ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 03, pp. 28118-28122, March, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

SEM BASED ANATOMICAL STUDIES IN STIPULAR EXTRA FLORAL NECTARY TISSUES IN *CASSIA ALATA* LINN

***Savitha Veeraragavan and Ramamoorthy, D.**

Department of Ecology and Environmental Sciences, Pondicherry University, Pondicherry

ARTICLE INFO

Article History:
Received 15th December, 2015
Received in revised form
20th January, 2016
Accepted 28th February, 2016
Published online 31st March, 2016

Key words:
EFN Gland,
Cassia Alata,
Size and Shape,
SEM Images-Nectar.

ABSTRACT

Presently an attempt is made to get detailed information on anatomical architect of the nectariferous tissues present in the ventrally folded stipules in *Cassia alata* using cross section of stipule and its SEM images. The anatomical studies revealed that nectar secreting tissues are present both on ventral and dorsal fold of the stipules located on both sides of petiole in *C. alata*. The cross section of the stipule show upper and lower ends made of epidermal tissues and secretary paranchymatous tissues in between. The paranchymatous cells are found to be loaded with nectar and spreading towards upper and lower epidermis. However, a closer observation on the SEM images it is noticed that amount of nectar is seen more on the lower end than upper side confirming the higher degree visitation of ants to the lower side of the stipule for nectar. The secretion is tested for sucrose using Benedict's solution after hydrolyzing the nectar. The nectariferous cells are devoid of any intracellular bodies as found in other nectaries. Therefore, it is presumed that nectar oozes out either through pores in the epidermis or by rupturing epidermal layer. This aspect requires further SEM studies to find out the way out for the nectar.

Citation: Savitha Veeraragavan and Ramamoorthy, D. 2016. "Sem based anatomical studies in stipular extra floral nectary tissues in cassia alata linn", *International Journal of Current Research*, 8, (03), 28118-28122.

INTRODUCTION

Extrafloral nectaries (EFNs) are nectar-secreting vascularized or non-vascularized structures not directly involved with pollination (Elias,1983;Koptur,1992a), which are especially common on leaves, petioles, young stems, stipules and reproductive structures (e.g. buds, calyx, inflorescence axis, flower peduncles, fruit) (Rico-Gray, 1989, 1993; Rico-Gray *et al*., 2004).The diversity of EFN shapes, nature of secretary glands and locations has been used for taxonomic purposes (Irwin and Barneby, 1982). Given such taxonomic value of EFNs (Bharathi Bhattacharya and Maheshwari, 1970; Lersten and Brubaker, 1987), and their role in ant-plant interaction, studies on anatomical features of these secretary glands /cells have become the concerned topic of taxonomists and ecologists. Moreover, the ecological importance of the EFNs and their interaction with insects, have motivated many authors to study the morphology, distribution and their anatomical architect of these structures in different plant taxa (e.g. Zimmerman 1932; Bentley 1977; Metcalfe and Chalk 1979; Elias 1983; Oliveira and Leita˜o-Filho 1987; Oliveira and Oliveira-Filho 1991; Koptur, 1992; Morellato and Oliveira 1994; McDade and Turner 1997).The extrafloral nectary (EFN) of *Hibiscus pernambucensis,* a native shrub species occurring in mangrove, occurs as furrows with a protuberant border on the abaxial surface veins of the leafblade. Each nectary consists of numerous secretory multicellular trichomes, epidermal cells in palisade-like arrangements and non-vascularized parenchyma tissue (Rocha and Machado, 2009). Bharathi Bhattacharyya and Maheshwari (1970), (Wrischer 1962), (Figier 1971), Tarkowska *et al*. (1981) and Davis *et al*. (1988) and Fahn (1990) who observed the presence of nectar-secreting glandular trichomes on the stipules of *Vicia faba*. As well as, Stpiczyñska (2000) noticed extrafloral nectaries on the stipules in *Vicia sativa* subsp. *angustifolia*, *V. sativa* subsp. *sativa*, *V. sepium* and *V. grandiflora*. The ultrastructure of secretory trichomes and their underlying epidermal and subepidermal cells of *Vicia faba* stipules has been studied in great detail by (Wrischer 1962), Figier (1971), Tarkowska *et al*. (1981) and Davis *et al*. (1988). Bharathi Bhatachria and Maheswari (1970) indicated based on phylogenetic clad that EFN is not present in *Cassia alata* But reports of Massazi *et al*. (2012) is contradictory to findings of Bharathi Bhatacharia and Maheswari (1970) quoting suitable evidence that *C. alata* has extra floral nectar secreting tissue

