Sugar content in nectar flowers of siratro (*Macroptilium atropurpureum* Urb.)

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ABSTRACT. Siratro (*Macroptilium atropurpureum* Urb.) is a forager with high nutritional values and excellent palatability, and its phenology is almost unknown. Aiming at improving the knowledge and understanding siratro pollinators and floral biology, the total sugar contents on its flowers nectar and the identification of these sugars were determined by, respectively, spectrophotometry and chromatography. The total sugar concentration varied from a maximum of 1.36 and 3.23 mg/flower and a minimum of 0.19 and 0.42 mg/flower. Results showed that the total sugar concentration is high at 8:30 a.m., when the flowers open, and varies slightly during the time the flowers keep open. The variations can be related to the number of insects that visit the flowers, especially bees that may collect pollen and nectar during the open period (8:30 a.m. to 4:30 p.m.). Through enzymatic analysis, data showed that siratro has only glucose in its composition.

Key words: *Macroptilium atropurpureum*, Fabaceae, siratro, phenology, spectrophotometric analysis, nectar.

RESUMO. Concentração de açúcares no néctar e visitantes florais do siratro (*Macroptilium atropurpureum* Urb.). Siratro (*Macroptilium atropurpureum* Urb.) é uma planta forrageira com alto valor nutricional e excelente palatabilidade, sua fenologia é pouco conhecida. Objetivando melhorar o conhecimento e compreensão dos polinizadores do siratro e sua biologia floral, o conteúdo de açúcares totais no néctar de suas flores e a identificação desses açúcares foram determinados por espectrofotometria e cromatografia, respectivamente. A concentração de açúcares totais variou de 1,36 a 3,23 mg/flor para o valor máximo e de 0,19 a 0,42 mg/flor para o valor mínimo. Os resultados mostraram que a concentração de açúcares totais é alta às 8h30min, quando as flores estão abertas e varia um pouco durante o tempo que as flores permanecem abertas. A variação pode estar relacionada ao número de insetos que visitam as flores, especialmente as abelhas que podem coletar pólen e néctar durante o período de antese (8h30min às 16h30min). Por meio de análise enzimática, foi verificado que o siratro possui somente a glicose na sua composição.

Palavras-chave: *Macroptilium atropurpureum*, Fabaceae, siratro, fenologia, análise espectrofotométrica, néctar.

Introduction

Siratro (*Macroptilium atropurpureum)* is a nutritional and highly palatable forager (Pupo, 1985). It also shows quicker growth than perennial soybean, forming a quantity of stems and leaves, highly appreciated by animals, in a short time (Pupo, 1985).

M. atropurpureum can be compared with soybean (*Glycine max* L. Merril) because, even being self-pollination and not benefiting from insects presence (Rubis, 1970; Morse e Carter, 1937), many varieties show an increase of production when visited by honeybees (Erickson, 1975; McGregor, 1976).

The amount of sugar secreted by flowers and consumed by pollinators makes the sugar concentration in flowers vary during their anthesis period. Floral nectars consist almost exclusively of sugar pure solutions, specially glucose, sucrose and fructose (Roberts, 1979). Traces of oligosaccharides may also be present (Harbone, 1998).

The content resulting from this balance,

associated with data about frequency of flower visitation by pollinators, allows estimating a nectar carbohydrate metabolism. To enable this evaluation it is important to determine accurately the sugar content. Thus, this research was carried out to estimate the concentration and determine which sugar is present in nectar.

Material and methods

M. atropurpureum flowers were collected in the Campus of State University of *Maringá*, *Paraná*, Brazil (23°55' lat., 51°57'long., 542 m altitude), with 1677 mm of mean annual rain precipitation and 22ºC as mean temperature.

The samples (five flowers per analysis) were collected in every other day, beginning at 8:30 a.m. and finishing at 4:30 p.m., in one-hour intervals. After collection, the floral nectar was extracted for 45 minutes in a 80 mL Becker with 25 mL distilled water and shaked every 5 minutes. The sugar extracts were eluted in chromatography in descending paper, with Whatmann n.1, with a standard mixture consisting of sucrose, glucose and fructose (1:1:1), with n-BuOH-toluene-pyridine-water eluent (5:1:3:3).

