

Flower characteristics and visitors of *Merremia macrocalyx* (Convolvulaceae) in the Chapada Diamantina, Bahia, Brazil

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Abstract

Merremia macrocalyx (Ruiz & Pav.) O' Donell (Convolvulaceae) is a common, native herb in open grasslands (Campos Gerais) of northeastern Brazil. At Pai Inácio Mountain, Chapada Diamantina, Bahia, Brazil (12° 27' 41" S, 41° 28' 34" W), we made daily observations from 6:30 a.m. until 5:30 p.m. from 25 April to 29 April, 2003 and on May 31, 2005. The stereomorphic corolla of the weakly scented flowers was 4.04 mm mean diameter, tube depth was 3.12 mm, and the mean diameter of the gullet was 1.17 mm. Anthesis is diurnal, between 06:30 a.m. and 07:45 a.m. and floral longevity varied between 9-10 h. Nectar secretion occurred from 7:00 a.m. to 11:00 a.m., and the standing crop of nectar per flower increased from 0.67 ml (7:00 a.m.) to 2.5 ml (09:00 a.m.) then declining. The nectar sugar concentration was low (22%). Bees were the most frequent visitors, and were classified into six morpho-functional categories. Their daily foraging pattern was synchronized with nectar availability, being concentrated between 8:30 and 10:30 a.m. The clumped distribution of the plants, flower size and easily accessible nectar all stimulate visitation, especially by generalist bee species. Eusocial bees (*A. mellifera*, *Geotrigona mombuca* and *Bombus morio*) and the solitary bee *Euplusia nigrohirta* (Friese, 1899) were the most frequent on the flowers and are the most likely potential pollinators, as suggested by their contact with floral reproductive structures and adhesion of pollen to their bodies.

Keywords: *Merremia macrocalyx*, Apoidea, flower biology, pollination, bees

Introduction

Merremia macrocalyx (Ruiz & Pav.) O'Donell is a twining herb or low-growing shrub in the morning glory family (Convolvulaceae Juss). Convolvulaceae comprises approximately 55 genera and 1600 to 1700 species worldwide (Austin, 1997a). Many species are widely distributed in tropical regions and can be encountered in tropical rain forests, savannas, prairies, and deserts. Their greatest diversity is encountered in the Americas and Africa. The genus *Merremia* Dennst. ex Endl. comprises about 70 species and is mostly distributed in tropical and subtropical regions (Deroin, 2001).

Flowers of Convolvulaceae are funnel-shaped, stereomorphic, often showy, and nearly always bisexual (Joly, 1993).

The perianth and androecial whorls are 5-merous. The sepals are usually distinct, but the corolla is strongly sympetalous, plaited, and often rotate or trumpet-shaped with inconspicuous lobes. The flowers of many species in Convolvulaceae attract a wide range of bee visitors (Austin, 1997b) and *M. macrocalyx* seems to be no exception.

Pollination is a key process for the conservation and maintenance of natural ecosystems (Kevan, 1991; Buchmann & Nabhan, 1996; Kearns et al., 1998; Kevan & Viana 2003), and understanding insect-plant interactions is essential for managing biodiversity. The Chapada Diamantina is noted for its floristic diversity and high levels of endemism (Stannard et al, 1995; Giulietti et al., 2000). Approximately 90% of the area of the Chapada Diamantina National Park is covered by Campo Rupestre and Gerais grassland vegetation (Funch, 1999), and both habitats support populations of *M. macrocalyx*. Campo Rupestre is dominated by herbs and shrubs that develop upon rock outcrops at elevations above 900 m (Giulietti & Pirani, 1988). Gerais are flat, open grassland valleys that develop on

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deep and sandy soils, at elevations between 1000-1500 meters (Giulietti et al., 1996).

Community level studies indicate that bees are important flower visitors and potential pollinators of many plant species (Faria, 1994; Martins, 1995; Viana et al., 1997; Pigozzo et al., 2006), including other species of *Merremia* (Maimoni-Rodella & Rodella, 1987; Willmott & Burquez, 1996; Kiill & Ranga, 2000, Kiill et al., 2000). This work provides the first record of flower visitors and the floral biology of *M. macrocalyx*.