****Corresponding author: Savitha Veeraragavan,***
Department of Ecology and Environmental Sciences, Pondicherry University, Pondicherry.

just on the ventral side of stipules embedded within the tissue of stipule. Keeping these contrasting finding on the presence of nectariferous tissues in *C. alata*, previously the authors have examined and confirmed the presence of secretary tissues in the stipules of *C.alata* (Savitha and Ramamoorthy, 2015). Presently an attempt is made to get detailed information on anatomical architect of the nectariferous tissues present in the ventrally folded stipules in *Cassia alata* using Cross section of stipule and its SEM images.

METHODS AND MATERIALS

Cassia alata, the candle bush, is an important medicinal shrub as well as an ornamental plant in the subfamily Caesalpinioideae. The plant is also known as, ringworm tree, or candle tree. The shrub stands 3–4 m tall, with leaves 50–80 cm long. The inflorescence looks like a yellow candle. The fruit, shaped like a straight pod, is up to 25 cm long. Its seeds are distributed by water or animals. *C.alata* is growing in the university campus as well as distributed both in urban and rural areas of Pondicherry town. Observations on the extra floral nectary substances/gland are made in relation to presence and visitation of ants into such plant parts. A thorough observation on the whole plant-from base to tip; of the each branch are made; similar observation was made in 10 healthy plants. To verify whether the modified stipular secretions are having sucrose as most of the floral nectar are rich in sucrose, Benedict's reagent is used after hydrolyzing the secretions in Dil.HCl. The isolated stipular sections are observed under Digital portable but computer guided microscope (USB Digital Microscope-CE FC) followed by cross sections of stipules and its SEM images to examine the secretary cells present in both of upper and lower side of the stipule. For SEM images the material were fixed in FAA then 70% ethyl alcohol, dehydrated with an ethanol series and critical-point dried using critical Point Drying Apparatus (FL 9496 Balzers). They were mounted onto stubs, sputter coated with gold and scanned with a Jeol SEM (JSM-T20) at 20 kV at central services lab., CIF

Fig.1 Cassia alata growing in the wild

RESULTS

Observation on 100 plants on lobed stipules of *Cassia alata* obviously revealed that *C .alata* possesses EFN tissues in the ventrally folded paired stipules located at the base of both sides of rachis. The modified stipular EFN tissues with their secretions are also visible under digital microscope at the ventral side of the stipular lobe (Fig.2-4).

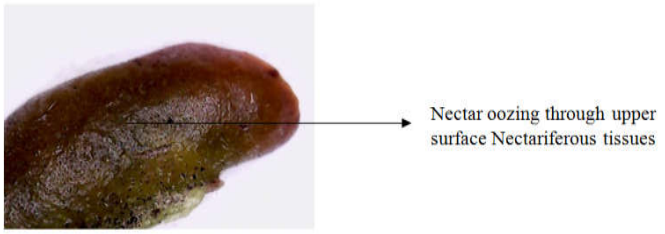

Fig. 2. Nectaries material on the uppr surface of secretary sipule under 500 x magnification

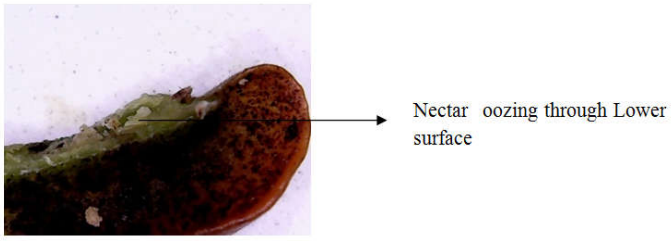

Fig. 3. Nectary secretions found on the lower surface of secretary sipule under 500 x magnification

