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Seasonal Extrafloral Nectar Production by *Laguncularia racemosa* (L.) C.F. Gaertn (Combretaceae) in Southeast Brazil

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Abstract: The aim of this study was to quantify the nectar production of extrafloral nectaries by *Laguncularia racemosa* leaves. Extrafloral nectaries are nectar-secreting glands not directly related with pollination and which attract diverse nectarivorous arthropods but most especially ants. During two years we investigated if the extrafloral nectaries of *Laguncularia racemosa* were morphologically variable and if the production of nectar was seasonal. Branches with five pairs of leaves of *L. racemosa* each were collected out in the mangrove of River Guaratuba (Bertioga, SP, Brazil; 23°45'12"S; 45°53'45"W) in five samplings from May 2008 to April 2010. Samples received only diffuse light and were analyzed 24 h after collection, when the presence or not of liquid in all five pairs of leaves per branch was recorded. The extrafloral nectaries near apical leaves produced nectar more frequently compared to other leaf pairs. There was a sharp decrease in the frequency of extrafloral nectar production from autumn to summer. The visitation of ants was recorded and could explain the observed seasonality of nectar production by Extrafloral nectaries because the fall is the period of flight dispersal of several herbivorous insects. This work demonstrated clearly that Extrafloral nectaries in *L. racemosa* are functional nectar-secreting structures that are able to attract nectarivorous insects such as ants and flies.

Key words: *Laguncularia racemosa*, extrafloral nectar, seasonality

INTRODUCTION

Nectaries can be classified as floral or extrafloral (Koptur, 1992a, b). These two types of nectaries can affect plant fitness by increasing pollination rate (Barth, 1985), or by decreasing plant damage caused by herbivores (Oliveira, 1997; Wackers and Bonifay, 2004).

Near 330 genera in more than 90 plant families have extrafloral nectaries whose function is to protect the plant against herbivores if they attract ants which will act as bodyguards (Bentley, 1976, 1977; Koptur, 1992a). But other nectarivores can be attracted including vertebrates (Cuatle and Rico-Gray, 2003).

In mangrove ecosystem on the southeastern coast of Brazil, the species *Rhizophora mangle* L. (Primack and Tomlinson, 1978) and *Hibiscus pernambucensis* Arruda (Cogni *et al.*, 2003) have extrafloral nectaries attract ants.

However, information about the presence of extrafloral nectaries in the white mangrove, *Laguncularia racemosa* (L.) C.F. Gaertn, have been documented in a controversial way (Kemmis and Lersten, 1984). Recently, Francisco *et al.* (2009) showed that there are five different types of glandular structures on leaves of *L. racemosa* - the salt-excreting on the leaf blade and

the sugar-secreting at the leaf base and nectar production by extrafloral nectaries was inferred from indirect evidence by the presence of ants.

This study aimed to quantify some of the morphological characteristics of the extrafloral nectaries present on the leaf petioles of *L. racemosa*. We show for the first time that they are functional structures capable of attracting nectarivorous arthropods and that nectar secretion varies seasonally.

MATERIALS AND METHODS

Field work was carried out in the mangrove of River Guaratuba (Bertioga, SP, Brazil), close to the BR101 highway (23°45'12"S; 45°53'45"W). Five samplings of *L. racemosa* were made in May, July, August and December 2008 and April 2010. Samplings were carried out between 0800 h and 1500 h with clear sky and during low tide. We collected 30 branches of *L. racemosa* from trees at least 1 m apart. Each branch was collected in order to keep intact the first five pairs of leaves and was individually kept in transparent numbered plastic bags. In the laboratory, the position of each leaf on the branch was specified by numbers and letters (Fig. 1A).

Samples received only diffuse light and were analyzed 24 h after collection, when the presence or not of liquid in all five pairs of leaves per branch was recorded. A spot test using Benedict reagent was used to confirm the presence of reducing sugars (Feigl and Anger, 1967; Assumpcao and Morita, 1968).

Forty-eight hours after samplings the morphological characteristics were described and quantified using a Leica Wild M3B stereomicroscope with a 40X magnification with calibrated ocular micrometer. Measurements were made on only one pair of apical leaves and on other of the fifth pair, in the opposite side, for example, 1a (left side) and 5b (right side).

The measurements were: diameter of the opening of nectaries, diameter of nectaries and the distance between them (when all were present) (Fig. 1A). Each distance between the nectaries 1, 2, 3, 4 was named a, b, c and d (Fig. 1C) to standardize the data collection.

