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Nectaries and reproductive biology of *Croton sarcopetalus* (Euphorbiaceae)

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Flower morphology, nectary structure, nectar chemical composition, breeding system, floral visitors and pollination were analysed in *Croton sarcopetalus*, a diclinous-monoecious shrub from Argentina. Male flowers have five receptacular nectaries, with no special vascular bundles, that consist of a uniserial epidermis with stomata subtended by a secretory parenchyma. Female flowers bear two different types of nectaries: inner (IN) and outer (ON) floral nectaries. IN, five in all, are structurally similar to the nectaries of male flowers. The five ON are vascularized, stalked, and composed of secretory, column-shaped epidermal cells without stomata subtended by secretory and ground parenchyma. In addition, ON act as post-floral nectaries secreting nectar during fruit ripening. Extrafloral nectaries (EFN) are located on petioles, stipules and leaf margins. Petiolar EFN are patelliform, stalked and anatomically similar to the ON of the female flower. Nectar sampled from all nectary types is hexose dominant, except for the ON of the female flower at the post-floral stage that is sucrose dominant. The species is self-compatible, but geitonogamous fertilization is rarely possible because male and female flowers are not usually open at the same time in the same individual, i.e. there is temporal dioecism. Flowers are visited by 22 insect species, wasps being the most important group of pollinators. No significant differences were found in fruit and seed set between natural and hand pollinated flowers. This pattern indicates that fruit production in this species is not pollen/pollinator limited and is mediated by a wide array of pollinators.

ADDITIONAL KEY WORDS: extrafloral nectaries – floral nectaries – insect pollination – nectar chemical composition – post-floral nectaries – self-compatibility – temporal dioecism.

INTRODUCTION

Plant–animal mutualisms encompass an enormous spectrum of relationships (Abrahamson, 1989) whose benefits to the plant may include protection, nutrition, pollination and seed dispersal (Price *et al.*, 1991). In general, plants use a suite of substances to reward animals that in turn offer a beneficial relationship; among them, nectar is by far the most widely used (Simpson & Neff, 1983).

Nectaries are organs of specialized tissue that secrete nectar. They are widespread among angiosperms and show a great diversity in shape, structure and function (e.g. Fahn, 1979; Elias, 1983; Smets, 1986). There are basically two nectary types – floral and extrafloral nectaries (EFN) – distinguished either by position or function (Elias, Rozich & Newcombe, 1975; Fahn, 1979; Elias, 1983; Smets, 1986). In general, floral nectaries are involved in pollination while EFN might protect vegetative and reproductive structures from herbivory and are visited by different animal guilds. In addition, there is a third type, less frequent and, accordingly, less studied: post-floral nectaries, i.e. floral nectaries where nectar production goes on after anthesis during fruit development (Faegri & van der Pijl, 1979; Keeler, 1981; Gracie, 1991).

The occurrence of several nectary types in the same species allows us to determine differences in their structure, chemical composition of the nectars produced and the role of the visitors of each type. These data may provide clues on the evolution of the plant–

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animal interactions. Members of Euphorbiaceae, including *Croton*, commonly have two nectary types in the same species, floral and EFN, that are usually morphologically different and have a distinct evolutionary origin (Bernhard, 1966; Webster, 1994a). The monophyletic genus *Croton* comprises at least 800 species in the tropics and subtropics (Webster, 1993, 1994b). In Central Argentina, we found a species that has not only floral and EFN, but also post-floral nectaries: *C. sarcopetalus* Müll. Arg. It is a diclinous-monoecious shrub common in Argentina with a distribution from Jujuy to Córdoba provinces, growing between 250–1300 m a.s.l. (Croizat, 1941). There is a previous study in this species on visitors of EFN and their role (Freitas *et al.*, 2000). Experimental data showed that there were no significant differences either in the degree of herbivory or in plant reproductive output between excluded and ant-patrolled branches. However, no data are available on nectary structure, nectar chemical composition, pollinators and reproductive biology. Thus, the aims of this paper are: (1) to determine lifetime, production and structure of the different flower types; (2) to compare the structures and nectar contents of floral and EFN; (3) to determine the breeding system; (4) to record floral visitors, observe their behaviour, and determine which ones are pollinators; (5) to understand the significance of the findings in the reproductive biology/plant–animal interaction of the species.

MATERIAL AND METHODS

Field work was carried out during 1995 in a Chaquean forest community, located in the Sierra Chica, Villa Warcalde, Córdoba Province, Argentina (31°20′S, 64°15′W). Vouchers (Bernardello *et al.*, 872 and 872 bis) are deposited at the Museo Botánico de Córdoba, Argentina.

Flowers to be sectioned were fixed in F.A.A., dehydrated through an ethyl alcohol/xylene series, and embedded in paraffin. Sections were cut at 9–12 μm thickness, mounted serially and stained with haematoxylin–safranin–Fast Green (Conn, Darrow & Emmel, 1960). Drawings were made using a camera lucida. To detect the presence of stomata, nectaries were cleared with NaOH (10% aqueous solution), washed with ethyl alcohol:water (3:1) and stained with Lugol solution (Johansen, 1940).

