Morpho-anatomy and ontogeny of the floral nectaries of *Cardiospermum grandiflorum* and *Urvillea chacoensis* (Sapindaceae)

Stella M. Solís¹,* & María S. Ferrucci²

¹) Facultad de Ciencias Exactas, Naturales y Agrimensura, Universidad Nacional del Nordeste, Instituto de Botánica del Nordeste (UNNE-CONICET), C.C. 209, 3400-Corrientes, Argentina (*corresponding author’s e-mail: estelissolis@yahoo.com)

²) Facultad de Ciencias Agrarias, Universidad Nacional del Nordeste, Instituto de Botánica del Nordeste (UNNE-CONICET), C.C. 209, 3400-Corrientes, Argentina

Received 20 May 2008, revised version received 22 Sep. 2008, accepted 12 Nov. 2008


The morphology, anatomy and ontogeny the floral nectaries of two species of Sapindaceae (Paullinieae), viz. *Cardiospermum grandiflorum* and *Urvillea chacoensis*, were studied using light and scanning electron microscopy. Both species are monoecious with oblique monosymmetric flowers. The floral nectaries are located at the base of the androgynophore. Vascular tissue supplying the nectaries consists of phloem. *Cardiospermum grandiflorum* has a posterior nectary with two lobes without any specially differentiated tissue, and with nectarostomata. In *Urvillea chacoensis* the nectary has four lobes; two posterior ones with distinctive nectariferous tissue with nectarostomata on the abaxial side, and two minor anterior ones that are histologically similar but lack nectarostomata. The nectaries in both species have a common base with the petals. Since the nectaries are macroscopically differentiated they are considered as structural nectaries.

Key words: floral morpho-anatomy, nectarostomata, nectary structure, nectary ontogeny, Paullinieae

Introduction

The family Sapindaceae comprises around 140 genera and 1600 species (Ferrucci 2005a), mainly from tropical and subtropical regions. *Cardiospermum* and *Urvillea*, both in the tribe Paullinieae, are characterized by unisexual, obliquely monosymmetric flowers, with a tetramerous corolla. Other important characters are the petals with a scale adnate to their adaxial basal surface, a unilateral nectary with two or four protruding lobes, and eight stamens of unequal lengths. The fruits are schizocarps or capsules (Radlkofier 1931–1934, Ferrucci 2000a, Weckerle & Rutishauser 2005).

The tribe Paullinieae displays the most advanced characters within the family such as the climbing habit, woody vines or, less frequently, herbaceous annual vines, and zygomorphic flowers with a unilateral disk modified into two or four prominent glands (Ferrucci & Anzótegui 1993). Other genera of Paulli-
nieae are *Serjania*, *Paullinia*, *Houssayanthus*, *Lophostigma* (subtribe Paulliniinae), and *Thinouia* (subtribe Thinouiniae) having a basal position in the tribe (Muller & Leenhouts 1976, Ferrucci & Anzótegui 1993, Ferrucci 2000b).

*Urvillea* comprises 17 species of woody vines native to tropical and subtropical areas of the New World (Ferrucci 2000a, 2006), and is recognized by its slightly inflated or flattened capsules with the central locules surrounded by marginal wings. *Cardiospermum* contains 14 species of herbaceous vines and, less frequently, sub-shrubs (Ferrucci 2000a), all of them present in the tropical Americas and three of them with a nearly cosmopolitan distribution. As a rule, *Cardiospermum* has inflated capsules, no wings or narrow marginal wings; it is the most heterogeneous genus within the tribe and is most closely related to *Urvillea* (Ferrucci 2006).

Among the reproductive characters of Sapindaceae, floral nectaries are particularly interesting since they are present in the entire family and possess systematic value at the generic or specific level (Radlkofer 1931–1934, Ferrucci 1993, 2000a). In an exhaustive review of the terminology used in the classification of the nectaries, Schmid (1988) recommended using the terms reproductive and extrareproductive, which are based on strictly positional and not on functional criteria. Considerable research regarding general nectary classifications has been carried out by e.g., Vogel (1977), Fahn (1979) and Smets (1986, 1988). In a systematic survey of floral nectaries Bernardello (2007) recognized that Schmid’s scheme is extremely practical to categorize nectaries.