The quantification of color was made on digital photographs of the structures obtained in the field and with corrected white balance. In five photos of each structure it was sampled more than a million pixels with results expressed as averages of the frequencies of the channels RGB using ImageJ software (<http://rsbweb.nih.gov/ij>). All statistical analysis was performed using Biostat v. 5 (Ayres *et al.*, 2007). In the analysis of nectar production, data was transformed to $\log_e (x+0.5)$.

RESULTS

The leaves of *L. racemosa* analyzed were: opposite, elliptic, petiolate without stipules, leathery but rather fleshy, glabrous, simple and entire. They were green and slight discolors (adaxial: R = 097, G = 119, B = 061; abaxial: R = 119, G = 140, B = 76) with elongated red petioles (R = 119, G = 65, B = 67; Fig. 2A) which is a diagnostic characteristic for the species.

Generally on the top, near the leaf blade of petiole there were two pairs of nectar secreting glands (Fig. 1B 2A). The number of glands in each petiole ranged from a minimum of two to four (paired or not; Fig. 2C). In the first four samplings (May 27, July 14, August 29 and December 8, 2008), the number of petioles with two nectaries was 55 (23.2%), with three was 52 (21.9%) and with four was 130 (54.9%).

The diameter of secretory hole of nectaries varied by an order of magnitude, between 0.08 and 0.87 mm (mean = 0.274 mm, sd = 0,0833 mm n = 789) and the overall diameter of the nectary ranged between 0.36 and 2.45 mm (mean = 1.327 mm, sd = 0,3066 mm; n = 783). The mean distance a between the nectaries was lower (mean = 0.56 mm) than the distance b (mean = 1.27 mm; sd = 0,5221 mm; n = 224), and these two smaller than the distances c (mean = 1.61

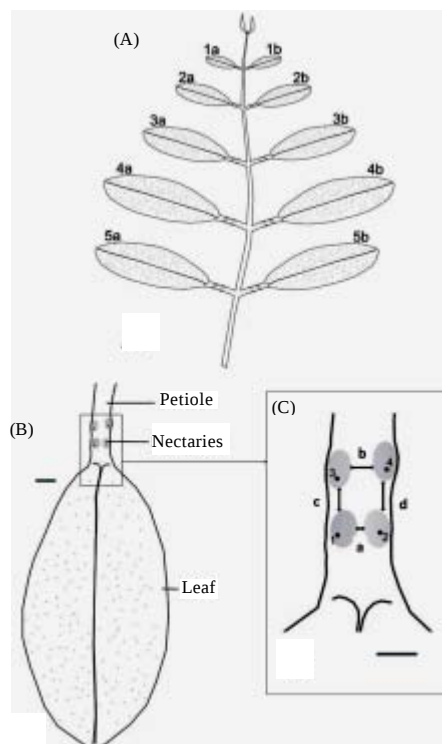


Fig. 1: (A) Topographic disposition of the five first pairs of leaves of *L. racemosa* in each branch; (B) Position of the four extrafloral nectaries in the basis of the leaf and (C) measurements between them. Scales in B and C = 2 mm

sd = 0,8151 mm; n = 154) and d (mean = 1.64 mm; sd = 0,8216 mm; n = 159). This asymmetry is due to the fact that the nectaries 1 and 2 are near the base of the leaf blade while the third and four are more distant. Several leaves showed also nectar producing trichomes on nectary protuberances or their neighborhood (Fig. 2E).

In the center of the gland there was a small opening where a fluid could be secreted. This liquid becomes translucent after the partial evaporation of water due to sugar crystallization (Fig. 2B) and was positive for the test of Benedict.

In the field it we found five species of ants on plants being one of the genera *Camponotus*, three *Pseudomyrmex* and one *Cephalotes* (Fig. 2D, G, H, I) and also a non-identified fly (Fig. 2F), all of them visiting the nectaries.