In cross-section of nectar gland, it constitute of simple secretary epidermal layer beneath the layer mass multilayered of parenchyma cells. (Fig.2). The nectariferous cells are embedded in the secretary parenchyma cells of both upper and lower side of the ventrally folded stipules; howver, the secretary/secretions are more at the inner side/lower side of the stipules under phase-contrast microscope seen. On the upper surface, in each of stipule each nectary consists of numerous secretory multicellular trichomes, epidermal cells in palisade-like arrangements and non-vascularized parenchyma tissue. The SEM images showing the internal cellular arrangement/nectariferous parenchymatous, is given as (Fig 5-7).

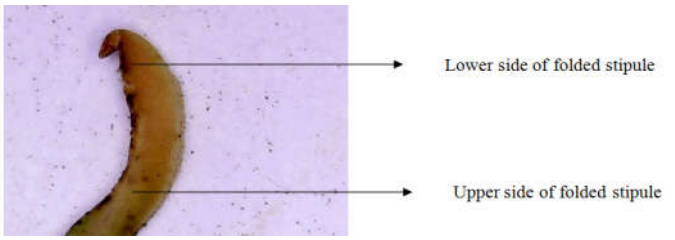

Fig. 4. Cross section of stipule showing secretary materials both upper and lower sides of folded stipule

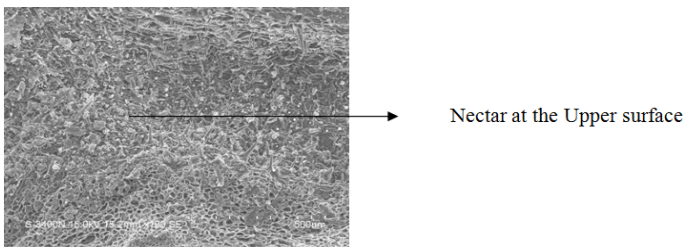

Fig.5 SEM image of upper surface of stipule

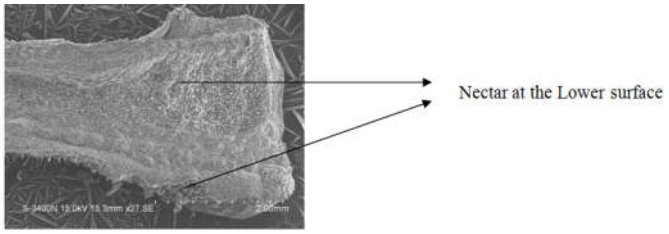

Fig. 6. SEM image of lower surface of stipule

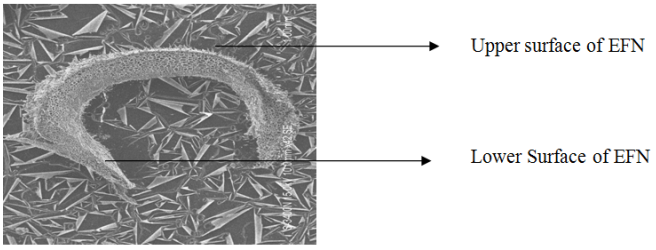

Fig.7 SEM image showing upper and lower surface of stipule with nectary substances (1.00mm)

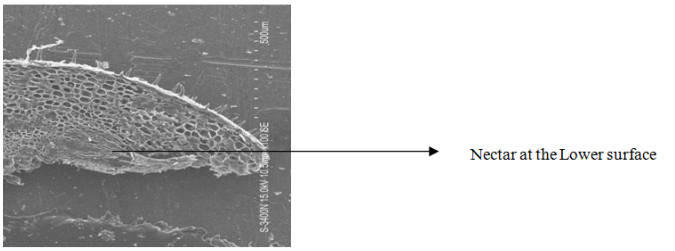

Fig. 8. SEM image showing upper and lower surface of stipule with nectary substances (500ɥm)