Nectar drops from each nectary type were placed on Whatman no. 1 chromatography paper and quickly dried. As female flowers have two types of nectaries, nectar was obtained separately from each type in the laboratory and with the assistance of a stereoscopic microscope immediately after collecting the plants in the field. Tests for amino acids, lipids, phenols, alkaloids and reducing acids were performed after Baker & Baker (1975). Sugar separation was accomplished by gas chromatography. Nectar was lyophilized and silylated according to Sweeley *et al.* (1963). Derivatives were then injected into a Konik KNK 3000-HRGS gas chromatograph equipped with a Spectra-Physics SP 4290 data integrator, a flame ionization detector and a OV 101 column (2 m long), 3% on Chromosorb G/AW-DMCS mesh 100–120. Nitrogen was the carrier gas (30 $ml^{-1}min^{-1}$) and the following temperature programme was used: 208°C for 1 min, an increase of 1°C min^{-1} until 215°C was reached, and then an increase of 18°C min^{-1} to 280°C which was maintained for 5 min. Chromatographic sugar analyses were made at least twice for each sample (except for nectaries of the female flower, analysed only once because of the small amount obtained). Carbohydrate standards (Sigma Chemical Co.) were prepared using the same method. The sugar ratio, as r = sucrose/fructose + glucose (Baker & Baker, 1983), and the hexose ratio, as hr = glucose/fructose, were calculated.

The receptivity of stigmata was tested using the hydrogen peroxide catalase activity method (Dafni, 1992). Breeding system treatments were performed after bagging buds with paper bags to exclude pollinators. One group of female flowers ($N = 46$) was left bagged until the end of the experiment to determine the eventual apomictic production of seeds. Two other groups of female flowers were hand-pollinated with pollen from male flowers of the same plant (geitonogamy, $N = 22$) or with pollen from different plants (xenogamy, $N = 119$). As control (open pollinated flowers), a group of female flowers ($N = 91$) was bagged when their stigmata were no longer receptive to equal the conditions of the other treatments and to prevent loss of seeds because of their explosive dehiscence. Geitonogamy, xenogamy, apomixis and control treatments were performed on the same seven plants. It was difficult to make the sample size of geitonogamous hand-pollinations equal to those of the other treatments because plants with male and female flowers at the same time were found only occasionally. Fruit set, seed set and seed mass were determined for all treatments.

Flower visitors were observed on a total of 37 days in two periods, March/June and October/December, because flowers are not produced in winter (July/September). Observations lasted *c.* 4 h per day (total of *c.* 150 h) and were made between 6:30 a.m. and 7:00 p.m. Visitors were photographed and observed for analyses of their behaviour. Insect specimens were collected to detect pollen on their bodies and to identify them. Insects that had *C. sarcopetalus* pollen in their pollen load and were detected on female and/or male flowers were considered to be pollinators.

A two-way mixed model ANOVA was used to examine

whether pollination treatments (xenogamy, geitonogamy and control), maternal plant, and the interaction of these factors influenced fruit set, seed set and seed mass. Maternal plant was treated as a nested factor. The assumptions of normality and homoscedasticity were met for all variables examined (Sokal & Rohlf, 1995). Bonferroni *a posteriori* testing was used to evaluate significant differences. The statistical program package SPSS (1992) was used for all these analyses.

RESULTS

INFLORESCENCES

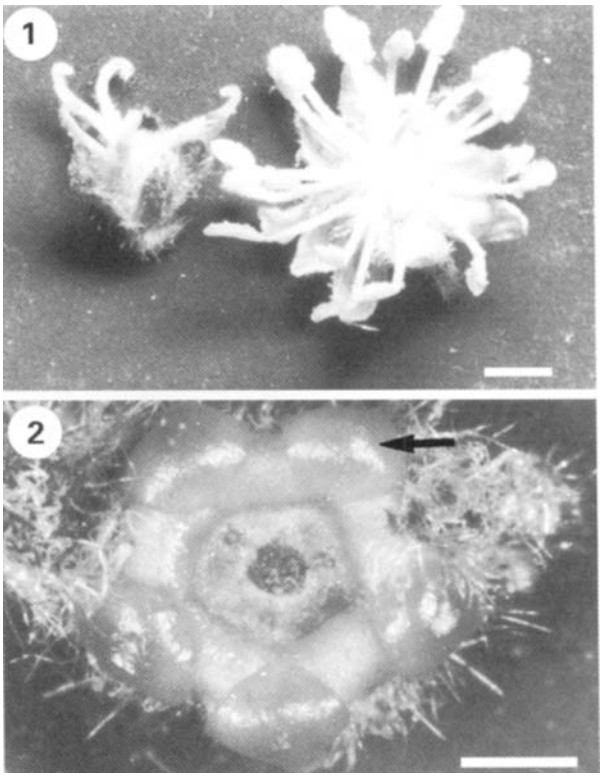
Croton sarcopetalus flowers are arranged in terminal racemes. During fruit development, some inflorescences showed newly produced male flowers among fruits. The number of flowers per inflorescence is variable (female flowers: mean = 10.9, range = 2–40; male flowers: mean = 25.3, range = 5–60, $N = 20$).

Within each raceme, female flowers are located at the base and opened first whereas male flowers are located at the apex and opened *c.* 1 day after the stigmata of the last female flowers were no longer receptive.

Within each individual plant, inflorescences were synchronous, i.e. at the beginning of the flowering period each plant had exclusively female flowers and, afterwards, only male flowers. Exceptions were found in only three of the 20 plants analysed (16.6%); those plants were older, bigger, very ramified and had more racemes (53, 85 and 146 *vs* a mean of 31.1 in the remaining 17 plants). These three bigger plants showed a short overlapping period (3–5 days) of flowers of both sexes: a few male flowers opened in a few inflorescences when the female period of the plant was ending, but never on the same racemes.

