

## **Preliminary data on: QUANTIFICATION OF THE CARBON SUMP EFFECT BY GUADUA (*Guadua angustifolia* Kunth)**

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### **INTRODUCTION**

In Colombia *Guadua angustifolia* Kunth (Poaceae: Bambusoideae) covers an approximate area of 51.521 has, 46.261 are spontaneous and 5,260 are cultivated. The optimal growing area is the Andean region where they form plant communities called "guadales". In the central-western region of the country, *G. angustifolia* has played an important role in the history, culture and economics. Records of its existence go as far back as the pre-Columbian times; today its growing area has been reduced to the river and creek basins. *Guadua angustifolia* was used by the settlers of the coffee region to build their towns, and by farmers as a substitute for lumber to build the facilities of their farms and other purposes. With this bamboo, farmers have been able to preserve water sources and have obtained additional income from selling it and savings by reducing construction costs.

*Guadua angustifolia* is considered to be one of the three largest species of bamboo, and one of the 20<sup>th</sup> worldwide most important because its physical-mechanical properties as mechanical strength of its wood which makes it an ideal construction material substituting the use of another traditional wood source in a large percentage. In addition, it could be industrialized in long life products such as furniture, crafts, agglomerates, laminates, floors, fabrics, etc (Londoño, 1998a).

*Guadua angustifolia* is a fast developing monocotyledon that grows up to 11 cm per day. Its optimal growth conditions are locations with an altitude between 500 – 1500 m, rainfall of 1200-2500 mm year<sup>-1</sup>, temperatures between 18 and 24 °C, relative humidity of 80 – 90 % (Londoño, 1998b).

The most outstanding aspects of research indicate that *G. angustifolia* emerges from the ground with an established diameter within a range of 6 - 22 cm, and reaches its full height in the first six months of growth. It achieves maturity between 4-5 years. The ideal composition of culms (stems) in a "guadual" has been estimated to be 10% new shoots, 30% young culms, 60% mature and over mature culms and 0% dry ones, with a plant density of 3,000 to 8,000 culms per hectare. The culms have an inverse relationship between density and diameter size (Londoño, 1998b; CORPOCALDAS, 1997; CVC, 2000). It has been reported that *Guadua* add 10 tons per ha<sup>-1</sup> year<sup>-1</sup> of biomass to the soil.

Dry matter accumulation was 76.6 tons including culms, branches and leaves when there was a total of 2,607 culms per hectare (De Wilde, 1993).

With an average production of 1,000 culms per hectare per year (Castaño, 1993) the total amount of *G.angustifolia* culm production for the four central-western coffee region departments, can be calculate to be 20,3 million of green stocked hollow culms per year, equivalent to 911,745 tonne per year in green condition (45 T-m/ha).

We did not find reports in the literature about growth as dry matter accumulation and distribution, total leaf area, leaf area emission and leaf area duration. In general, we could not find any analysis of classical growth that would allow us to carry out mathematical modeling. We did not find data on leaf photosynthetic activity or whole plant gas exchange measurements for Guadua.

Renewable biomass grown as a carbon sump could be handled in short rotation shifts, using biomass for long life consumption goods production and for construction. That would in turn generate local job sources and income for the poorest sectors of the population in a quick and continuous way. Natural areas of non disturbed forests, generally associated to fragile ecosystems, could also be preserved from deforestation, which would guarantee their existence and to allow preservation of biodiversity in their areas of influence.

The main objective of this research is to develop a growth model for photosynthesis and biomass accumulation in the aerial and underground portion of *Guadua angustifolia*. We present the data collected during the first months of this research for several aspects related with dry matter accumulation, leaf area emission and enzyme activities for Rubisco and PEPC the most important enzymes for carbon assimilation in plants with C<sub>3</sub> and C<sub>4</sub> metabolism.

## **METHODS**

### **Location**

Field work was carried out outdoors at the central Colombian coffee region (Caldas, Quindío and Risaralda departments), and laboratory research at Centro Nacional de Investigaciones de Café, CENICAFE (Chinchiná, Colombia). Headquarters.

