Morphological characterization of Gliricidia sepium *and bromatological composition and sugar proportion in its flowers*

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ABSTRACT

The study was conducted at the Pasture and Forage Research Station Indio Hatuey, in order to determine the morphological characteristics of *Gliricidia sepium*, as well as the bromatological composition and sugar proportion in its flowers. Twenty of 165 *G. sepium* trees were sampled, which represented 12 % of the total, and each tree constituted a replication. The experimental design was completely randomized and the following morphological variables were measured: total plant height, stem diameter at breast height (DBH) and crown width. In addition, the number of flowers per tree and per hectare was estimated. In the collected flowers the bromatological composition was determined: DM, CP, CF and Ca, moisture and Klason lignin. The mean values were: DBH, 44,7 cm; height, 4,85 m; 295 racemes, with 34 flowers –as average– each, per tree and an estimated total of 10 030 flowers per tree and 8 986 880 flowers per hectare. The DM was 8,69 %; CF, 32,57 %; Klason lignin, 28,8 %; CP, 7,04 %; and Ca, 0,99 %. The simple sugars detected were: glucose (4,82 %) and fructose (10,36 %), while sucrose showed values of 1,73 %. It is concluded that all the sampled *G. sepium* trees were typical of the species. The DM percentage of their flowers was low, but the content of CF, CP, Klason lignin and calcium was high. Fructose was the most abundant sugar in the extract and sucrose was the scarcest.

Key words: chemical composition, flowers, *Gliricidia sepium*

INTRODUCTION

Gliricidia sepium (Jacq.) Kunth ex Walp. is a tree which belongs to the subfamily *Faboideae*, family *Leguminoseae* or *Fabaceae*, order Fabales (Sablón, 1985). In Cuba, the genus *Gliricidia* is monotypical, because *G. sepium* is its only representative species. From the edaphic point of view, it tolerates a wide range of soils, from pure sands to deep black vertisols, with a pH from 4 to 7. However, it has been observed to have little survival on lands with bad internal drainage, as well as on extremely acid soils, with high aluminum content (Barreto, 1990).

According to Simons (1996), *G. sepium* shows adequate growth at temperatures between 20,7 and 29,2 ºC; although it could reduce its growth and be defoliated if they are lower than 15 ºC. In addition it is a heliophilous plant which is affected when there are other plants that compete with it for the light.

At present it is considered a multipurpose tree, due to its usefulness depending on the phenotype, chemical composition and the edaphoclimatic conditions under which it grows. The *G. sepium* plants are usually used as transitory or permanent shade, as living post and in weed control in cacao, coffee and tea crops. The extract of its leaves has allelopathic effects, for which it influences the germination and growth of some plants (Rodríguez *et al*., 1994). Its flowers are edible for man, and are very useful for honey production and as ornament; while the seeds and bark, pulverized and mixed with rice, show rodenticide qualities (Roig, 1974).

According to Sardiñas (2009), this species is naturalized in all the Cuban geography. Nevertheless, its use has been mainly limited to posts for fences in livestock production areas and as shade in coffee plantations, and it is almost inexistent in silvopastoral systems, in which *Leucaena leucocephala* prevails. It is not used in the production and use of green manures either.

Most of the studies about *G. sepium* have been aimed at such structures as the root, stem and leaves. Yet, the bromatological components and the sugar content in typical flowered trees practically have not been studied. For this reason, the objective of this research was to determine the morphological characteristics of *G. sepium* trees, as well as the bromatological composition and sugar proportion in the flowers.

MATERIALS AND METHODS

Location. The trial was conducted in the Pasture and Forage Research Station (EEPF) Indio Hatuey, located at 22º 48' and 7" North latitude and 79º 32' and 2" West longitude, at 19 m.a.s.l., in the Perico municipality, Matanzas province, Cuba (Academia de Ciencias de Cuba, 1989).

Edaphoclimatic conditions. Table 1 shows the main climate characteristics in the experimental area during the flower collection and morphological characterization of the trees.

The soil has plain topography, with a slope from 0,5 to 1,0 %; and it is classified, according to Hernández *et al.* (2003), as lixiviated Ferralitic Red, hydrated ferruginous nodular humic, of fast desiccation, clayey and deep on limestone, with a slightly acid pH (6,2-6,4).

Procedure. Twenty of the 165 *G. sepium* trees present in the fence of the main entrance to the institution were sampled, for which the sample size used represented 12 % of the total, and each tree constituted a replication. The experimental design was completely randomized and the following morphological variables were measured: height, stem diameter at breast height and crown width.

The quantity of flowers was determined from the total number of racemes in the selected branches. For such purpose, five racemes were chosen per tree (1,7 % of the total) and the quantity of flowers in each was counted, in order to estimate the yield per tree and per hectare. For this calculation a planting frame of 3,0 x 3,7 m was considered.

During the first morning hours 400 g of flowers were manually collected, at different heights, in the 20 selected trees. They were put in polyethylene bags, which were introduced in ice to prevent the enzymatic degradation that normally occurs in plant samples. Then, they were transferred to the laboratory of biotechnology of the EEPF Indio Hatuey, where they were processed.