MALE FLOWER

These flowers are dish-shaped (Figs 1, 2), pentamerous, actinomorphic, measure 7–10 mm in diameter, and have hairy pedicels *c.* 2 mm long. Sepals are *c.* 2 mm long, free, hairy and green. Petals are free, delicate, slightly concave and greenish yellow with two deep green longitudinal lines, and are about the same size as the sepals. The androecium is represented by 15 to 20 free yellowish green stamens *c.* 3 mm long. Anthers are light yellow, basifixed, introrse and bilocular, and dehisce through longitudinal slits. Flowers last around 1 day, usually opening during the morning and falling late in the afternoon; sometimes, they open in the afternoon lasting until the afternoon of the following day. A sweet odour is detected at the beginning of flower opening.



Figures 1 & 2. *Croton sarcopetalus.* Fig. 1. Female and male flowers, respectively. Fig. 2. Floral nectaries of female flowers, arrow shows an outer nectary. Scale bars: 1 = 2 mm, 2 = 500 μm.

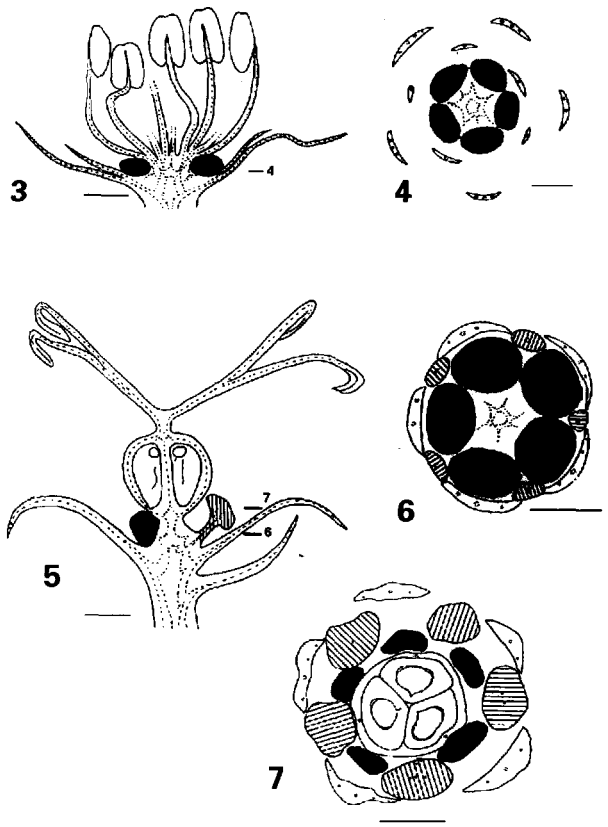
FEMALE FLOWER

The main difference from female flowers is that the corolla is absent in male flowers (Figs 1, 5). Flower diameter is *c.* 4 mm. Sepals are *c.* 3 mm long, green, elliptical, sub-equal and hairy. The three-carpellar ovary is sessile, hairy, and light green to brownish green. Each carpel has one epitropous ovule with axilar placentation (Fig. 7). The three whitish styles are bifid (Fig. 5) and *c.* 4 mm long. Stigmata are located above the bifurcation of the styles. These flowers last for 3–4 days and their opening occurs at any time during the day. No odour was detected.

MALE FLOWER NECTARIES

Five receptacular nectaries are located opposite the sepals (Figs 3, 4). Each sessile gland (Fig. 9) is cylindrical with two superior lobes that have a depression where nectar accumulates. Anatomically, they have a uniserial epidermis subtended by a secretory parenchyma (Fig. 9). Epidermal cells bear a thin cuticle. Stomata were detected on the apical portions of the lobes through which nectar seems to exude.

The secretory parenchyma has up to 20 layers of



Figures 3–7. *Croton sarcopetalus* Fig. 3. Schematic longitudinal section (LS) of male flower showing nectaries in black. Fig. 4. Schematic cross-section of 4 in Fig. 3 showing nectaries in black. Fig. 5. LS of female flower showing inner (black) and outer (diagonal hatched) nectaries. Figs 6 & 7. Cross-sections of female flower at levels 6 and 7 in Fig. 5. Symbols as in Fig. 5. Scale bars: 3, 4 = 800 μm, 5–7 = 500 μm.

isodiametric cells (Fig. 9). Epidermal and parenchymatous cells possess large nuclei, dense granular cytoplasm and many small vacuoles. Idioblasts with calcium oxalate druses are frequent in the parenchyma. The nectary does not have a special supply, but vascular bundles from the pedicel are located near the nectary base (Fig. 9). Non-articulate laticiferous ducts are observed near the vascular system (Fig. 9).

FEMALE FLOWER NECTARIES

Two different types of nectaries (Figs 2, 5–7), here designated as inner (IN) and outer nectaries (ON), were detected. Each flower has five nectaries of each type alternately distributed: IN opposite the sepals and ON alternating with them (Figs 2, 5–7).

The IN are similar to the male flower nectaries: cylindrical glands with no special vascular supplies (Fig. 8), composed of a secretory epidermis and many layers of parenchyma (Fig. 8). Nectar is probably exuded through the stomata.