### **Plant material and growth conditions:**

#### **1. New sowing with nursery plantlets**

*Guadua angustifolia* plantlets were cultivated under nursery conditions during 3 months. Then, they were transplanted to the field with a distance of 5 m between plants and 5 m between rows, for a total plot area of 5000 m<sup>2</sup>. Three plots were established at Balsora Farm (La Tebaida - Quindío) (04° 26 N, 75° 50 W, 1150 m of altitude), San Jorge Farm (Pereira - Risaralda) (04° 49 N, 75° 50 W, 1240 m of altitude) and Naranjal Central Research Station (Chinchiná – Caldas) (04° 59 N, 75° 39 W, 1400 m of altitude).

Up to date, two destructive random samples have been taken from each plot and the following variables have been recorded: number and length of culms, total foliar area using a leaf area measurement system (Delta T device). Rhizomes and fine roots were carefully extracted from the ground and washed with tap water.

Fresh tissue was dried (80 °C) up to constant weight. Sample density was determined following water displacement of both fresh and dry tissue picked from lower, middle and upper culm portion using a calibrated container.

With the information collected every 3 months, until 5-year-old, we expect to construct at the end of the research a growth curve and determine the potential carbon sequestration by guadua from a new plantation following classical growth analysis and mathematical modeling.

## **2. Growth of *Guadua angustifolia* from new shoots in established guadua communities (guadales).**

At two sites: Naranjal and San Jorge we have selected and labeled 100 renewals in established guadales, which have been measured using the following methodology:

Each month until six months old, 5 new shoots are selected randomly. At present only 2 random samples have been picked for measuring the following variables: Total length, diameter for each section (the culm have been cut in three similar length sections), fresh weight, and 10 cm culm subsamples for dry weight determination. Also we have determined culm leaves and rhizome fresh and dry weight.

## **3. Activities of Ribulose 1,5 bisphosphate carboxylase – oxygenase (Rubisco) and Phosphoenol pyruvate carboxylase (PEPC) from foliage leaves tissue.**

One g of foliage leaves tissue was ground in a chilled mortar with 8 ml of modified Palmer (1986) extraction buffer 0.05 M Tris-HCl, pH 8.0, 0.35 M sorbitol, 0.005 M EDTA, 0.005 M  $\beta$ -mercaptoethanol, and 3 % w/v PVP-40. The macerate was filtered through 4 layers of cheesecloth and an aliquot was taken for total chlorophyll determination (Wintermanns and de Mots 1965). The remaining filtrate was centrifuged in an Eppendorf 541-C microcentrifuge at 4°C and 8000 rpm for 8 min. One ml of supernatant was filtered through a Biorad Econopac 10DG column equilibrated with elution buffer (0.1 M HEPES-KOH, pH 7.5, 0.0005 M EDTA, 0.01 M magnesium acetate, 0.005 M DIECA, 5% v/v glycerol, 0.05% Triton X-100, 0.02 M  $\beta$ -mercaptoethanol, 0.005 M DTT, and 3 % (w/v) PVP-40) (Angelov *et al.* 1993). The eluate was also used to determine the total soluble protein concentration (Bradford 1976).

**PEPC and Rubisco assays:** PEPC activity was determined in a Perkin-Elmer Lambda 3B spectrophotometer with 1 ml of 0.1 M HEPES-KOH, pH 8.0, 0.0005 M EDTA, 0.001 M MgCl<sub>2</sub>, 0.001 M DTT, 0.01 M NaHCO<sub>3</sub>, 0.0002 M NADH, and 5 units of pig heart MDH. Fifty  $\mu$ l of the extract were incubated during 10 min at 35°C and the reaction was

started with 0.0004 M of PEP (Angelov *et al.* 1993). Rubisco activity was determined in 1 ml of 0.1 M HEPES-KOH, pH 7.8, 0.0005 M EDTA, 0.01 M MgCl<sub>2</sub>, 0.001 M DTT, 0.005 M phosphocreatine, 0.0002 M NADH, 0.01 M NaHCO<sub>3</sub>, 0.001 M ATP, 2 units of phosphoglycerate phosphokinase, 2 units of glyceraldehyde 3-phosphate dehydrogenase, and 1 unit of creatine phosphokinase, as modified from Usuda (1984). The enzyme (50 µl of the extract) was incubated for 10 min at 25 °C and the reaction was started with 0.0005 M RuBP (Angelov *et al.* 1993). In both cases, enzyme activity was determined following NADH oxidation at 340 nm with an extinction coefficient of 6.22 x 10<sup>3</sup> M (Segel 1976).