DISCUSSION

The Genus *Cassia* Linn. comprises 600 species occurring mostly in the tropics and subtropics, especially in India. *Cassia* is the only genus in Cassieae in which some species are characterized by the presence of extrafloral nectaries on the petiole, leaf-surface and rachis (Bharathi Bhattacharyya, 1970) and found distributed widely around the world, and more common in tropical than in temperate environments (Pemberton 1988, Oliveira and (Leitao-Filho 1987; Oliveira and Oliveira-Filho 1991). They are found on several different vegetative plant parts (e.g. leaves), as well as on inflorescences (e.g. pedicels) and the outside of the outer floral organs not directly involved in pollination (e.g. sepals). Fahn (1990) mentioned the presence of nectar-secreting glandular trichomes on the stipules of *Vicia faba*. The detailed studies on the EFN glands of *Vicia faba* have been done by (Devis and Gunning (1991, 1992) and Davis *et al*. (1988). Morphologically, EFNs represent a heterogeneous multitude of secretory structures, from simple glandular trichomes and cryptic secretory tissue embedded within EFN-bearing plant parts to conspicuous, complex vascularized or non-vascularised glands, all of which produce and secrete nectar (Elias and Gelband, 1976; Fahn, 1979; Schmid, 1988; Vogel, 1997;Bernardello, 2007). One such type of EFN tissue embedded within EFN-bearing plant parts-stipule has been reported in *Cassia alata* (Savitha & Ramamoorthy, 2015). In *Cassia alata*, the presence of actual gland is reported to be absent (Bharathi Bhatachriaa and Maheswari 1970) but reports of Marazzi *et al*., 2012) with relevant evidences claimed that extra floral nectar secreting tissues are present in *C.alata* just on the ventral side of stipules at each node. Keeping these contrasting finding on the presence of EFN in *C.alata* our previous studies confirmed the presence of nectariferous cells in the ventrally folded stipule located at the base of each rachis (Savitha and Ramamoothy 2015). In the present study, the nectar secretion by those stipules are confirmed through cross section and SEM images of the stipules as well as chemical testing for the presence of nectar using Benedict's reagent. Cross section and SEM images of stipules revealed obviously that *C.alata* possesses EFN secreting tissues in the ventrally folded paired stipules located at base of rachis one on each side. The upper and lower sides of the stipule are having whitish substances secreted and sent out side directly. (Fig 6&7) show the close up view of the surface (upper) and lower surface with nectar substances. SEM images clearly show the nectar present more at the lower end and lesser in the upper end. The field observation also in support of presence of more nectar in the lower side of the stipule by witnessing ants mostly attended/visited lower side of the stipule for nectar than the upper side.

Present observation are conformity with the reports of (Stpicznska 2000) that stipules with nectariferous tissues are present in four *Vicia* taxa, *V.sativa* subsp.*angustifoin lia* in *Hibiscus pernambucensis* (Rocha, 2009) and in *Croton urucurana* (Leandro Fretas 1999). Simple anatomy consisting of an upper/outer epidermis immediately with a mass of small, closely-packed parenchyma cells are found in *Chamaecrista fasiculata* and *Senna hepecarpa* (Lenore T. Durkee, 1999) could also be cited here. One of the notable feature understood from SEM images of the EFN secretary cells that these secretary cell or the parenchmatous tissues are not having any vacuoles, droplets or vesicle as found in *Croton urucurana* (Leandro Freitas 1999). *Hibiscus pernambucensis* (Rocha and Machado, 2009) *Croton urucurana* (Leandro Freitas 1999) or starch grains as in *Ricinus communis* (Kalman and Gulyas,1974).

SEM images further clearly revealed that multilayered parenchymatous tissue present in the middle of the stipule is not having any intra or inter cellular bodies or structures. Need for conduct of nectar is necessary generally when there is a nectar gland; but in *C.alata* the the secretary tissues are modified cells of stipule. Further, it is also clear from reports of (Kupicha 1976) that members of the genus *Vicia* the stipules are with nectariferous spots on abaxial surfaces in subgenus *Vicia.* (Stpiczyñska 2000) studied extrafloral nectaries located on the stipules in four *Vicia taxa*; *V.sativa* subsp. angustifolia, *V. sativa* subsp. *sativa*, *V. sepium* and *V. grandiflora*. The nectariferous tissues consisted of secretary hairs building of four cells of head, one stalk and basal cell and 2-3 layers of subepidermal cells. But in *C.alata* the upper epidermis is single layered with or with non secretary trichome followed by multilayered parenchymatous tissue and terminal fold, with rich secretary cells (Fig.7). SEM images further underlines absence of cuticle rupture which indicate that the cuticle is permeable to nectar, as pointed for a number of other species (Stpiczynska *et al.* 2005).