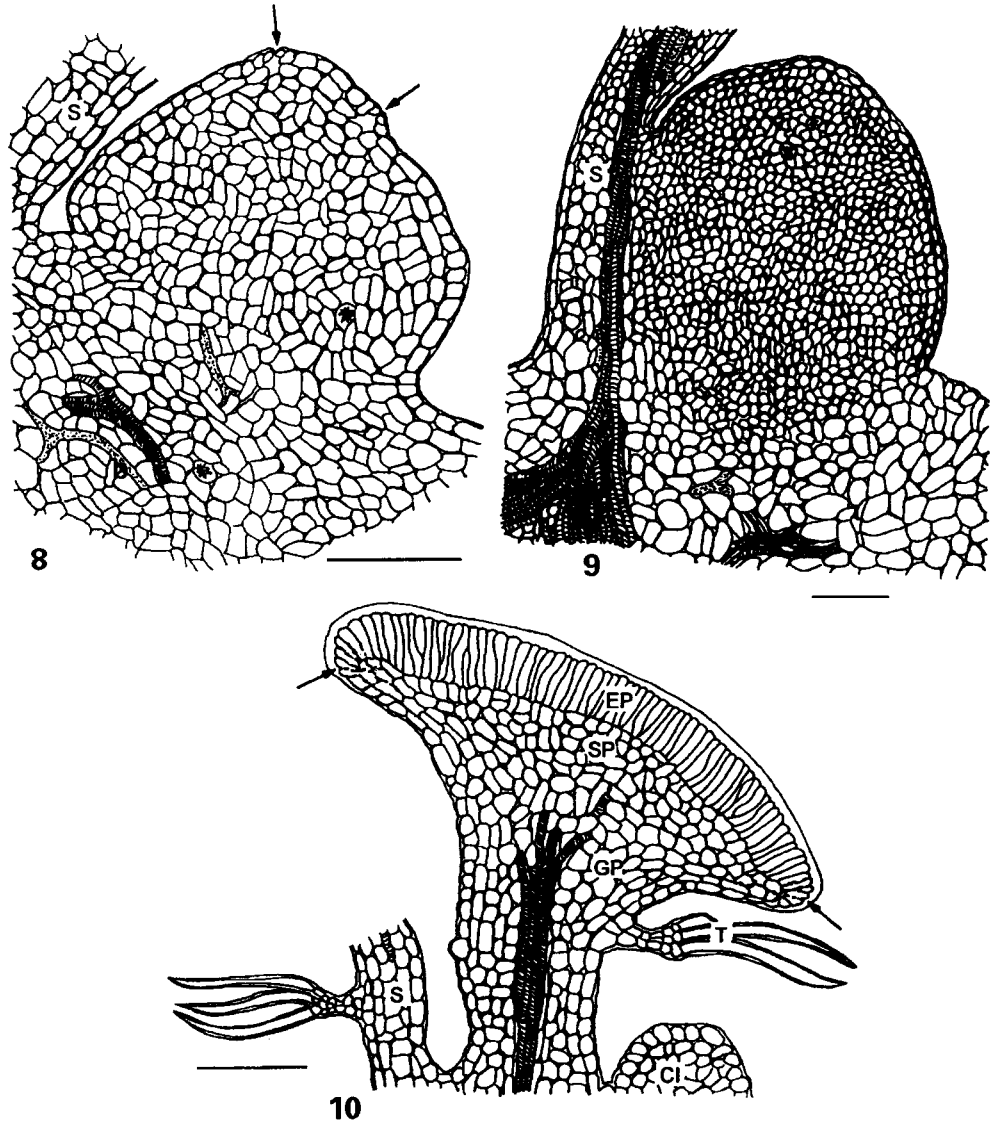
The ON are patelliform and stalked and similar in size to the IN (Fig. 10), consisting of a secretory epidermis subtended by secretory parenchyma and ground parenchyma. Brachysclereids with simple pits are present laterally (Fig. 10). The epidermis is composed of a layer of column-shaped cells covered by a thick cuticle (Fig. 10). Stomata are absent and nectar seems to be exuded through cuticular channels (Freitas, 1997). Secretory parenchyma has up to eight layers of isodiametric small cells. The secretory cells of both epidermis and parenchyma are characterized by large nuclei, several small vacuoles and dense cytoplasm. Stalk and basal parts of the ON head are composed of ground parenchyma (Fig. 10), a tissue comprising many layers of highly vacuolate comparatively larger cells. In contrast to the IN, a vascular system with xylem and phloem derived from collateral bundles located in the floral pedicel irrigates the ON ground parenchyma reaching the basal cells of the secretory parenchyma (Fig. 10). Laticiferous ducts are located near the vascular tissue inside the ON (Fig. 10). As these glands continue to secrete nectar during fruit development, they additionally function as post-floral nectaries.

EXTRAFLORAL NECTARIES

Extrafloral nectaries (EFN) are found on different plant parts: 2–8 glands are usually found on the adaxial surface of the petiole distal portion (Fig. 11) while there are 2–4 glands on the stipules. EFN on the leaf margins vary from none to more than 10 glands per leaf. Both stipule and leaf EFN are diminutive and produce small amounts of secretion, even in cultivated plants that were regularly watered.

Petiolar EFN are patelliform, stalked and have their own vascular supply (Fig. 11). They are composed of a secretory epidermis with column-shaped cells covered by a thick cuticle and subtended by several layers of secretory and ground parenchyma; in addition, sclerenchymatous cells are found near the edges of the glands (Fig. 12). Petiolar EFN are anatomically similar to the ON of the female flowers and also exude nectar through cuticular channels (Freitas, 1997).

In spite of the differences in position and size among EFN, they have similar structure (Figs 12–14). EFN on stipules (Fig. 14) and leaf margins (Fig. 13) show a columnar epidermis with a thick cuticle and a secretory parenchyma. However, they may not have ground parenchyma layer or stalk, and their shape may be globose instead of patelliform.