### **Electrophoretic PEPC detection on non denaturing polyacrilamide gel**

One g of leaf tissue from *Saccharum* spp cv POJ 2878, *Guadua angustifolia* and *Coffea arabica* L. were grinded and extracted as described above. Electrophoresis of 25 µg of total protein per lane was performed on a non denaturing 8.0 % polyacrilamide gel under 150 V and 40 mA during 1.5 hours at 4 °C. After electrophoresis the gel was incubated 15 min with 20 ml buffer [Tris-HCl 0.1 M pH 8.0, MgSO<sub>4</sub> 0.03 M, KHCO<sub>3</sub> 0.04 M, 100 mg PEP (monocyclohexilammonium salt)]. Oxalacetate formation on the PEPC gel bands was revealed adding 60 mg Fast Violet B salt after incubation (Vidal *et al.*, 1976). Red brick bands appeared after 20 min. No molecular weight markers were used in this experiment. The other gel was stained for total proteins with Coomassie R 250.

## **RESULTS and DISCUSSION**

### **Growth characteristics of *G. angustifolia* in two different farms.**

#### **1. New sowing with nursery plantlets**

At San Jorge, three months after planting plantlets of *G. angustifolia* reached 19.6 stems (6.5 stems month<sup>-1</sup>) meanwhile at Balsora 8 months after planting stem number reached 49 (6.1 stems month<sup>-1</sup>). It seems that shoots developing have the same rate in both places. The same behavior is registered for leaf developing because no difference is found for the leaf apparition in both places (29.3 leaves month<sup>-1</sup> at San Jorge vs. 30.25 leaves month<sup>-1</sup> at Balsora). Nevertheless, leaf area grew more readily at Balsora (325.4 cm<sup>2</sup> month<sup>-1</sup> vs. 256.5 cm<sup>2</sup> month<sup>-1</sup> at San Jorge). This result can be explained because leaf area growth rate must be more rapid after three months after planting. Last result is agreed with the leaf dry weight growth rate. In effect, at Balsora, presumably samples were chosen when guadua reaches the maximum rate of growth (1.38 g leaf tissue month<sup>-1</sup> vs. 1.16 g leaf tissue month<sup>-1</sup> at San Jorge). Differences between growth behavior of whole plant dry weight (6.63 g month<sup>-1</sup> at San Jorge vs. 5.88 g month<sup>-1</sup> at Balsora) can be explained because growth of dry weight of roots at Balsora is lower than at San Jorge. Eight months after planting, specific leaf area growth is 50% lower at Balsora than at San Jorge three months after planting (1.28 cm<sup>2</sup> g<sup>-1</sup> month<sup>-1</sup> vs. 2.56 cm<sup>2</sup> g<sup>-1</sup> month<sup>-1</sup>). No differences for another variables are found in this experiment (Table 1).

## 2. New shoots in established guadua communities (guaduales).

Culm height at San Jorge grows  $1.53 \text{ m month}^{-1}$  (Table 2). It seems that at San Jorge the new shoots grow using dry matter stored in the roots because after a month root dry weight is  $323.7 \text{ g}$  lower. Meanwhile, dry matter of stems and culm leaves is higher. No differences are found for culm diameter, root tissue and stem tissue density after a month of age at San Jorge. Height growth rate at Naranjal is faster than at San Jorge but this variable is measured in guadua of different ages. Remarkably, at Naranjal, root and leaf culm dry matter grows faster than stem dry matter during the time of observation. As registered for San Jorge, no differences are found for culm diameter, root tissue and stem tissue density.