The morphology, anatomy, and development of floral nectaries in Sapindaceae have been little studied. Leinfellner (1958) studied the scales of the petals in two species of Paullinieae: *Serjania cuspidata* and *Cardiospermum corindum* (as *C. canescens*). Petals with ventral elaborations are mostly associated with a nectary. Except for Sapindaceae, there are few petal elaborations in Sapindales, and the ventral part of the scale has the function of a nectar cover (Endress & Matthews 2006a, 2006b). Weckerle and Rutishauser (2005) studied the gynoecium, fruit and seed structures and ontogeny in selected species of Paullinieae. Further studies on other tribes of the family are those of Ronse Decraene et al. (2000) and Weckerle and Rutishauser (2003).

The aim of this study is to present a comparative analysis of the morpho-anatomy and development of nectaries of *Cardiospermum grandiflorum* and *Urvillea chacoensis*. The number of stomata on the surface of the nectaries among male and female flowers in both species were also studied. The width and length of stomata were measured in order to detect if there are differences between male and female flowers and between the two species. This study will also contribute to the interpretation of the floral characters in the tribe.

**Material and methods**

Fresh samples fixed in FAA (formaldehyde–acetic acid–alcohol) were used for the anatomical research.

The ontogenetic study revealed that bud length was a reliable proxy to determine the age of a flower. In *C. grandiflorum*, the buds were classified as: Stage A: buds 44 × 70 µm; Stage B: buds 64 × 80 µm; Stage C: buds 118 × 140 µm; Stage D: buds 300 × 240 µm. In *Urvillea chacoensis*: Stage A: buds 40 × 40 µm; Stage B: buds 80 × 98 µm; Stage B: buds 80 × 98 µm; Stage C: buds 120 × 144 µm; Stage D: buds 320 × 210 µm.

The study is based mainly on light-microscope observations. Longitudinal and transverse serial sections of flowers and buds in different stages were prepared according to standard techniques. Permanent microscope slides were made by dehydration through an ethanol series, with a rinsing pre-impregnant (González & Cristóbal 1997). For infiltration in paraffin, the technique of Johansen (1940) was applied, and the material was later embedded in Histoplast®. Sections of 10–12 µm were made with a rotary microtome and stained with Astra blue-safranin (Luque et al. 1996) before mounting them with synthetic Canada Balsam.

Observations and photography were performed using a Leica MZ6 stereomicroscope and a Leica DM LB2 binocular microscope, both equipped with digital cameras.

The SEM micrographs were obtained with a JEOL 5800 LV scanning electron microscope.
operating at 20 KV. Preserved material was dehydrated and then immersed in CO₂ for critical-point drying before coating, and sputter-coated with gold palladium.

To detect the presence of stomata, nectaries of five male and five female flowers from both species were cleared with NaOH (10% aqueous solution), washed with ethyl alcohol-water (3:1), and stained with Lugol solution (Johansen 1940). Their diameter, length and width were measured using a graduated ocular. Thirty nectarostomata in male and female flowers were observed in both species. A two-way ANOVA followed by the Tukey test was used to evaluate intra- and interspecific differences in lengths and widths of stomata. Calculations were carried out using InfoStat (InfoStat 2008).


**Results**

Comparative floral structures of Cardiospermum grandiflorum and Urvillea chacoensis are described based on anthetic flowers, the anatomical structure of the nectary, and its ontogeny.

**Cardiospermum grandiflorum** (Fig. 1A and B)

**Morphology**

Male and hermaphrodite flowers, functionally...
female, are arranged in racemiform thyrses and are whitish, (5.5)6–9(–17) mm long, with two conspicuous nectaries in both floral types. The calyx has two comparatively shorter and suborbicular external sepals, and two internal obovate and petaloid sepals. The corolla has two posterior and two anterior petals with scales, which are symmetric in the posterior petals and asymmetric in the anterior ones; the deflexed appendage of each scale is comparatively long and reaches the base of the petal. In the inner part, two horn-like posterior nectary lobes are situated on the androgynophore base, are whitish, 1.25–3.5 mm high, and protected by the scale appendage of the posterior petals (Figs. 1C and 2C). The androgynophore is pilose and with eight stamens in the upper part, connivent at the base and of unequal length (three higher anterior, two median lateral and three shorter posterior) are inserted. In male flowers, the gynoecium is reduced to a rudimentary pistillode of 1 mm in length. Female flowers have eight short stamens with indehiscent anthers. The gynoecium is eccentric, 3-carpellate with a single campylotropous ovule per carpel, a trigonous-ellipsoidal or villous ovary, and a filiform style longer than the stigma.