Of the total 1,500 sampled leaves, 404 (26.9%) produced nectar. The number of nectaries which produced nectar ranged from ten (five pairs) to one (only one nectary) but anyone can produce nectar. There was a logarithmic decreasing trend in the nectar production

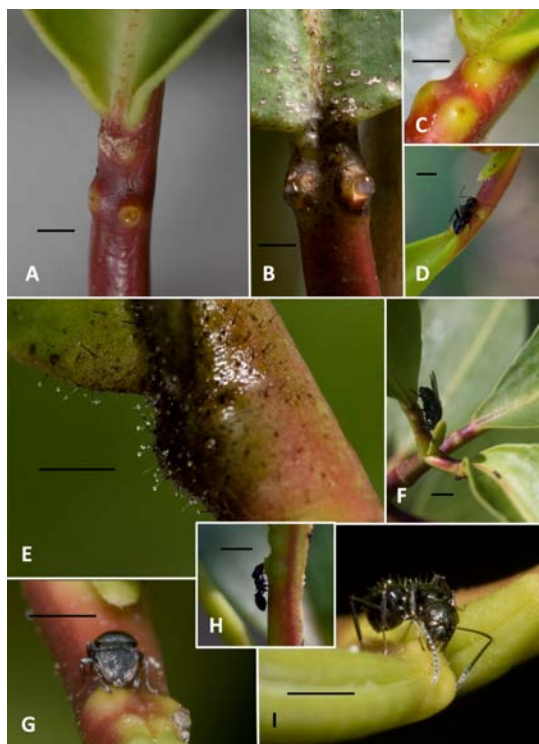


Fig. 2: (A-B-C) Details of the extrafloral nectaries in the basis of the leaves of *L. racemosa* showing the reddish coloration of the petioles (A) a leaf with a pair of extrafloral nectaries; (B) a leaf with a pair of extrafloral nectaries, one with a opalescent drop of nectar due to partial evaporation of water; (E) nectar producing trichomes in the region of extrafloral nectaries (F) Fly foraging in a extrafloral nectary; (G-H) ants of genus *Cephalotes* and of genus *Camponotus* (D, I) foraging on extrafloral nectaries. Scale bar = 2 mm

from nectaries of pair 1 to nectaries of the pair 5 ($R = 0.97$; $p < 0.05$).

The two samplings of autumn (May 27, 2008 and April 21, 2010) showed 60.4% of nectaries producing nectar followed by two winter samplings (July 14 and August 27, 2008) with 33.7% with summer sampling with the lowest value. There was a highly significant difference in total production of the five pairs of nectaries between the three seasons ($F_{2,297} = 47.8281$, $p < 0.0000001$) that showed a linear decay from autumn to summer ($R = 0.96$; $p < 0.05$; Fig. 3). Also the higher production by nectaries of pair 1 may be associated to the protection of young leaves complemented by the presence of nectarivores in this pair.

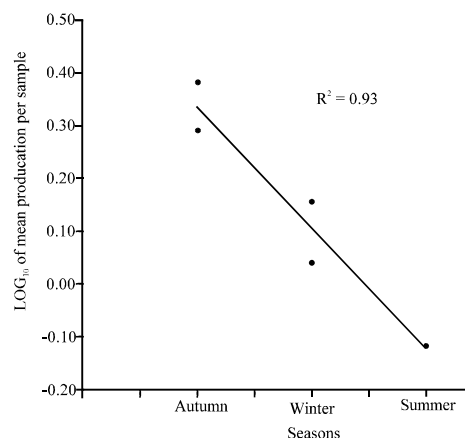


Fig. 3: Variation of the nectar production in branches of *L. racemosa* from autumn to summer with data transformed to $\log_{10}(x)$

DISCUSSION

Francisco *et al.* (2009) showed only one pair of the nectary glands in their material from Venezuela which can be an indicative of a possible geographical variation in the number of nectaries in this species or a characteristic of local populations. This can be strengthened by the proportion of petioles with less than four nectaries in our material together with their great variation of distances and diameters which may be due to the instability of this trait.

In Texas, seasonality was found also in *Catalpa speciosa* (Bignoniaceae) which increases the nectar production in the extrafloral nectaries from June to September to attract ants to inhibit females of the moth *Ceratomia catalpae* (Lepidoptera: Sphingidae) to oviposit on its leaves (Stephenson, 1982). Seasonality in nectar production by nectaries was found also by Diaz-Castelazo *et al.* (2004) in several plants of Veracruz (Mexico) and by Robbins and Miller (2009) in *Opuntia stricta* (Cactaceae) in Florida. Also the higher production by nectaries of pair 1 may be associated to the protection of young leaves complemented by the presence of nectarivores in this pair. Although leaves of *Laguncularia* also have the capacity of salt excretion (Hogarth, 1999; Sobrado, 2004; Francisco *et al.*, 2009) this is not accomplished by the glands in petiole basis.

To clarify a controversial point we state that leaves of *Laguncularia* have glands in the basis of petiole which are functional nectar-secreting structures (extrafloral nectaries) which are able to attract nectarivorous insects such ants and flies.