Figures 8–10. Floral nectaries of *Croton sarcopetalus*. Fig. 8. Schematic LS of an inner nectary of a female flower, arrows indicate stomata. Fig. 9. Schematic LS nectary of a male flower. Fig. 10. Schematic LS outer nectary of a female flower; arrows indicate sclerified cells. Abbreviations: S = sepal, EP = palisade epidermis, SP = secretory parenchyma, T = trichome, GP = ground parenchyma, CI = nectary of the inner cycle. Scale bars: 8 = 150 μm, 9, 10 = 120 μm.

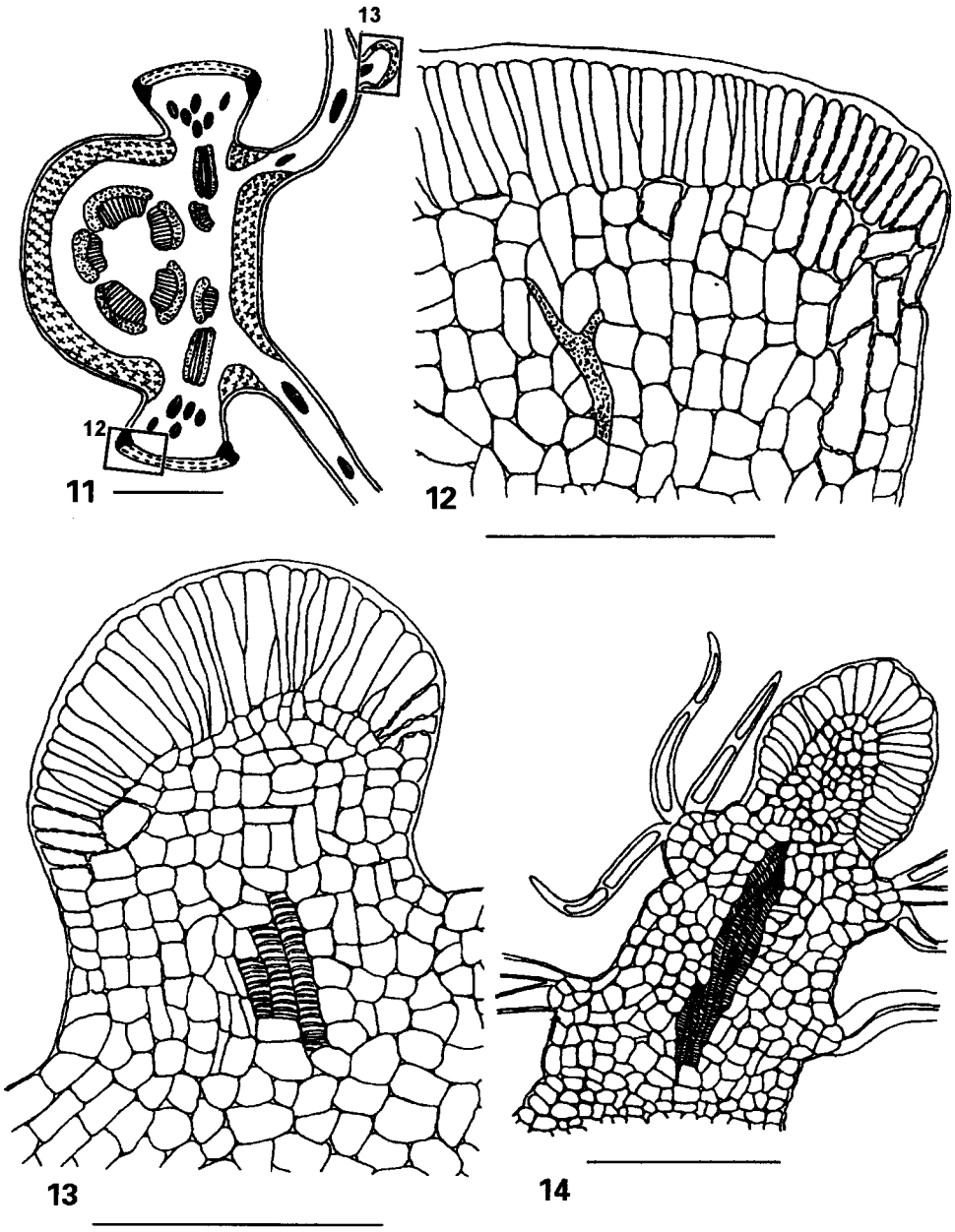
NECTAR

All nectary types produced small amounts of highly viscous nectar (*c.* 1 μl, concentration: 60–80%). The sugars in the nectar were mainly monosaccharides with a predominance of glucose over fructose in all nectary types (Table 1). The only exception was the post-floral nectar that mostly had sucrose; of the hexoses present there was more fructose than glucose (Table 1). In addition to sugars, reducing acids and amino acids were always detected. Amino acids were found in low quantities (histidine scale 1–2; Baker & Baker, 1975). Phenols were exclusively found in extrafloral nectar whereas lipids and alkaloids were never detected.

BREEDING SYSTEM

The species is self-compatible, as shown by the results of hand-pollination treatments (Table 2). In addition, no apomixis was detected in 46 experimental flowers treated.

A two-way mixed model ANOVA revealed that fruit and seed sets obtained from the different treatments (xenogamy, geitonogamy and control) were similar (Table 3). Seed mass showed significant differences



Figures 11–14. Extrafloral nectaries of *Croton sarcopetalus*. Fig. 11. Schematic LS petiole cross-section with a portion of leaf blade showing two petiolar nectaries and a nectary of a leaf margin. Fig. 12. Detail of a petiolar nectary, indicated 12 in Fig. 11. Fig. 13. Nectary of a leaf margin, indicated 13 in Fig. 11. Fig. 14. Partial cross-section of a stipule with an apical nectary. Hatchings in Fig. 11: dashes = palisade epidermis, crosses = colenchyma, stripes = xylem, dots = phloem, black = sclerenchyma. Scale bars: 11 = 600 μm, 12–14 = 140 μm.

between treatments (Table 3): seeds obtained by geitonogamous crosses had lower mass than seeds from xenogamous crosses or from control flowers (Table 2). There were also no differences in fruit set, seed set and seed mass among maternal plants and none of the interaction terms was significant (Table 3).