## 3. Chlorophyll and protein content

Leaf tissue chlorophyll content was higher in *Coffea arabica* ( $4.37 \text{ mg Chl g}^{-1}$  fresh weight) than in *Saccharum* spp ( $3.11 \text{ mg Chl g}^{-1}$  fresh weight) and *Guadua angustifolia* ( $2.62 \text{ mg Chl g}^{-1}$  fresh weight). These results are similar to those registered in literature which show that consistently chlorophyll content in Poaceae is lower than in broad leaf dicots (López et al, 2000) (Fig. 1a). On the other hand, it is expectable that chlorophyll content in herbaceous monocots be higher than in the woody ones. Protein content in leaf tissue was determined following Bradford (1976). Leaf tissue protein content of *G. angustifolia* ( $25.37 \text{ mg g}^{-1}$  fresh weight) is higher than those of *Saccharum* spp. ( $16.96 \text{ mg g}^{-1}$  fresh weight) and *C. arabica* ( $14.06 \text{ mg g}^{-1}$  fresh weight). No data about leaf protein content of *G. angustifolia* are found in the literature. Nevertheless, in spite of this, it is amazing that in more than four different experiments carried out, consistently protein content in *G. angustifolia* leaves be higher than in *C. arabica* and *Saccharum* spp. Differences in protein content between *C. arabica* and *Saccharum* spp. are in agree with data reported by López et al (2000) for *C. arabica* (C<sub>3</sub>) and *Zea mays* (C<sub>4</sub>) (Figure 1b).

## Enzyme activities of PEPC and Rubisco

Carboxylating activity of PEPC in leaf tissues of sugarcane (C<sub>4</sub>) ( $32.76 \text{ } \mu\text{mol PEP g}^{-1}$  fresh weight  $\text{min}^{-1}$ ) is very high but it drops into the range observed for C<sub>4</sub> plants. Surprisingly, PEPC activity in leaf extracts of *G. angustifolia* ( $3.49 \text{ } \mu\text{mol PEP g}^{-1}$  FW  $\text{min}^{-1}$ ) represents 10.6 % of PEPC activity in sugarcane. This value is very high if we suppose that *G. angustifolia* is a C<sub>3</sub> plant. Similar values are reported for C<sub>3</sub>-C<sub>4</sub> intermediate plants. Nevertheless, such high activities of PEPC in *G. angustifolia* not necessarily can be taken as a criterion to include it into the C<sub>3</sub>-C<sub>4</sub> intermediate plants. On the other hand, *C. arabica* PEPC activity values are similar to those found in C<sub>3</sub> plants (under 1% of C<sub>4</sub> plant PEPC activity) (Figure 1c). No significative differences for Rubisco activities were found between sugarcane, *G. angustifolia* and coffee, although Rubisco activity in sugarcane is unusually high for a C<sub>4</sub> species (Figure 1d). Sugarcane PEPC/Rubisco activity ratio (5.22) is typical for a C<sub>4</sub> species. The same ratio in *G. angustifolia* (0.54) is higher than in *C. arabica* (0.15), a C<sub>3</sub> species (Figure 1e). This result can be explained if we assume that *G. angustifolia* PEPC activity is the product of a more active form of the enzyme in the leaf tissue of this species.

Behavior of PEPC activity on polyacrilamide gel is more conspicuous. In effect, results of two experiments show that PEPC activity is detected on the gel only for sugarcane and *G. angustifolia* lanes meanwhile no PEPC activities were detected for *C. arabica*, ( $C_3$  species)(Figure 2). Nevertheless, the relative position of both PEPC bands in the gel suggests that *G. angustifolia* PEPC molecular mass is higher than the molecular mass of sugarcane PEPC (~ 400000 Da). This result presumably can explain the observed differences in PEPC activity between the three species.

## LITERATURE CITED

Angelov, M.N., Sun, J., Byrd, G.T., Brown, R.H., Black, C.C. 1993, Novel characteristics of cassava *Manihot esculenta* Crantz, a reputed  $C_3$ - $C_4$  intermediate photosynthesis species. *Photosynthesis Research*, vol. 38, pp. 61-72

Bradford, M.M., 1976, A rapid and sensitive method for the quantitation of micrograms quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, vol. 72, pp. 248-254

Castaño, F. 1993. La silvicultura de la guadua en Colombia. Pp. 69-72 in *Memorias del primer Congreso Mundial Bambú/Guadua*, Pereira, Colombia, 8-15 Agosto 1992. SENA, Bogotá.

Corpocaldas. 199?. La Guadua. Planta emblema de Caldas. Propagación, manejo y utilización. p. 12.

Corporación autónoma regional del valle del cauca - CVC. 2000, Taller sobre manejo y utilización del recurso Guadua. Proyecto Guadua CVC – BID. Subdirección de patrimonio ambiental grupo bosques. p. 6.

De Wilde, A. 1993, Informe final proyecto Guadua – CARDER, subproyecto granja experimental La Pedrera. p. 23.