Secretory fluids that accumulate in subcuticular spaces may be eliminated via cuticular pores, cuticle rupture, or in more permeable cuticular regions, as described for other types of nectaries and secretory trichomes (Fahn, 1979). In P. crucis, the secretor fluids accumulate in the subcuticular space, indicating that the nectar must be released through cuticle rupture. As reported by several authors, the accumulation of secretion below a lifted cuticle (which later ruptures) is a general feature for EFNs (McDade and Turner, 1997**,** Nepi, 2007 and Picazo *et al*., 2008). However, as cuticular ruptures or detachments were not observed, we do not think that this is the case for the species studied here. Because the epidermis here was deprived of stomata and contained microchannels, nectar may be released through these microchannels as reported for other botanical families (Freitas *et al*., 2001, Koteyeva, 2005, Stpiczyńska *et al*., 2005 and Weryszko-Chmielewska and Bożek, 2008). We are also compelled to believe that the main site of nectar exudation is the central area where thin-walled epidermal cells are found. The presence of thin-walled epidermal cells would weaken the barrier against nectar release. Moreover, (Francino *et al*. 2006) reported the presence of cuticular pores at the centre of the EFN concavity which may be the sites for nectar exudation in C. trichopoda**.** Further, (Marrazi *et al* 2012) have found out two distinct kinds of EFNs existing in two unrelated clades within *Senna* (*Cassia*). 'Individualized' EFNs (iEFNs), located on the compound leaves and sometimes at the base of pedicels, display a conspicuous, gland-like nectary structure, are highly diverse in shape and characterize the species-rich EFN clade. The stipule blade may be glabrous or covered by trichomes and is strongly asymmetric and cordate, forming one lobe that is modified in colour and thickness and includes the secretary tissue (Fig 2-3). Two distinct kinds of EFN morphologies exist in Senna, which is interpreted in relation to their degree of individualization, i.e. morphological differentiation and specialization, with respect to the organ that bears them. The newly discovered, cryptic EFNs are non-individualized EFNs (non-iEFNs), i.e. stipules, bracts and sepals that are EFN-bearing organs, in which specific zones differentiate into nectar-producing structures. Such structures consist of nectariferous tissue embedded in the coloured stipule lobe. Presence of simple type of secretary tissues of ventrally embedded non-vascularised stipular lobe with in *C.alata* probably imply primitive nature of EFN in the evolution particularly in members of family Cassiaceae.

Acknowledgement

We thank Dr. A. Yogamoorthi and Authors are thankful to the department of Ecology and Environmental Science, Pondicherry University for providing laboratory facilities in order to laboratory assistants for their help in getting images from the phase-contrast microscope and photography and also extend their thanks to technicians of CIF of our university who helped in getting SEM images.

REFERENCES

Bentley B.L. 1977. Extrafloral nectaries and protection by pugnacious bodyguards. *Annual Review of Ecology and Systematics*, 8, 407–427.

Bernardello, G. 2007. A systematic survey of floral nectaries. In: Nicolson, S.W., Nepi, M., Pacini, E. (Eds.), Nectaries and Nectar. Springer, Dordrecht, pp. 19–128.

Bharathi Bhattacharyya &Maheswari J.K. 1970. Studies on Extrafloral nectaries of the Leguminales.Vol.37, B, No. 2.

Cecilia Di´Az-Castelazo1, Victor Rico-Gray1, Fernando Ortega and Guillermo A ´ Ngeles. Morphological and Secretory Characterization of Extrafloral Nectaries in Plants of Coastal Veracruz, Mexico., Annals of Botany 96: 1175–1189, 2005.

Davis, A.R. and B.E.S. Gunning, 1991. The modified stomata of the floral nectary of Vieux *faba*L. Stomatal number and distribution as selection criteria for breeding for high nectar sugar production. ActaHortic., 288: 329-334.