The fresh material was washed with distilled water and dried on filter paper to eliminate the liquid excess. Then, a 300-g sample was sent to the laboratory to determine the bromatological composition: dry matter (DM), crude protein (CP), crude fiber (CF) and calcium (Ca), according to the AOAC (1990). The sample was weighed on a Sartorius analytical scale. The remaining 100 g were frozen with liquid nitrogen and stored at -20ºC until the moment of their use.

Table 1. Climate indicators during the evaluation period.

Year	Month	Rainfall (mm)	Mean temperature (°C)	Mean relative humidity (%)
2010	March	20,9	19,7	77
	April	108,3	23,3	74
	May	62,1	26,3	77
	June	161,3	28,0	79
	July	259,7	26,7	84
	August	226,0	26,8	85
	September	315,2	26,2	86
	October	104,9	24,4	86
	November	73,7	21,7	81
	December	6,9	17,1	79
2011	January	51,0	20,3	78
	February	0	21,3	75
	March	6,8	21,6	70
Cumulative		1 267,6	-	-
Annual average			23,7	79,3

The moisture content was discerned through the standard method for the determination of total solids in the biomass (Sluiter, Hyman, Payne and Wolfe, 2008). Klason lignin was determined as the residue of the analytical acid hydrolysis in the biomass samples (Sluiter *et al*., 2011).

For the chromatographic determination of the sugars present in the flower maceration a Young Lin HPLC system (Republic of Korea) was used. Glucose, fructose and sucrose were separated in an ICSep COREGEL-87 H355 column (7,8 x 300 mm) at 60 ± 1 ºC; for such purpose, deionized water was used –as mobile phase– at a flow of 0,4mL/minute. These sugars were detected with a differential refractometer (RID, YL 9170). The software for data acquisition Clarity (YL 9100 HPLC, USA) was used as interface for the data analysis, the result attainment and the system control.

Statistical processing. The standard mean and error, obtained through the standard deviation, were used.

RESULTS AND DISCUSSION

Table 2 shows the quantitative indicators of the tree characterization. The mean of the stem diameter at breast height (DBH) was 44,7 cm. This value is within the range reported by Álvarez and Varona (1988), who defined that *G. sepium* is a small, deciduous and fast-growing tree, with a DBH between 40 and 70 cm. Pennington and Sarukhán (2005) and Elevitch and Francis (2006) coincide in stating that the diameter of this species varies between 25 and 60 cm, although it is normally small (30 cm).

Height reached an average of 4,85 m, which coincides with the report by Mora (1983); this author described *G. sepium* as a perennial plant that can reach 4 to 5 m of height. These results indicate that the sampled trees are typical of the species, in terms of DBH and height, and that they were in full growth.

According to Standley and Williams (1964) and Morton (1981), the *G. sepium* flowers are grouped in 5-10–cm long racemes, densely flowered. In this study, from the estimation of the number of racemes per branch, a mean of 295 racemes per tree was obtained.

It is known that the inflorescence of *G. sepium* appears in axillary racemes. In this indicator 34 flowers were found as average, which coincides with the range reported by Cordero and Boshier (2003). These authors described the flowers as papillionate (typical of the subfamily *Faboideae*) and arranged in short racemes that curve upwards, up to 15 cm long, with 30 to 100 flowers in each.

The total of estimated flowers per tree was 10 030, which is considered adequate due to the profuse flowering that characterizes this species. On the other hand, the total flower estimate per hectare was 8 986 880. This value was related to the genotypic characteristics of the plant, and to the favorable edaphic and climate conditions during the study (table 1), because the soil as well rainfall, relative humidity and mean temperature contributed to the creation of a propitious environment for plant growth and flower production.

In this sense, the annual accumulation of rainfall in the evaluation period was 1 267,6 mm (see table 1), which is in correspondence with the optimum annual rainfall range established by Glover (1989) for *G. sepium* (900 mm-1 500 mm). In addition, the temperature –which, as it is stated in literature, is the climate element that interacts the most as limiting factor for this species– was 23,7 ºC, and was comprised in the range reported by this author (20-29 ºC).

The soil characteristics in this study coincide with the edaphic demands of *G. sepium*, according to the statement by Libreros (1992). This author explained that the plant grows on soils with diverse texture (from loam-sandy to clayey) and well drained, and that it prefers moderate to high fertility soils and with a pH range between 5,5 and 7,0.

Regarding the bromatological composition, the dry matter percentage of the flowers was low (8,69 %) probably because of the presence of very soft and turgid petals. On the other hand, the crude fiber value (32,57 %) was high. This could have been associated –to some extent– to the inclusion of the flower sepals in the sample and to the high lignin

Table 2. Quantitative indicators of the botanical characterization.

Stadigraph	DBH (cm)	Height (m)	Crown diameter (cm)	Number of racemes	Number of flowers/raceme	Total of flowers/tree	Total of flowers/ha
Mean	44,7	4,85	372,35	295	34	10 030	8 986 880
SE ±	3,71	0,25	24,94	20,13	1,53	-	-

percentage in the flower (28,8 %) as a product of the acid hydrolysis. These values are higher than those obtained by Gomes (2010), who reached 22,7 % when determining the average Klason lignin in ten legumes.