Edwards, G.E., Ku, M. 1987, Biochemistry of  $C_3$  -  $C_4$  intermediates. In *The Biochemistry of Plants*. V.10. Ed. P.K. Stumpf & E.E. Conn. Academic Press, New York. pp. 276-325.

Londoño, X. 1998a, Evaluation of Bamboo Resources in Latin America. Final report of project No. 96-8300-01-4 for International Network for Bamboo and Rattan. p. 19 –35.

Londoño, X. 1998b, A Decade of Observations of a *Guadua angustifolia* Plantation in Colombia. *Journal of American Bamboo Society*. vol. 12, pp. 37 – 43.

Lopez, Y., Riaño, N., Mosquera, P., Cadavid A., Arcila, J. 2000, Activities of phosphoenolpyruvate carboxylase and ribulose-1,5-bisphosphate carboxylase/oxygenase in leaves and fruit pericarp tissue of different coffee (*Coffea* sp.) genotypes. *Photosynthetica*, vol. 38 no. 2, pp. 215-220

Vidal, J., Cavalie, G., Gadal, P. 1976, Etude de la phosphoenol-pyruvate carboxylase du haricot et du sorgho par electrophorèse sur gel de polyacrylamide. Plant Science Letters. Vol. 7, pp. 265-270

Wintermanns, J.F.G.M., De Mots, A. 1965, Spectrophotometric characteristics of chlorophyll and their pheophytins in ethanol. Biochemistry and Biophysics Acta. Vol.109, pp. 448-453

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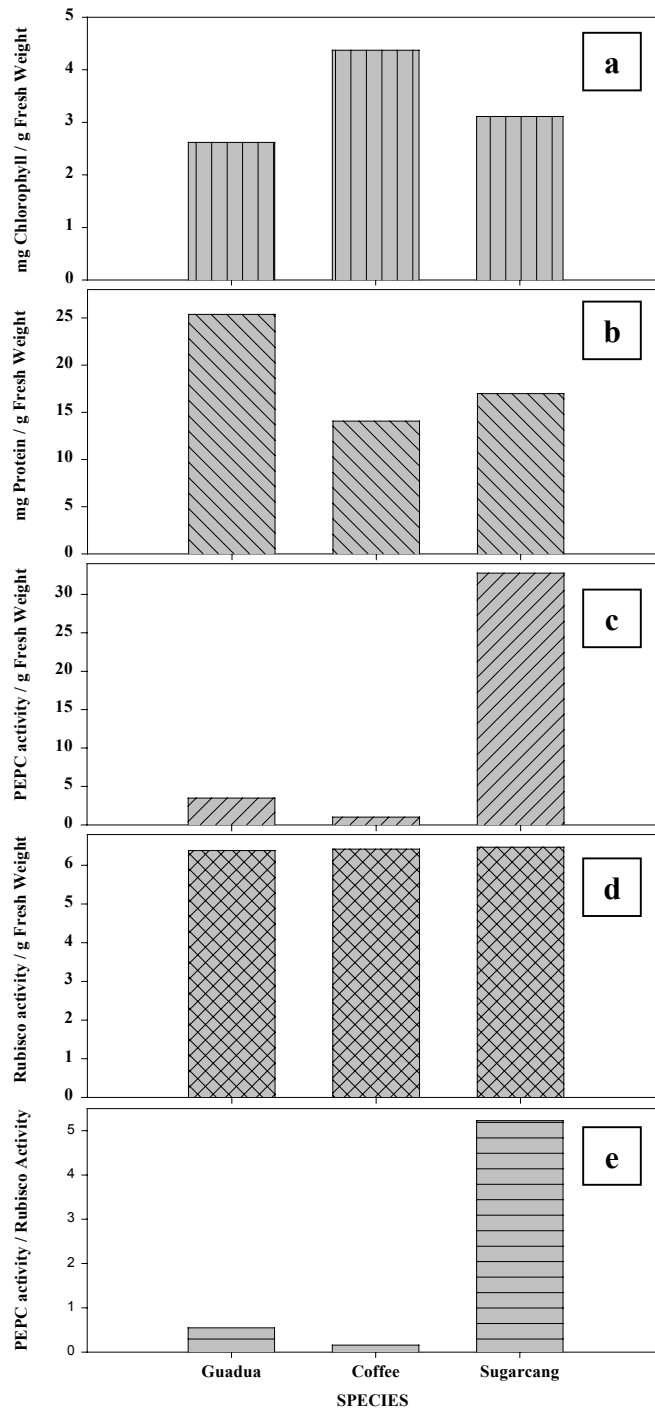
**Table 1.** Growth characteristics of *Guadua angustifolia* plantlets in two different farms.