FLOWER VISITORS AND POLLINATORS

The honeybee (*Apis mellifera*) and 21 native insect species (in Coleoptera, Diptera, Hymenoptera and Lepidoptera) were observed visiting *C. sarcopetalus* flowers (Table 4, Figs 15–18). Visitors were more frequently observed during the warmest hours of the day in the

Table 1. Nectar sugar composition in *Croton sarcopetalus*. Values are means ± SD; *N*, number of sampled plants from which the nectar was obtained (number of flowers); r, sugar ratio; hr, hexose ratio; IN, inner nectary; ON, outer nectary; EFN, extrafloral nectary

Nectary type	*N*	Sugar composition			r	hr
		Sucrose	Fructose	Glucose		
Male flower nectary						
Pre-anthesis stage	3 (12)	12.8 ± 3.70	30.6 ± 8.05	56.6 ± 4.36	0.147	1.850
	2 (9)	4.1 ± 0.81	37.7 ± 0.17	58.2 ± 0.65	0.043	1.544
Mean		8.4 ± 6.15	34.2 ± 5.02	57.4 ± 1.13	0.095	1.697
Anthesis stage	2 (7)	1.9 ± 1.13	40.1 ± 4.67	58.0 ± 3.54	0.019	1.445
	1 (5)	12.5 ± 1.56	33.2 ± 0.84	54.3 ± 2.38	0.143	1.633
	1 (7)	0	36.1 ± 3.22	63.9 ± 3.22	0	1.770
Mean		4.8 ± 6.09	36.5 ± 4.03	58.7 ± 4.97	0.054	1.616
IN of female flower						
Anthesis stage	2 (18)	0	24.1	75.9	0	3.149
	2 (14)	0	33.5	66.5	0	2.000
Mean		0	28.8 ± 6.65	71.2 ± 6.65	0	2.472
ON of female flower						
Anthesis stage	2 (18)	0	25.5	75.5	0	2.961
Post-floral stage	4 (18)	61.1 ± 6.15	27.0 ± 6.70	11.8 ± 0.64	1.575	0.437
	3 (15)	49.5 ± 3.69	44.6 ± 4.77	5.9 ± 1.07	0.980	0.132
	2 (9)	40.0 ± 2.28	53.4 ± 0.87	6.6 ± 1.40	0.667	0.124
Mean		50.2 ± 10.57	41.7 ± 13.44	8.1 ± 3.22	1.008	0.194
Petiolar EFN	2 (7)	7.9 ± 0.96	31.4 ± 1.91	60.6 ± 2.86	0.086	1.930

Table 2. Fruit set, seed set, and seed mass (means ± SD) of hand pollination treatments and naturally pollinated (control) flowers of *Croton sarcopetalus*. Lowercase letters indicate the Bonferroni test results (see the analysis in Table 3)

Treatment (*N* = number of inflorescences used)	Fruit set (numbers of fruits/ flowers)	Seed set (numbers of seed/ ovules)	Seed mass (mg)
Xenogamy (*N* = 15)	0.67 ± 0.20 (80/119)	0.50 ± 0.22 (180/357)	[a]8.11 ± 1.88
Geitonogamy (*N* = 5)	0.54 ± 0.27 (12/22)	0.35 ± 0.36 (23/66)	[b]4.38 ± 0.99
Control (*N* = 12)	0.63 ± 0.34 (57/91)	0.42 ± 0.19 (114/273)	[a]8.12 ± 1.77

spring (October to December). Most visitors (20 spp.) were observed foraging for nectar and five of them also foraging for pollen (Table 4). A group of six insect species was apparently not involved in pollen transfer because of the absence of pollen of *C. sarcopetalus* in their pollen loads (Table 4). The remaining species were observed visiting male and/or female flowers, had pollen grains of *C. sarcopetalus* on their bodies, and can be considered as pollinators (Hymenoptera and Coleoptera species indicated with an asterisk in Table 4). These species mainly transport pollen grains on the ventral portion of the cephalothorax; the only exception was the small *Dialictus* sp. which carried pollen only on the dorsal portion of the cephalothorax. In addition to honeybees, the wasp *Brachygastra augusti* (Fig. 15) and the beetle *Astylus rubricostatus* (Fig. 17) were the most frequent native pollinators in the area of study.