The crude protein percentage (7,04 %) was acceptable, which could have been associated to the physiological process of circulation of organic substances in the plant, because according to Vázquez and Torres (2006) they are elaborated in the leaves –for example, the nitrogen compounds aspartic acid, threonine, serine, leucine, valine, phenylalanine, asparagine, glutamine and α-aminobutyric acid– and can be transported towards the roots or other active growth centers, such as the terminal meristem of the stem, flowers or growing fruits, where they are generally found in a higher concentration and number.

With regards to calcium, being the flowers the first step in fruit formation, a relatively high value (0,99 %) was to be expected; this mineral is extremely important, because it participates in the formation of cell membranes and lipid structures, and intervenes in other processes.

The sugar contents found in the aqueous extract of the *G. sepium* flowers macerated at room temperature are shown in table 3. The highest peaks were detected in the simple sugars (glucose and fructose), with 4,82 % and 10,36 %, respectively (fig. 1). Fructose was the most abundant monosaccharide in the extract, and sucrose, the most scarce disaccharide (1,73 %).

Such results coincide with those obtained by Galetto and Bernadello (2003) in the nectar of native *Fabaceae* from Argentina. These authors stated that the sugar composition is dominated, mainly, by monosaccharides; while sucrose is absent or very scarce.

On the other hand, the nectar production varies according to the floral conditions of each plant, influenced by climate, sun intensity and, in general, the edaphoclimatic conditions of a particular zone (Salamanca, Serra and Quijano, 1999).

Table 3. Sugar content present in the aqueous extract of G. sepium flowers.

Retention time (minutes)	Aream (mV.s)	Name of the compound	g/L	%
9,925	12,023	Unidentified	-	-
13,817	58,588	Unidentified	-	-
15,625	726,129	Sucrose	14,46	1,73
19,042	1 729,609	Glucose	5,19	4,82
22,142	62,308	Unidentified	-	-
25,325	12 463,396	Fructose	31,08	10,36

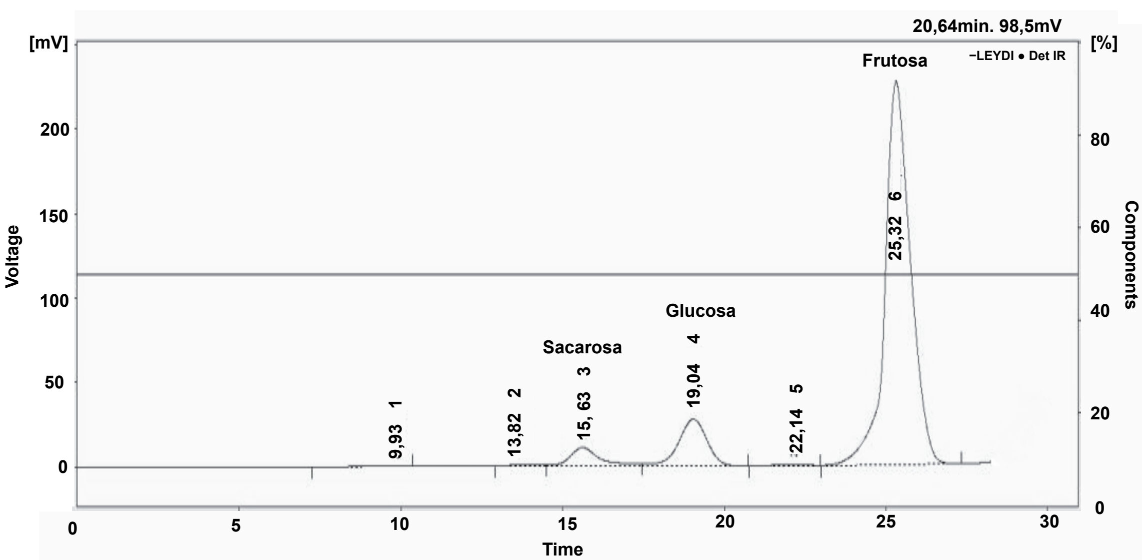

Figure 1. Chromatogram –by HPLC– of the sugars present in the aqueous extract of *G. sepium* flowers.

The content of sugars detected in the maceration of flowers constitutes an important datum for beekeepers in general, because bees prefer nectaries of high sugar concentration which contain, along with sucrose, the two monosaccharides (glucose and fructose). Thus, it is explained that bees are usual visitors of the melliferous tree *G. sepium*, because the nectar is the main source from which honey is originated.

IT IS CONCLUDED THAT:

- All the sampled *G. sepium* trees are typical and in full growth, because they show the morphological characteristics described for this species.
- The sampled *G. sepium* flowers had a low dry matter percentage; nevertheless, the crude fiber, crude protein, Klason lignin and calcium percentages were high.
- The presence of monosaccharides (glucose and fructose) and disaccharides (sucrose) was detected in the extracts of macerated flowers at room temperature. Fructose was the most abundant sugar in the extract, and sucrose, the scarcest.

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