FARM	Age after planting	Stem	Leaves	Leaf area	Leaf D.W.	Stem D.W.	Root D.W.	Whole Plant D.W.	Leaves/Stem	Specific leaf area	Leaf D.W. ratio	Stem D.W. ratio	Root D.W. ratio
	Months	No.	No.	cm <sup>2</sup>	g	g	g	g	No.	cm <sup>2</sup> g <sup>-1</sup>	g g <sup>-1</sup>	g g <sup>-1</sup>	g g <sup>-1</sup>
<b>San Jorge</b>	3	19.6	88	769.6	3.5	4.1	12.3	19.9	4.4	7.7	0.1	0.2	0.7
<b>Balsora</b>	8	49	242	2571.4	11.04	13.82	22.22	47.1	4.8	10.24	0.18	0.24	0.58

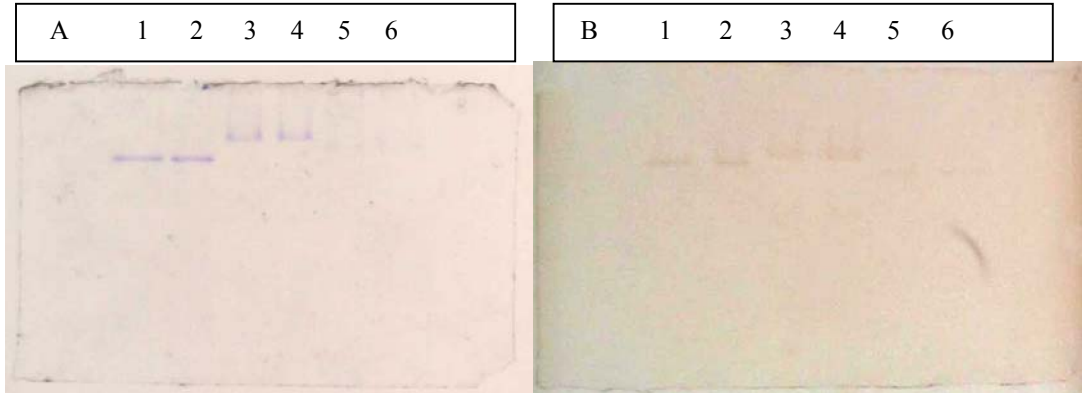
**Table 2.** Growth characteristics of *Guadua angustifolia* new shoots in two different farms.

FARM	Time	Height	Root D.W.	Stem D.W.	Leaf culm D.W.	Leaf D.W. ratio	Stem D.W. ratio	Root D.W. ratio	Culm Diameter	Root tissue density	Stem tissue density
		m	g	g	g	g g <sup>-1</sup>	g g <sup>-1</sup>	g g <sup>-1</sup>	Cm	g cm <sup>-3</sup>	g cm <sup>-3</sup>
<b>San Jorge</b>	1	0.28	2039.2	71.6	355.6	0.78	0.03	0.19	9.83	1.06	1.0
	2	1.81	1715.5	1404.4	518.3	0.44	0.42	0.15	10.8	1.01	0.98
<b>Naranjal</b>	1	1.67	2896	1352.9	355.6	0.62	0.3	0.08	11.84	0.99	0.97
	2	3.93	3076.6	4270	1111.8	0.37	0.5	0.13	11.93	1.01	1.02





**Figure 1a** – Leaf chlorophyll content; b – Leaf protein content; c – Leaf PEPC activity; d.- Leaf Rubisco activity; e – PEPC / Rubisco ratio, in *Guadua angustifolia*, *Coffea arabica* L. and *Saccharum* sp.



**Figure 2.** (A) Native polyacrylamide gel electrophoresis stained with Coomassie R-250. (B) Native polyacrylamide gel electrophoresis stained with Fast Violet, specific for PEPC activity. Lanes 1,2 Sugarcane; lanes 3,4 Guadua; lanes 5,6 Coffee.