Anatomical structure of the nectary (Fig. 2).

Epidermis. The nectary is covered with quadrangular or sub-polygonal epidermal cells; the cuticle has thin epicuticular striae. Nectarostomata are anomocytic and are concentrated at the apex, while they are scarce at the nectary base. Trichomes with ornamented cuticle are scattered over the entire surface (Fig. 2F). In transverse section, the epidermis is one-layered with a thin cuticle and with sub-quadrangular cells, the outer wall slightly convex, and the nucleus in parietal position. Nectarostomata are located at the level of the other epidermal cells; a sub-stomatal chamber is absent (Fig. 2B and E). Trichomes are unicellular.

Parenchyma and phloem. The nectary contains several layers of small, uncolored, isodiametric cells with thin walls, and minute intercellular spaces. Abundant idioblasts with calcium oxalate crystals in the form of druses are found towards the periphery. Numerous phloem strands extend from the base to the upper portion of the nectary (Fig. 2D). Between 6–12 phloem strands reach the apex (Fig. 2B). These strands are composed of sieve tubes, small companion cells, and phloem parenchyma cells of polygonal outline with prominent nuclei (Fig. 2D).

Nectary ontogeny

Stage A. In transverse section, the bud exhibits four sepals, two external and one anterior well developed, while the posterior sepal is still developing. At both sides of the posterior sepal, two posterior petal primordia are present. Nectary tissue is not visible (Fig. 3A–C).

Stage B. Two small protuberances, the incipient nectary glands, are differentiated at the base of the androgynophore (Fig. 4A).

Stage C. The nectary lobes have enlarged, and nectarostomata and simple hairs are present from the base to the apex of the glands (Fig. 4B and C). Beneath the epidermis there is parenchymatous tissue with thin cell walls, dense granular cytoplasm, small vacuoles, and a relatively large nucleus. Sieve tubes are present.

Stage D. In a mature bud, both nectariferous structures located on the base of the androgynophore are fully developed (1.25–3 mm high), with nectarostomata most abundant at the apex and sparse at the base. Between the bud stages C–D a progressive maturing of the nectary is observed (Fig. 4D–F). The nectary persists in the fruit although no secretion takes place.

Urvillea chacoensis (Fig. 1D and E)

Morphology

The flowers are fairly similar to those of Cardiospermum grandiflorum, so only the differences are described here. The flowers are arranged in racemiform thyrses, the partial inflorescences with short peduncles. Flowers are 4–7 mm long. The calyx is pentameric with two short, ovate external sepals and three obovate internal ones, the two anterior sepals slightly connate at the base; aestivation in bud is quincuncial. The nectary, in male and female flowers,
is fragmented into four lobes: two ovoid, obtuse posterior lobes and two similar but smaller anterior ones (Figs. 1F and 4J). The androgynophore is glabrous, except for a ring of hairs on the androecium base where eight stamens of unequal length are inserted. The ovary is villous, trigonous or obovoid, and the style is generally shorter than the stigma.

**Anatomical structure of the nectary (Fig. 5)**

**Epidermis.** The nectary is covered with quadrangular or sub-rectangular epidermal cells. The cuticle has fine striae. Nectarostomata are anomocytic (Fig. 5F). In transverse section, the epidermis is one-layered with a thin cuticle and with uniform rectangular cells, with granular cytoplasm and large nuclei. Nectarostomata are present only in the abaxial lobe of the posterior nectary glands; the substomatal chambers are reduced (Fig. 5E). No nectarostomata are observed on the surface of anterior glands.

**Parenchyma and phloem.** The nectary contains isodiametric cells, with thin walls, and without intercellular spaces; a dense cytoplasm with abundant reddish granular content, and a large nucleus. Numerous idioblasts with druses are distributed in the tissue (Fig. 5A and B). In the central part of the nectary where the petal is inserted, the subnectary parenchyma consists of larger cells with bigger vacuoles and less dense cytoplasm; idioblasts with granular cytoplasm content are also present.
The vascular traces supplying the posterior nectaries come from one of the central ectophloic stele, four or five phloem strands, radiating to supply the abaxial lobe of each nectary gland. These strands branch again till they reach the three or four subepidermical layers (Fig. 5B and D). The phloem strands are composed of sieve tubes, companion cells and parenchymatous cells. The anterior nectary lobes are non-vascularized (Fig. 5C).