Material and Methods

Study Site and plant species: A population of *Merremia macrocalyx* was found beside the road access to Pai Inácio Mountain (12° 27'41"S, 41° 28' 34"W), in the Chapada Diamantina, Bahia, Brazil at an elevation of approximately 1060 m. Mean annual precipitation in the region ranges from 800 to 1200 mm, mostly between November and March (Giulietti et al., 1996). Voucher specimens were deposited in the Alexandre Leal Costa Herbarium of the Universidade Federal da Bahia, and the Herbarium of the Universidade Estadual de Feira de Santana.

Floral morphology and biology: The diameter of the exposed corolla, and the depth and diameter of the corolla tube (measured at the widest part) were recorded from a arbitrarily selected flower sample (n = 20 flowers). Observations on flower biology were made from 25 April to 29 April, 2003. Flower anthesis, longevity and changes, such as in color, stigma and anther positions, pollen presentation and stigma receptivity, during the flowers' lifespan were noted.

The anthesis daily pattern was recorded on April 28, 2003 by counting open flowers every 15 minutes, from 6:00 a.m. to 5:30 p.m., among 28 marked buds in a 1 m x 2 m rectangular sample plot randomly selected within the population. Stigma receptivity was checked at hourly intervals, from 7:30 a.m. to 5:30 p.m. using the peroxidase reaction (Dafni et al., 2005). Five flowers randomly selected from the population were tested every hour (n = 55 anthers). Pollen viability was assessed every hour, from 6:30 a.m. to 5:30 p.m. (one anther per flower, n = 12 anthers), using neutral red (Dafni et al., 2005). The same method was used to locate the nectaries (n = 3 flowers).

Nectar standing crop was measured in open flowers (n = 12 flowers/hour, n = 84 flowers/day) at two hours intervals from 7:00 a.m. to 5:00 p.m. Sugar concentrations were also measured with a refractometer (ATAGO, range 0 to 90%), with the same flower samples.

Floral visitors: floral visitors were sampled using an insect net (diameter = 30.5 cm) from April 25 to 29 2003. Bee specimens were identified and deposited in the Insect Collection of the Laboratório de Biologia e Ecologia de Abelhas (LABEA), at the Universidade Federal da Bahia, Brazil.

The number of insects visiting flowers in the 1 m x 2 m plot with 28 flowers of *M. macrocalyx* was recorded for periods of 15 minutes at 2-hour intervals, from 6:30 a.m. to 4:30 p.m. Additional sampling and complementary observations of bee behavior were made from 10:00 a.m. to 12:00 p.m. on May 31, 2005 in the same population. Bee behaviors, such as place of landing, contact with the anthers and stigma, resources collected (pollen or nectar) were recorded. Whenever possible, the number

of flowers visited and duration of single visits to individual flowers were recorded.

Morpho-functional categories. Bee specimens were categorized into morpho-functional groups, based on standards defined by Roubik (1989) and Michener (2000): a) method of obtaining the resources; b) specialties such as: adaptations for perforating the corolla and for oil-collection; c) method of pollen gathering; d) method of pollen transport; e) body pilosity; f) length of glossa; and g) body length and thoracic width.

Bee body size considered total body length and thoracic width. Five size classes were used: Large mass: total body length > 14.0 mm and thoracic width > 6.0 mm; medium robust mass: total body length 10.0 mm to 14.0 mm and thoracic width 4.0 mm to 6.0 mm; medium thin mass: total body length 10.0 mm to 14.0 mm and thoracic width 3.0 mm to 4.0 mm; medium to small mass: total body length 7.0 mm to 10.0 mm and thoracic width 2.0 mm to 3.0 mm; small mass: total body length no more than 7.0 mm and thoracic width < 2.0 mm.

Bees were also grouped into five categories by glossal length: Very long: > 12.0 mm, long: 7.0 mm to 12.0 mm, intermediate: 3.0 mm to 6.9 mm, short: < 3.0 mm, short bilobate: < 3.0 mm.

Results

Floral morphology and biology: The flowers *Merremia macrocalyx* are grouped in auxiliary dichasia and are bisexual and lightly scented. Buds, flowers and fruits sometimes occurred in the same inflorescence. The mean diameter of the white, fully opened, stereomorphic corolla was 4.04 mm (SD = 0.17 mm), tube depth was 3.12 mm (SD = 0.12 mm), and the mean diameter of the gullet was 1.17 mm (SD = 0.08 mm). Anthers are five, yellow, and the ovary has four ovules.