The total sugar content in floral nectar was determined by spectrophotometry (Dubois *et al.,* 1956), which allows the determination of the total sugar even in very small quantities. The method consists in a preparation of a glucose stock (Merck®) solution as standard. This stock solution is prepared diluting 250 mg of glucose with DI water in a volumetric balloon. From the stock solution dilutions from 10 up to 100 μg/mL, with 10 μg/mL increments, were prepared in a 50 mL volumetric balloon. After appropriate dilution, 1 mL phenol and 2.5 mL concentrated sulfuric acid were added to 1 mL of each sample. A Varian Spectrophotometer Cary Model 1E UV-VIS was used for the spectrophotometric readings. Ten readings at 490 nm were taken 45 minutes after the brown color resulting solution, using a 1 cm optical path glass cuvette. Linear regression was obtained from the standard dilutions based on the average of 10 readings for each point. The obtained curve is given by the following equation:

$Y = -7.47222 + 57.6797X$

Where Y is the absorbance and X is the solution concentration. The obtained correlation coefficient is r= 0.9991.

Results

The total sugar contents per flower collected are shown in Figure 1. These data represent the nectar total sugar contents variation in relation to the day hours in which the flowers were open. Data show that the higher sugar concentration in nectar occurs during the flowers opening time, 8:30 a.m., and the closing time is after 4:30 p.m. and they produce sugar all night long without the visit of pollinating insects. During the first hours after flower opening there is a decrease in the sugar contents. After 11:30 a.m., the contents start to rise again, reaching a maximum at 12:30 a.m. In the subsequent period, there are small fluctuations in the contents, tending to decrease when the flowers closing time approaches. During the analyses, data showed that nectar secretion stopped after its removal.

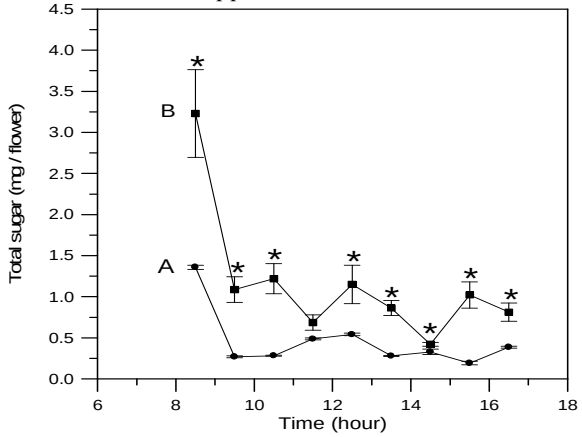

Figure 1. Total sugar contents in nectar of siratro flowers along two-day period (A= total sugar concentration; B=Glucose concentration).

Figure 1 indicates Tukey's test significant values, for each time, in which the total sugar concentration in nectar was estimated. It may be seen that only at 12:30 a.m. this value was not significant. When the two curves were compared, the results were not significant. Table 1 represents the values of total sugar concentration, as well as its respective standard error.

Table 1. Mean concentrations of total sugar in siratro flowers at different periods.

Time (h)	Sugar (mg/flower)	
	20/05/2000	27/05/2000
	$\overline{X}$ ± SE	$\overline{X}$ ± SE
08:30	1.3600 ± 0.0251	3.2300 ± 0.5324
09:30	0.2689 ± 0.0134	1.0875 ± 0.1562
10:30	0.2826 ± 0.0068	1.2200 ± 0.1852
11:30	0.4858 ± 0.0125	0.6862 ± 0.0926
12:30	0.5401 ± 0.0152	1.1500 ± 0.2349
13:30	0.2800 ± 0.0056	0.8620 ± 0.0906
14:30	0.3280 ± 0.0328	0.4220 ± 0.0226
15:30	0.1912 ± 0.0213	1.0222 ± 0.1609
16:30	0.3855 ± 0.0117	0.8131 ± 0.1111

$\overline{X}$ (mean concentration of flower sugar content) and SE (standard error).

Chromatography in paper showed that the nectar from siratro is constituted exclusively of glucose, as may be seen in Figure 2.

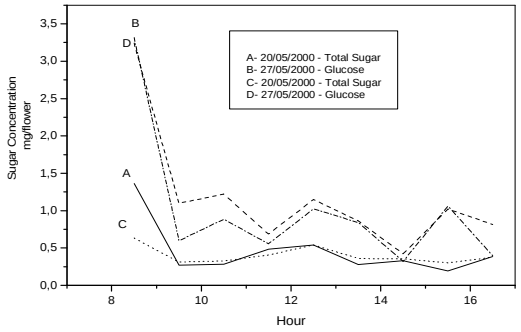

Figure 2. Total sugar and glucose contents in nectar of siratro flowers along two-day period.

The total number of visiting bees in siratro flowers in each time of the experiment may be seen in Table 2. These results demonstrate that the most intensive flower visitation time was between 11:30 a.m. and 1:30 p.m., coinciding with the nectar concentration variation. At 12:30 a.m. there was a decrease of the number of visiting bees, coinciding with the time of nectar sugar content increase. Afterwards, the number of visiting bees increased, with subsequent decrease of sugar concentration in nectar.