Females of *Brachygastra augusti* chiefly searched for nectar and were the most abundant and main pollinators of *C. sarcopetalus* during the spring. Males also visited the flowers but were less frequent. When visiting male flowers, these wasps moved around the flower to take nectar from each individual nectary

Table 3. Results of two-way mixed-model ANOVA for fruit set, seed set and seed mass among pollination treatments (xenogamy, geitonogamy and control) and maternal plants ($N=7$) of *Croton sarcopetalus*. Maternal plant was treated as a nested factor

Variable	Source	df	MS	*F*	*P*
Fruit set	Pollination treatments	2	0.05	1.31	0.30
	Maternal plant	6	0.10	1.93	0.10
	Treatments × plant	8	0.07	1.80	0.15
	Error	31	0.07		
Seed set	Pollination treatments	2	0.05	1.22	0.32
	Maternal plant	6	0.09	2.10	0.12
	Treatments × plant	8	0.04	0.98	0.48
	Error	31	0.05		
Seed mass	Pollination treatments	2	24.13	10.52	0.002
	Maternal plant	6	5.89	2.57	0.07
	Treatments × plant	8	2.22	0.97	0.49
	Error	31	4.60		

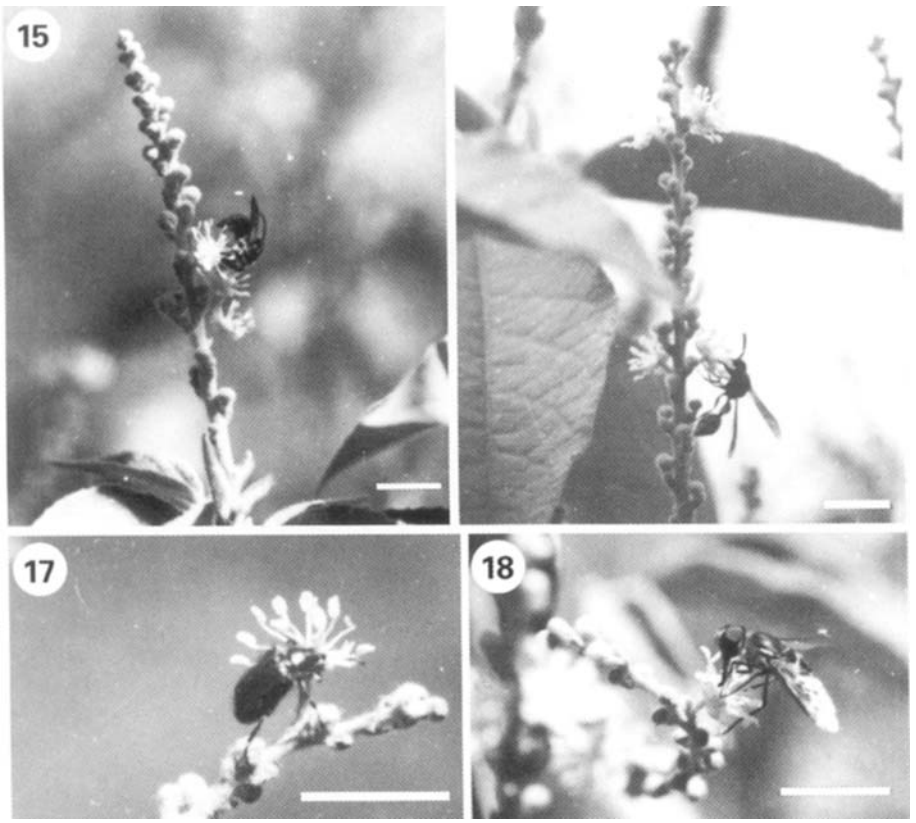
Table 4. Insect flower visitors in *Croton sarcopetalus* in Central Argentina. *, species considered as pollinator; +, observed; −, not observed; N, nectar; P, pollen; ?, undetermined

Order	Family	Species	Visited flower		Pollen load	Searched reward
			male	female		
Coleoptera	Cerambycidae	*Ommata* sp.	+	+	No	N
		Sphecomorpha sp.*	+	−	Yes	N
	Chrysomelidae	*Diabrotica speciosa**	+	−	Yes	N, P
	Dasytidae	Unidentified	+	−	No	?
	Melyridae	*Astylus rubricostatus**	+	+	Yes	N, P
		Astylus sp.	+	+	No	N
	Mordellidae	*Mordellistoma* sp.	+	−	No	N
Diptera	Bombyliidae	Unidentified	+	−	No	N
Hymenoptera	Apidae	*Apis mellifera**	+	+	Yes	N, P
	Halictidae	*Augochlora* sp.*	+	−	Yes	N, P
		Dialictus sp.*	+	+	Yes	N
	Megachilidae	Unidentified*	+	−	Yes	P
	Eumenidae	Unidentified sp. 1*	+	+	Yes	N
		Unidentified sp. 2*	+	+	Yes	N
	Sphecidae	*Ammophila* sp.*	+	+	Yes	N
		Bicyrtes sp.*	+	+	Yes	N
	Vespidae	*Brachygastra augusti**	+	+	Yes	N, P
		*Polistes canadensis**	+	+	Yes	N
		*Polybia ignobilis**	+	−	Yes	N
		*Polybia occidentalis**	+	+	Yes	N
	Formicidae	*Crematogaster scelerata*	+	−	No	N
Lepidoptera	Lycaenidae	*Thecla eurytulus**	+	−	Yes	N

getting pollen on their ventral surface and, when visiting female flowers, pollen would be transferred to stigmata. A few times we observed these insects feeding on pollen directly on anthers. In addition, males of *B. augusti* were seen feeding on EFN and post-floral nectaries frequently during the day although mainly at dawn.

Astylus rubricostatus was observed on flowers during the overall study period, being the only native pollinator observed in autumn. These beetles foraged on nectar and pollen and a large quantity of pollen was transported in their hairy bodies, mainly ventrally.

Polybia occidentalis, *Bicyrtes* sp. and a species of Eumenidae sp. 1 may be considered occasional pol-



Figures 15–18. Floral visitors in male flowers of *Croton sarcopetalus*. Fig. 15. *Brachygastra augusti*. Fig. 16. Eumenidae, sp. 2. Fig. 17. *Astylus rubricostatus*. Fig. 18. Fly of family Bombyliidae. Scale bars = 10 mm.

linators, while *Polybia canadensis*, *Dialictus* sp., *Ammophila* sp. and another species of Eumenidae sp. 2 (Fig. 16) are rare pollinators.