**Nectary ontogeny**

**Stage A.** In transverse section, the bud shows meristematic cells with a prominent nucleus and reddish cytoplasm, located at both sides of the central stele between the two primordia of the anterior internal sepals and the posterior internal one (Fig. 3D). These cells undergo anticlinal and periclinal divisions, constituting the point of origin of the petals and development of the nectary lobes (Fig. 3E and F).

**Stage B.** Petal primordia with a swollen base belonging to the initial development of the nectary are differentiated (Fig. 4G and K).

**Stage C.** The initial formation of the two posterior nectary lobes is evident, but the two anterior ones cannot be distinguished yet. The globose nectary lobes are seen in the petal basal-internal part; the scale of the petal has also developed (Fig. 4H and L). In the longitudinal section, the nectary lobes are covered with quadrangular epidermis cells with a large nuclei; numerous nectarostomata are distinguished in the abaxial side of the posterior nectary lobes (Fig. 5A). The secretory tissue, small cells with dense cytoplasm, is present below the epidermis (Fig. 5B). Sieve tubes are present.

**Stage D.** In a mature bud, the four nectary lobes, two posterior and two anterior ones, are fully developed and are recognized by their
shape, size and internal structure. Each nectary lobe is protected by the petal scale. The four nectary lobes persist in the fruit although they are not functional, (Fig. 4I–M).
Differences in length and width of stomata

In *C. grandiflorum*, female flowers had a high number of stomata, ranging from 11 to 20, whereas in male flowers their number ranged from 9 to 13. In *U. chacoensis*, there was no difference in the number of stomata between the types of flowers. These differences were not statistically tested. The lengths of stomata differed significantly among species (two-way ANOVA: $F_{1,596} = 363.37, p = 0.0019$), but not between sexes; with the stomata of *C. grandiflorum* being significantly longer ($21.81 \pm 3.42 \mu m$) than the stomata of *U. chacoensis* ($17.13 \pm 2.57 \mu m$) (Tukey test: $p < 0.0001$).

The widths of stomata differed significantly among species (two-way ANOVA: $F_{1,596} = 228.21, p < 0.0001$), but not between sexes. The stomata of *C. grandiflorum* were significantly wider than the stomata of *U. chacoensis* ($16.84 \pm 2.77 \mu m$ and $13.64 \pm 2.42 \mu m$, respectively) (Tukey test: $p < 0.0001$).

Discussion

The floral nectaries in *C. grandiflorum* as well as in *U. chacoensis* can be considered structural nectaries as defined by Zimmermann (1932), who distinguished structural from non-structural nectaries.

In both species, the nectaries belong to the axial type of Smets (1988) and Smets and Cre-sens (1988). In *U. chacoensis* the four nectary lobes are initiated simultaneously with the petal primordia from a common meristematic group of cells. The two posterior nectaries are initiated first and later the anterior ones develop; this pattern of nectary development is widespread.
in Paullinieae. On the other hand, in *C. grandiflorum* the two nectary lobes arise simultaneously with the posterior petal primordia from a common meristematic group of cells. The nectary lobes and the petals are disconnected, and the nectariferous tissue is located at the base of the androgynophore. So, the nectaries are not on the petals but are topographically associated with them. It is interesting that in the tribe this feature is shared with few species of *Cardiospermum*, such as *C. anomalum* belonging to the sect. *Carphospermum*, and *C. integerrimum* and *C. heringeri* of the sect. *Ceratadenia*. The reduction in the number of nectary lobes as well as an independent position of them in relation to petals, are floral structural features that could be interpreted as apomorphic characters. Among the other species of Paullinieae these features were not found. Ronse Decraene *et al.* (2000) in their study of floral structure in *Koelreuteria paniculata* (Sapindaceae, Koelreuterieae) mention that in the ontogeny of the flower a short androgynophore is formed and that the area at the base of the androgynophore differentiates as a continuous nectary disk, although stomata are lacking. The presence of stomata in floral nectaries has been reported in more than 75 families of angiosperms (Davis & Gunning 1993). Nectarostomata occur mainly in exposed floral nectaries (Endress 1994). Fahn (1979) mentions that nectaries exuding nectar through stomata generally present a well-differentiated nectariferous tissue. Such modified stomata that have lost the capacity to open and close (Davis & Gunning 1992) were termed nectarostomata by Ronse Decraene and Smets (1991). In both of the studied species, the vascular tissue, consisting entirely of phloem strands, supplies nectar that is exuded via nectarostomata. However, a secretory nectariferous parenchyma is evident only in *U. chacoensis*.