Davis, A.R. and B.E.S. Gunning, 1992. The modified stomata of the floral nectary of Viciafaba L. 1. Development, anatomy and ultrastructure. Protoplasm a, 166: 134-152.

Davis, A.R., R. L. Peterson and R.W. Shuel, 1988. Vasculature and ultrastructure of the floral and stipularnectaries of Viciafaba(Leguminosae). Can. J. Bot., 66: 1435-1 448.

Doak, P., Wagner, D. & Watson, A. 2007. Variable extrafloral nectary expression and its consequence in quaking aspen. Canadian Journal of Botany 85:1-9.

Doucet, Arnaud, Nando De Freitas, and Neil Gordon. "An introduction to sequential Monte Carlo methods." *Sequential Monte Carlo methods in practice*. Springer New York, 2001. 3-14.

Elias T S 1983. Extrafloral nectaries: their structure and distribution. In *The Biology of Nectaries* (eds B. Bentley and T. Elias), pp. 174-203. Columbia University Press, New York

Elias T.S., Gelband H. 1976. Morphology and anatomy of foral and extrafloral nectaries in Campsis (Bigoniaceae) American Jouranal of Botany, 63, 1349-1353.

Fahn, A., 1990. Plant Anatomy. 4th Edn.,Pergamon Press, Oxford, pp: 175-178.

Fahn, A.1979 . Secretory tissues in plants. Academic Press, London, UK.

Fernando lopez-Anido & Jose Vesprini. 2007. Extrafloral nectaries in *Cucurbita maxima* Sub.*andreana* (Naudin) Filov. Cucurbit Genetics Cooperative Report 30:38-42.

Irwin, Howard S., and Rupert C. Barneby. "The American Cassiinae: a synoptical revision of Leguminosae tribe Cassieae subtribe Cassiinae in the New World." *Mem. New York. Bot. Gard* 35.1 (1982): 2.

Joecildo Francisco Rocha and Silvia Rodrigues Machado. Anatomy, ultrastructure and secretion of *Hibiscus pernambucensi s*Arruda (Malvaceae) extrafloral nectary. Revista Brasil. Bot., V.32, n.3, p.489-498, 2009.

Kalman, F. and S. Gulyás. 1974. "Ultrastructure and mechanism of secretion in extrafloral nectaries of Ricinus communis L." *Acta biol. szeged* 20: 57-67.

Koptur S. 1992. Extrafloral nectary-mediated interactions between insects and plants. In: Bernays E. (Ed.), Insect–Plant Interactions. CRC Press, Boca Raton: 81–129.

Kupicha, F. K. 1976. The infrageneric structure of Vicia. *Notes R. Bot. Gard. Edinb*, *34*, 287-326.

Leandro Freitas and Adelita A. S. Paoli. 1999. Structure And Ultrastructure Of The Extrafloral Nectaries Of *Croton*

Urucurana Baill. (Euphorbiaceae)., Bol. Bot. Univ. sao Paulo 18: 1-10.

Lenore T. Durkeel, Matthew H. Haber, Lisa Dorn, And Ann Remington 1999. Morphology, Ultrastructure, and Function of Extrafloral Nectaries In Three Species of Caesalpiniaceae. Jour. Iowa Acad. Sci. 106(4):82-88.

Lersten, Nels R., and Curt L. Brubaker. "Extrafloral nectaries in Leguminosae: review and original observations in Erythrina and Mucuna (Papilionoideae; Phaseoleae)." *Bulletin of the Torrey Botanical Club* (1987): 437-447.

Machado S.R, Morellato L.P.C, Sajo M.G. and Oliveira P.S 2008. Morphological patterns of extrafloral nectaries in woody plant species of Brazilian cerrrado. Plant biology, 10: 660-673.

Marrazi *et al*. 2012. Diversity and evolution of a trait mediating ant–plant interactions: insights from extrafloral nectaries in Senna (Leguminosae) Ann.Bot.doi. 10.1093/aob/mcs226

McDade L.A., Turner M.D. (1997) Structure and development of bracteal nectary glands in Aphelandra (Acanthaceae). *American Journal of Botany*, 84, 1–15.

