was supported by the lower  $\alpha$ -amino-N and non-structural carbohydrate concentration in leaves and roots of nodulated seedlings and juveniles when compared to non-nodulated ones at similar SI. Based on present results, the carbohydrate requirement for nodule growth in seedlings at SI-10 to SI-12 was estimated to be around 1.3 mmol/g leaf dry wt. The strong sink strength of nodules has been reported in *Vigna unguiculata* (Izaguirre-Mayoral et al., 1992b), *Phaseolus vulgaris* (Izaguirre-Mayoral et al., 1994) and in seedlings of *Gliricidia sepium*, a native tree of tropical forests (Thomas et al., 1991).

With the onset of the nodulation process seedlings and juveniles changed from  $\alpha$ -amino-N producers to ureide metabolizing individuals. In nodulating seedlings at SI-10 to SI-12 the high ureide concentration in the different compartments must be the result of an active nitrogen fixation in nodules and the rapid export of the N-products to aerial organs. In contrast, the high  $\alpha$ -amino-N concentration in non-nodulated seedlings and juveniles could be partially explained by a switch from sucrose to amino-acid synthesis mediated by the activation of protein kinases (Plaut et al., 1987, Champigny and Foyer, 1992), since understorey plants have very low leaf glutamine synthetase activity (Medina and Cuevas, 1994). On the other hand, the high nitrate concentration in shoots of non-nodulating seedlings and juveniles at SI-6 to SI-15 may point toward an induction of the nitrate reductase as an alternative way to fix soil nitrogen in the absence of nodulation. However, this proposed metabolic nitrate reduction in non-nodulated seedlings and juveniles was not supported by: a) the predominance of  $\text{NH}_4^+$  in the soil, an inhibitor of nitrate reductase (Botella et al. 1993), b) the preferential uptake of  $\text{NH}_4^+$  by soybean plants grown in a pH 5 solution containing 2:1  $\text{NH}_4^+:\text{NO}_3^-$  ratio (Raper et al., 1991), c) the low nitrate reductase activity reported in understorey forest plants (Stewart et al., 1993, Medina and Cuevas, 1994), and d) the apparent mortality of all non-nodulated individuals. Thus, it is tempting to speculate that under the cloud forest conditions the growth of *D. guianensis* is fully dependent on the establishment of the symbiotic nitrogen fixation process.

The marked dependence of seedlings and juveniles on the symbiotic nitrogen fixation was also ascertained by the high RAU values (>90%) detected at the early stages of plant development and by the significantly higher allocation of dry mass to above rather than to below-ground organs in nodulated when compared to non-nodulated individuals at SI-10 to SI-15. Alterations in dry

mass allocation brought about by nodule formation has been reported in seedlings of *Alnus rubra* (Arnone and Gordon, 1990). Nevertheless, these dependence of the plants on the nitrogen fixation process apparently diminished progressively with growth. At SI>20 cm RAU was reduced to about 60% in spite of the increasing nodule mass in juveniles and of the massive clusters of nodules detected on roots of all adults sampled. This observed ontogenetic decrease in the RAU values was ascribed to an increase in the nitrate concentration with increasing plant height rather than to a decrease in the amount of ureide detected in nodules and shoots of plants at SI>26 cm. The progressive inadequacy of the symbiotic nitrogen fixation in meeting the requirements for nitrogen with increasing age has been demonstrated in the understorey species *Acacia pulchella* and *A. alata* (Hansen and Pate, 1987). However, there is a possibility that the assumed insufficiency of the symbiotic process to meet the nitrogen requirements of adult plants is the result of an underestimation of RAU due to the dilution of the ureides along the stems. This point requires further investigation.

From the present data four major conclusions can be drawn: 1) *D. guianensis* might be considered as a shade-tolerant species because: a) nodulated seedlings as well as juveniles were confined to the understorey stratum of the forest; b) adults either unable to reach nearest trees or fallen from higher branches grew intertwined with other species in the understorey stratum; and c) adults growing vertically on tress still developed leaves in the lowest strata of the forest. This classification was further supported by the photoinhibition undergone by seedlings and juveniles subjected to full sunlight (data not shown) and by the high RAU values detected in nodulated plants at SI-10 to SI-17; 2) according to RAU values this species can be classified as a good N<sub>2</sub>-fixer, with symbiotic nitrogen fixation rates similar to that of native legumes collected in a neotropical savanna (Sicardi de Mallorca and Izaguirre-Mayoral, 1993) and to that reported for the understorey legume tree *Bossiaea laidlawiana* (Grove and Malajczuk, 1992); 3) *D. guianensis* might also be categorized as an Al-accumulator species based on the high Al concentration in mature leaves (>1,000 ppm) (Cuenca et al., 1990). In this species, the pattern of Al accumulation mainly in roots and leaves and the partial exclusion of this ion from nodules may be the probable mechanisms underlying the efficiency of the symbiotic nitrogen fixation process under the deleterious soil conditions of the cloud forest; and 4) from the homogeneous external and ultrastructural morphologies of nodules formed in seedlings, juveniles and adults it might be inferred that plants are nodulated by only one type of *Bradyrhizobium* cells. In native legumes, the coinfection of roots by different rhizobial strains usually causes the concomitant appearance of determinate and indeterminate nodules with contrasting external colors. This proposed low diversity of native



rhizobial strains in the forest soils could be a consequence of the small abundance of forest legumes (Sicardi de Mallorca and Izaguirre-Mayoral, 1994).

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