Anthesis was diurnal, primarily between 6:30 a.m. and 7:45 a.m. The flowers were open only on the day of anthesis and, between 3:30 p.m. and 4:30 p.m. the corolla close in spiral. The corolla tube was directed horizontally, facilitating the bees' entry and serving as a landing platform for them. Anthers and styles were usually positioned at the lower side of the corolla tube. The green calyx was persistent, bearing the developing fruits. Pollen availability and stigma receptivity occurred immediately after anthesis. The stigma remained receptive until the corolla collapsed. Pollen viability attained 100% in flowers sampled from 6:30 a.m. to 3:30 p.m., but was strongly reduced after 3:30 p.m.

Nectar was present in a disk in the center of the corolla tube, around the base of the ovary. Nectar was secreted mostly in the morning, although its volume varied greatly during the day: 1.6 ml at 7:00 a.m., 2.5 ml at 9:00 a.m. and 0.67 ml at 11:00 a.m. Very little nectar was offered in the afternoon. Sugar concentration of the nectar was approximately 22%.

Flower visitors: Bees comprise almost all the flower visitors on *M. macrocalyx*, although a few other insects, such as Lepidoptera, Diptera and other Hymenoptera (ants and wasps), were seen. Twenty-two bee species from 16 genera in Apidae and Halictidae were captured (Tab. 1). The Apidae were more diverse both at the generic (13 genera) and specific (18 species) levels than were Halictidae, represented by 4 species and 3 genera (Tab. 1). Nine morpho-functional categories were

Table 1 - Flower visiting bees of *Merremia macrocalyx* (Convolvulaceae) collected in 2003 and 2005, at the Pai Inácio Mountain, Chapada Diamantina, Bahia, Brazil.

Family/ Species	Number of collected bees		
	Male	Female	Total
APIDAE			
<i>Ancyloscelis apiformes</i> (Fabricius, 1793)	2	3	5
<i>Apis mellifera</i> Linnaeus, 1758	0	27	27
<i>Bombus (Fervidobombus) morio</i> (Swederus, 1787)	0	12	12
<i>Centris (Centris) sp.</i>	0	1	1
<i>Centris aenea</i> Lepeletier, 1841	0	1	1
<i>Centris caxienseis</i> Ducke, 1907	0	2	2
<i>Ceratina (Crewella) cf. asuncionis</i> Strand, 1910	0	3	3
<i>Ceratina sp.</i>	0	1	1
<i>Epicharis (Epicharitides) iheringi</i> Friese, 1899	5	3	8
<i>Epicharis bicolor</i> Smith, 1854	7	0	7
<i>Euglossa fimbriata</i> Rebêlo & Moure, 1995	1	1	2
<i>Euplusia danielis</i> (Schrottky, 1907)	1	0	1
<i>Euplusia nigrohirta</i> (Friese, 1899)	2	14	16
<i>Exomalopsis (Exomalopsis) analis</i> Spinola, 1853	0	1	1
<i>Geotrigona mombuca</i> (Smith, 1863)	0	13	13
<i>Ptilothrix plumata</i> Smith, 1853	3	0	3
<i>Thygater (Thygater) analis</i> (Lepeletier, 1841)	1	0	1
<i>Trigona spinipes</i> (Fabricius, 1793)	0	2	2
HALICTIDAE			
<i>Augochloropsis callichroa</i> (Cockerell, 1900)	0	2	2
<i>Augochloropsis sp.</i>	0	1	1
<i>Augochlora sp.</i>	0	3	3
<i>Dialictus sp.</i>	0	1	1

identified. The **Ebm** category (see Tab. 1), with 4 species, showed the highest specific diversity (Tab. 2). The most frequent bees (*Apis mellifera*, *Euplusia nigrohirta*, *Bombus morio* and *Geotrigona mombuca*) were included in **Ebm** and **Tam** (see Tab. 1).

In 2003, the peaks of bee visitation were between 8:30 and 10:30 a.m. while nectar availability was high. However, in 2005, when the weather was cooler, cloudier and windier, the number and diversity of visitors intensified only after 10 a.m., and only *Apis mellifera* and *Bombus morio* visited *M. macrocalyx* flowers before 10 a.m.