DISCUSSION

The floral nectary is a conspicuous feature of many plant families (e.g. Fahn, 1979; Schmid, 1988; Smets, 1986; Endress, 1994). However, it is less frequent that a species, such as *Croton sarcopetalus*, shows all the different types of nectaries: floral, extrafloral, and post-floral. In the Euphorbiaceae (Webster, 1994a), the floral nectary can be either continuous or five-segmented, as in *C. sarcopetalus*. This is the first report of two morphologically distinct series of floral nectaries in the same flower type, as we found for the female flower.

EFN on leaves and stipules are common and typical of some *Croton* sections and their origin is probably polyphyletic (Webster, 1993). However, data on their structure are scarce (Schnell, Cusset & Quenum, 1963; Jose & Inamdar, 1989; Freitas & Paoli, 1999). Petiolar nectaries seem to be the nectaries most characteristic of the genus because those found in *C. sarcopetalus* are similar to previous reports for other species (e.g. *C. amabilis*, *C. aubrevillei*, *C. refractus*, *C. glandulosus*, *C. macrostachyus*: Schnell *et al.*, 1963; *C. bonplandianus*: Jose & Inamdar, 1989; *C. urucurana*: Freitas & Paoli, 1999).

Post-floral nectaries have been reported in a few species of families such as Acanthaceae, Loasaceae and Rubiaceae (Faegri & van der Pijl, 1979; Keeler, 1981; Gracie, 1991). The system found in *C. sarcopetalus* is different because only one of the two nectaries of the female flowers (ON) functions as post-floral nectary.

Ganeshaiah & Shaanker (1988) detected EFN on pedicels of female flowers in *C. bonplandianus* that began to secrete nectar after fertilization and reached the maximum secretion at fruit maturation. This fact and the similarity of the structure of post-floral nectaries to pedicelar EFN in *C. sarcopetalus* could indicate that post-floral nectaries had an extrafloral origin as pedicelar nectaries that migrated to the floral receptacle.

Nectar may be considered as phloem fluid modified during secretion (Fahn, 1979). In a broad sense, the sucrose-dominant phloem fluid (pre-nectar) is converted into a mixture of sucrose, fructose and glucose, probably by invertase action inside the secretory cells (Findlay, 1988). Considering nectary diversity (vas-

cularized or non-vascularized, floral or EFN) and the different floral visitors found in *C. sarcopetalus*, variations in nectar composition can be expected. However, in all nectaries except post-floral ones the nectar was dominated by hexoses. In addition, although there are anatomical differences, ultrastructural aspects of secretory cells and nectar secretion are similar between floral and EFN (Freitas, 1997).

Post-floral nectaries are somewhat different. The high proportion of sucrose found in their nectar may be due to a failure in pre-nectar conversion inside the dictyosomes of the aged ON. This can be inferred because, at the ultrastructural level, secretory tissues of ON showed cells with inactive dictyosomes and some senescent cells at the post-floral stage in contrast with the previous stage of floral anthesis (Freitas, 1997). Thus, the sugar composition of the different nectaries may reflect ultrastructural traits (mechanisms of nectar production, cell age) of the glands rather than structural aspects or ecological function.

Croton sarcopetalus is a monoecious and self-compatible species. Self-compatibility is present within the genus, considering data from *C. floribundus*, *C. priscus*, *C. bonplandianus* and *C. suberosus* (cf. Reddi & Subba Reddi, 1985; Domínguez & Bullock, 1989; Passos, 1995). In *C. sarcopetalus*, artificial geitonogamy reduced the mean seed mass compared with control or xenogamous seeds. The lighter geitonogamous seeds could be a manifestation of inbreeding depression. Nevertheless, opportunities for natural geitonogamy are rare in this species because normally each individual has only female or male flowers at a given time, i.e. it has temporal dioecism (Cruden & Hermann-Parker, 1977) and most insect visitors would allow cross-pollination. Temporal dioecy is a mechanism to promote xenogamy, as an alternative to self-incompatibility and dioecy (Cruden & Hermann-Parker, 1977; Cruden, 1988). In *C. floribundus* and *C. priscus*, also self-compatible species, self-pollination is rare due to temporal dioecism as well (Passos, 1995).

It is not infrequent that only a small fraction of the ovaries produced by a flowering plant become fruits, leaving an apparent floral excess that makes no contribution to seed set (e.g. Stephenson, 1981; Sutherland, 1987; Lee, 1988; Burd, 1998). Although about half of the treated flowers of *C. sarcopetalus* failed to set fruit, no significant differences were found in fruit and seed set between natural and hand pollinated flowers. This pattern indicates that fruit production in this species is not pollen/pollinator limited. Consequently, the wide array of pollinators attracted are effective in transferring pollen and the plants seem to develop all the fruits that each can bear. The observed female flower over-production may allow increased fruit set during occasional but unpredictable years of resource abundance (Ehrlén, 1991) in the face of losses to herbivores, weather, etc., or may provide a larger pool from which superior fruits can be selectively mature (Stephenson, 1981; Burd, 1998). Although we did not find evidence of ant protection that would imply a gain in the reproductive output (Freitas *et al.*, 2000), the role of EFN would be of significance when the circumstances listed above determine an increase in natural fruit set. Nevertheless, a long-term study is necessary to evaluate carefully the variables and factors that influence the reproductive biology of *C. sarcopetalus* and to determine the role of its three nectary types.

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