Metcalfe C.R. and Chalk L. 1979. Anatomy of the Dicotyledons. Claredon Press, Oxford: 276 pp.

Morellato L.P.C., Oliveira P.S. 1994. Extrafloral nectaries in the tropical tree Guareamacrophylla (Meliaceae). *Canadian Journal of Botany*, 72, 157–160.

Murrell, D.C., R.W. Shuel and D.T. Tomes, 1982. Nectar production and floral characteristics in birds foot trefoil *(Lotus corniculatus*L.). *Can. J. Plant Sci.,* 62: 361-371.

Nepi, Massimo, and Ettore Pacini, eds. *Nectaries and nectar*. Springer, 2007.

Oliveira P.S. and Leita˜o-Filho H.F. 1987. Extrafloral nectaries: their taxonomic distribution and abundance in the woody flora of cerrado vegetation in Southeast Brazil. Biotropica, 19, 140–148.

Oliveira, P.S. and Oliveira-Filho, A.T. 1991. Distribution of extraflora lnectaries in the woody flora of tropical communities in Western Brazil. In Plant-Animal Interactions: Evolutionary Ecology in Tropical and Temperate Regions (eds P.W. Price, T.M. Lewinsohn, G.W. Fernandes and W.W. Benson), pp. 163-175. John Wiley & Sons, New York.

Pemberton, R.W. 1988. The abundance of plants bearing extra-floral nectaries in Colorado and Mojave desert communities of southern California. *Madroño*, 35: 238-46.

Picazo-Tadeo, Andrés J., Francisco J. Sáez-Fernández, and Francisco González-Gómez. 2008. "Does service quality matter in measuring the performance of water utilities?." *Utilities Policy* 16.1: 30-38.

Rocha and Machado. Anatomy, ultrastructure and secretion of *Hibiscus pernambucensi s*Arruda (Malvaceae) extrafloral nectary. Revista Brasil. Bot., V.32, n.3, p.489-498, 2009.

Schmid, R. 1988. Reproductive versus extra-reproductives nectaries-historical perspective and terminological recommendations. Botanical review 54: 179-232.

Schoonhoven, L.M., van Loon, J.J.A. and Dicke, M. 2005. Insect–plant interactions, 2nd edn. New York: Oxford University Press.

Stpiczyńska, M., K. L. Davies, and A. Gregg. 2005. "Comparative account of nectary structure in Hexisea imbricata (Lindl.) Rchb. f.(Orchidaceae)."*Annals of Botany* 95, no. 5: 749-756.

Stpiczyrnska, M. 2000. Structure of the extrafloral nectaries of *Vicia*(L.) Fabaceae. Acta Agrobotanica, 53: 5-13.

Tarkowska, Jadwiga A. "Relations between ultrastructure of mitotic spindle and chromosome translocation." *Acta Societatis Botanicorum Poloniae* 50.4 (1981): 585-594.

Vogel S. 1997. Remarkable nectaries: structure, ecology, organophyletic perspectives. I. Substitutive nectaries. Flora 192: 305–333.

Voznesenskaya, Elena V. *et al*. 2005. "Differentiation of cellular and biochemical features of the single-cell C4 syndrome during leaf development in Bienertia cycloptera (Chenopodiaceae)." *American journal of botany* 92.11: 1784-1795.

Wa¨ckers, F.L. and Bonifay, C. 2004. How to be sweet? Extrafloral nectar allocation by Gossypium hirsutum fits optimal defense theory predictions. Ecology 85: 1512–1518.

Weryszko-Chmielewska, Elżbieta, and M. Bozek. 2008. "Structure of trichomatous nectaries in flowers of Lonicera kamtschatica [Sevast.] Pojark." *Acta Agrobotanica* 61.1.

Wrischer, M. 1962. "Electronic microscope examination of the floral nectaries of Vicia faba." *Acta. Bot. Croat* 20.21: 75-94.

Zimmerman J. 1932. U¨ ber die extrafloren nektarien der Angiospermen. Beihefte Botanisches Zentralblatt, Abt A, 49, 99–196.

****