There is no vascular tissue in the nectaries of some species. In other species, vascular bundles supplying nectaries are phloic, xylie, or xyliphloic. The strictly phloic nature of the vascular supply of the nectaries of our two species is consistent with that of most nectaries (Frey-Wyssling 1955, Schmid 1975, Fahn 1979).

*Cardiospermum grandiflorum* has a nectary with only two hornlike lobes, with the most stomata distributed at the apex, whereas *U. chacoensis* has four nectary lobes, two posterior ones with stomata restricted to the abaxial side, and two anterior ones where stomata are absent. A similar situation is present in *Rosmarinus officinalis*, in which the nectary is tetralobed with the anterior lobe longer than the others; only the longer lobe has stomata. This is interpreted as an adaptation to the horizontal position of the flower in the Lamiaceae, in which the nectar produced by the whole nectary flows through the intercellular spaces to the longer lobe, from which it is released by stomata (Zer & Fahn 1992).

In *C. grandiflorum* and *U. chacoensis* the position, structure, size and histology of the floral nectaries is similar in male and female flowers. Significant differences in length and width of stomata were, however, found between species: in *C. grandiflorum* they are larger than in *U. chacoensis*; the former had a higher number of stomata in female flowers than in male ones, whereas in the latter there was no difference between the floral types.

The nectaries of both species correspond to the mesenchymatous nectary type (Vogel 1977). According to the classification of floral nectaries proposed by Smets (1986), they have persistent nectaries, a character considered as apomorphic in eudicots. The nectaries here studied belong to the axial persistent type (Smets & Cresens 1988, Smets 1989). Weckerle and Rutishauser (2003) mention the presence of a persistent extrastaminal flower nectary disk in *Averrhoidium* (Sapindaceae, Doratoxyleae). From an evolutionary point of view the persistent nectaries are concentrated in the medium- and higher-evolved groups of the dicots (Endress 1994).

Fahn (1979) proposed a topographic classification of the floral nectaries, later adapted by Schmid (1988) and Bernardello (2007). Following the previous classification, the nectaries analyzed here correspond to the receptacular extrastaminal type.

A distinctive feature in Sapindales is the presence of a nectary disk where nectar is hidden by the stamens (Endress 1994, Judd *et al.* 1999). According to Gadek *et al.* (1996), the receptacular nectary could represent an important morphological synapomorphy for the order. In Sap-
indaceae, the floral nectary may have a different shape, it may appear either as a disk (simple or double), or it may be unilateral as a half-disk or fragmentated into four or two parts. Its position is extrastaminal except in Dodonaeae, where in D. viscosa it occupies an intrastaminal position in female and in perfect flowers, but is absent from male flowers (Ferrucci 2005b). In Sapindaceae, the main evolutionary differentiation in the position of the nectaries is centrifugal. Dodonaea belongs to the subfamily Dodonaeoideae, which gives in many respects the impression of being an assemblage of relicts (Muller & Leenhouts 1976). In the subtribe Paulliniinae, the shape, number and with less significance the indumentum of the nectary lobes in a flower are of diagnostic value. These characters are variable among species of the genus, and in some cases are used for recognizing the species. In Cardiospermum, one of the three sections, the sect. Ceratadenia, is characterized by the presence of hornlike nectary lobes. In subtribe Thinouinae, the receptacle disk is annular and continuous, a character that might be correlated with the actinomorphic floral structure.

Acknowledgements

We are deeply grateful to Peter Endress for critically reading an early version of the manuscript. We thank Rudolf Schmid and one anonymous reviewer for helpful suggestions on the manuscript. We are also indebted to Ana González for her technical assistance with the digitations of images, to Mercedes Avanza for her help with statistic analyses and to María Pierpaoli for improving the English version. Financial support for our research was provided by the Universidad Nacional del Nordeste (PI 038/06) and by the Agencia Nacional de Promoción Científica, Tecnológica y de Innovación (ANPCyT-UNNE, PICTO 00096).

References