Bees foraged for both nectar and pollen on flowers of *M. macrocalyx*, especially the social bees *A. mellifera* and the Meliponini. These bees usually visited several flowers in the patch *M. macrocalyx* before leaving. Vibratile pollen collecting was performed by *Bombus* and Centridini, spreading pollen grains over the bees' bodies. Large bees, such as *B. morio*, used the lowermost lobe of the corolla as a landing platform, contacting the stigma and the anthers sternotribically. *B. morio* females spent from 3 s to 5 s in a single flower (3.38 ± 1.19 ; $n=8$) and visited more than one flower in the *M. macrocalyx* population before leaving. *B. morio* females were seen grooming, transferring pollen to their corbiculae. Medium sized bees, such as *Apis mellifera*, landed on the upper part of the

corolla lobe before crawling into the corolla tube for nectar collection. When collecting pollen, *A. mellifera* touched the anthers and stigmas nototribically with their heads.

Discussion

Merremia macrocalyx is an important food resource for nectar-feeding insects in the Campo Rupestre, being visited by a diversity of bees with varying anatomical, behavioral and functional features. The flowers of other species of *Merremia* are visited and pollinated by assemblages of insects, mostly bees, including *A. mellifera*, *Trigona spinipes*, *Ancyloscelis apiformis*, and *Exomalopsis* spp. Other species in the genus (e.g. *M. palmeri*, in Mexico) are pollinated by Sphingidae (Willmott & Burquez, 1996), *M. cissoides* and *M. aegyptia* are mostly pollinated by bees in Brazil (Maimoni-Rodella & Rodella, 1987; Kiill & Ranga, 2000). Bee species are also mentioned as flower visitors and pollinators of other genera in Convolvulaceae (Austin, 1978, 1997; Maimoni-Rodella et al., 1982; Maimoni-Rodella, 1992; Kiill & Ranga, 2003).

The patchy distribution of *M. macrocalyx* population and its showy bloom may serve as a long distance attractant (Ohashi & Yahara, 1998). The early morning pattern of floral nectar secretion is synchronized with the foraging activities of some

Table 2 - Morpho-functional classification of bee species visiting *Merremia macrocalyx* at Pai Inacio, Chapada Diamantina, Bahia, Brazil.

Morpho-functional categories	Code	Resource gathering		Specialities	Pollen uptake				Pollen transportation				Body hair		Body size				Glossa length					
		parasite	non-parasite		first leg	ventral scopa	mandibles	vibration	corbicula	Scopa (hind femur and tibia)	ventral scopa	Scopa (femur, middle trocater and hind coxae)	Dense	Sparse	large	median robust	median thin	median to small	small	Very long	Long	Intermediate	Short	Short bilobated
Euglossina and <i>Bombus morio</i>	(Ebm)		X			X	X					X	X						X	X				
<i>Trigona spinipes</i>	(Ts)		X	mandibles robust and matched for eating or perforating floral parts	X			X															X	
other Trigonini and <i>Apis mellifera</i>	(Tam)		X	Legs and hairs adapted for oil-collection	X			X															X	
Centridini	(Ct)		X					X																
<i>Exomalopsis analis</i>	(Ex)		X					X																
<i>Ceratina</i>	(Cr)		X					X															X	
Augochlorini	(Ag)		X					X															X	
<i>Dialictus Ptilothrix</i> , <i>Thygater</i> and <i>Ancylloscelis</i>	(Di)		X					X															X	
	(Pta)		X					X															X	

bee species. The assemblage of bees showed two types of pollination: *B. morio* and *E. nigrohirta* perform sternotribic pollination but *A. mellifera* and *Geotrigona mombuca* made dorsal contact, with its head touching the plant's reproductive organs, causing nototribic pollination. In terms of morphological features of flowers and frequency of the flower visitors, *B. morio*, *E. nigrohirta*, *Geotrigona mombuca* and *Apis mellifera* are potential pollinators at the study site, given their medium and large size, their high probability of touching both the anthers and stigmas. The combination of the two pollination mechanisms and the assemblage of bees could result in a synergistically higher efficiency in pollination than if only one type of pollination by a single species occurred (Bagnara & Vincent, 1988).

Because the flowers are homogamous, insect visitation may result in self-pollination, but until the breeding system has been elucidated the plant dependence on cross-pollination or insect-mediated self-pollination remains unknown. *Merremia cissoides* and *M. aegyptia* are self-compatible (Maimoni-Rodella & Rodella 1987; Kiill & Ranga, 2000) but *M. palmeri* is self-incompatible (Willmott & Burquez, 1996).

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