The next step is to determine if ants in fact protect the leaves against herbivores.

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REFERENCES

- Assumpcao, R.M.V. and T. Morita, 1968. Manual of Solutions, Reagents and Solvents. Standardization, Preparation, Purification. Editora Edgard Blucher Ltd., Sao Paulo.
- Ayres, M., D.L. Ayres and A.S.S. Santos, 2007. Bioestat: Statistical Applications in Biomedic Science Areas. Society Mimirua Civil, Brasil.
- Barth, F.G., 1985. Insects and flowers: The Biology of a Partnership. Princeton University Press, Princeton, New Jersey, ISBN-13: 9780691083681.
- Bentley, B.L., 1976. Plants bearing extrafloral nectaries and the associated ant community: Interhabitat differences in the reduction of herbivore damage. *Ecology*, 57: 815-820.
- Bentley, B.L., 1977. Extraoral nectaries and protection by pugnacious bodyguards. *Ann. Rev. Ecol. Syst.*, 8: 407-427.
- Cogni, R., A.V.L. Freitas and P.S. Oliveira, 2003. Interhabitat differences in ant activity on plant foliage: Ants at extrafloral nectaries of *Hibiscus pernambucensis* in sandy and mangrove forests. *Ent. Exp. Applied*, 107: 125-131.
- Cuautle, M. and V. Rico-Gray, 2003. The effect of wasps and ants on the reproductive success of the extraoral nectaried plant *Turnera ulmifolia* (Turneraceae). *Functional Ecol.*, 17: 417-423.
- Diaz-Castelazo, C., V. Rico-Gray, P.S. Oliveira and M. Cuautle, 2004. Extrafloral nectary-mediated ant-plant interactions in the coastal vegetation of Veracruz, Mexico: Richness, occurrence, seasonality and ant foraging patterns. *Ecoscience*, 11: 472-481.
- Feigl, F. and V. Anger, 1967. Spot Tests in Organic Analysis. 7th Edn., Elsevier Publishing Company, Amsterdam.
- Francisco, A.M., M. Diaz, M. Romando and F. Sánchez, 2009. Morphoanatomical description of leaves glands types in white mangrove *Laguncularia racemosa* L. Gaertn (f.). *Acta Microscopica*, 18: 237-252.
- Hogarth, P.J., 1999. The Biology of Mangroves. Oxford University Press, Oxford, England, USA..
- Kemis, J.R. and N.R. Lersten, 1984. Petiolar glands in Combretaceae: Review of past ambiguities and an anatomical description of the sunken gland of white mangrove (*Laguncularia racemosa*). *Am. J. Bot.*, 71: 34-35.
- Koptur, S., 1992a. Extraoral Nectary-Mediated Interactions between Insects and Plants. In: *Insect-Plant Interactions*, Bernays, E. (Ed.). Vol. 4. CRC Press, Boca Raton, Florida, USA., pp: 81-129.
- Koptur, S., 1992b. Plants with extrafloral nectaries and ants in everglades habitats. *Florida Entomol.*, 75: 38-50.
- Oliveira, P.S., 1997. The ecological function of extrafloral nectaries: Herbivore deterrence by visiting ants and reproductive output in *Caryocar brasiliense* (Caryocaraceae). *Functional Ecol.*, 11: 323-330.
- Primack, R.B. and P.B. Tomlinson, 1978. Sugar secretions from the buds of Rhizophora. *Biotropica*, 10: 74-75.
- Robbins, M. and T.E.X. Miller, 2009. Patterns of ant activity on *Opuntia stricta* (Cactaceae), a native host-plant of the invasive cactus moth, *Cactoblastis cactorum* (Lepidoptera: Pyralidae). *Florida Entomol.*, 92: 391-393.
- Sobrado, M.A., 2004. Influence of external salinity on the osmolality of xylem sap, leaf tissue and leaf gland secretion of the mangrove *Laguncularia racemosa* (L.) Gaertn. *Trees Structure Function*, 18: 422-427.
- Stephenson, A.G., 1982. The role of the extrafloral nectaries of *Catalpa speciosa* in limiting herbivory and increasing fruit production. *Ecology*, 63: 663-669.
- Wackers, F.L. and C. Bonifay, 2004. How to be sweet: Extrafloral nectar allocation by *Gossypium hirsutum* fits optimal defense theory predictions. *Ecology*, 85: 1512-1518.