Table 2. Total number of bees visits in siratro flowers from 8:30 a.m. to 4:30 p.m.

Time (h)	Number of bee visits
08:30	5
09:30	7
10:30	8
11:30	14
12:30	11
13:30	17
14:30	2
15:30	0
16:30	4

Discussion

Variations in the siratro nectar sugar content measured along the day were observed (Figure 1). This variation is probably associated with the intensity of foraging by honeybees, which is directly related to the nectar quantity and quality (Heinrich, 1979; Hagler, 1990), or to its sugar composition (Waller, 1972; Abrol and Kapil, 1991). Cruden *et al.* (1983) suggested that the maximum nectar accumulation occurs before or at the beginning of pollinator activity. Such fact can be verified in siratro, since the highest sugar concentration was found at 8:30 a.m. (Figure 2), time in which the bee visitation started.

Results showed that siratro does not have new nectar secretion during the day, after its collection. Such characteristic was observed by Del Río and Burquez (1986) when studying *Mirabilis jalapa*; they did not detect nectar secretion induction after its removal from the flower. However, in *Pongania pinnata* Vent. and *Parkinsonia aculeata* L. an increase in nectar secretion according to the flower age was observed (Jain and Dhingra, 1991).

In siratro, the sugar quantity variations found in nectar coincide with the period of most intensive bee visitation. However, it was not possible to demonstrate significant correlation between the number of visiting bees and the nectar sugar concentration. This result agrees with Silva and Dean (2000) who, studying the sugar concentration in onion flower (*Allium cepa* L.) nectar, did not observe significant correlation between visiting bee numbers per umbel. But, the mean quantity of nectar produced by both umbels and individual florets, had significant positive correlation with the number of bee visits (Silva and Dean, 2000).

The *Erythrina crista-galli* (Fabaceae) flowers open from three to four days and produce nectar with dominance of hexose with chemical composition and constant concentration (ca. 22%) during all flowering period (Galleto *et al.,* 2000). The present experiment also verified that, when the flowers open, more than 50% of the total nectar was already available for pollinators.

Siratro nectar has only hexose glucose in its sugar composition (Figure 2), however in most plants a mixture of glucose, fructose and sucrose, with extremely variable concentrations, is detected. Percival (1961) found out that the Cruciferae nectars are almost exclusively constituted by a mixture of fructose and glucose. In 1983, Baker and Baker verified that Cruciferae nectar has hexose dominance [sucrose/ (glucose + fructose) <0.1].

Phytolacca dioica (Phytolaccaceae) produces its nectar overnight and it is constituted by monosaccharides with glucose dominance (fructose $\overline{X}$ = 29.7%, glucose $\overline{X}$ = 70.2%) and a small amount of amino acids (Bernardello *et al.,* 1993).

The study of 25 canola varieties carried out by Kevan *et al.* (1991) demonstrated that 23 of them had 0.95 or more glucose: fructose rates in their nectars. In that study, the authors verified that only three varieties had glucose in smaller quantities and none of the samples had detectable quantities of sucrose.

Davis *et al.* (1998) studied nine species from five Brassicaceae tribes. They verified that the nectar produced by lateral nectary chambers had a comparatively higher glucose/fructose rate (usually

1.0-1.2) than the one from the median nectary chambers (0.2 –0.9) in the same flower. Comparatively, less sucrose was detected in any nectar sample from *Matthola bicornus* (Sibth. *Et* Sm.) DC., that had only lateral nectary chambers and produced dominant exudates-sucrose.

Waller (1972) suggested that the nectar composed predominantly by sucrose attracts honey bees. The nectar siratro flowers do not have sucrose in its composition, only glucose. The flowers of *P. aculeata* and *P. pinnata* studied by Jain and Dhingra (1991) have glucose-rich nectar and receive solitary bees as visitors. Vieira *et al.* (2002) reported that in Brazil, there are six families of bees and they have found five in *Maringá* region (South of Brazil – *Paraná* State) visiting the flowers of this plant: Apidae (56%), Megachilidae (12%), Halictidae (16%), Andrenidae (12%) and Anthophoridae (4%). The same authors also reported in 2001, that the honeybees *Apis mellifera* were not seen in siratro flowers, nevertheless, the bees of genus *Euglossa* were seen frequently.

Analyses of the total sugar concentration in siratro nectar may allow the suggestion that this forager can be considered an excellent nectar source for bees.

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