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Pollination efficiency on *Ipomoea bahiensis* (Convolvulaceae): morphological and behavioural aspects of floral visitors

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ABSTRACT. Pollination depends on morphological and behavioural adjustments between visitors and plants. Some plant species as *Ipomoea bahiensis* (Convolvulaceae) provide nectar and pollen to visitors and occur in anthropic areas, therefore becoming an important source of resources for the maintenance of native pollinating insects. However, what is the efficiency of each floral visitor species for the pollination of this plant species? What morphological and behavioural characteristics determine the pollinators? In this regard, this study evaluated the *I. bahiensis* flower and visitor morphology in a semi-arid area and the foraging behaviour of these floral visitors. Also, the efficiency rate of potential pollinators was quantified. Bees and butterflies visited the flowers of *I. bahiensis*, but the bees *Melitoma* spp., *Apis mellifera* (Linnaeus, 1758), and *Pseudaugochlora pandora* (Smith, 1853) were the most frequent visitors. These species presented medium-sized and compatible with the floral tube width. In addition to the size, these bees presented behaviour that favoured the contact with the reproductive structures of the flower, as evidenced by the efficiency test of the flower visits. Although butterflies often collect nectar from the flowers, they do not present characteristics that could result in pollination. Hence, medium-sized bees played the role of efficient pollinator of *I. bahiensis*.

KEYWORDS. *Apis mellifera*, *Melitoma*, *Pseudaugochlora pandora*, Bee-flower interaction.

Pollination depends on the adjustment between the traits of flowers and their visitors. Morphology and behaviour are key traits to mediate contact of visitors with the flower anthers and stigma during the collection of resources, therefore essential aspects on pollination efficiency (Machado & Sazima, 1987; Parra-Tabla & Bullock, 2003; Pinto-Torres & Koptur, 2009).

Some plant families have flowers with important traits, such as odour, shape, size and colour, that ensures specialized pollination and increase pollination efficiency. For example, pollinators for flowers with tube lengths that match proboscis length on a plant-pollinator mutualism in the interaction between a long-tongued fly [*Prosoeca ganglbaueri* Lichtwardt, 1910 (Nemestrinidae)] and its primary floral food plant [*Zaluzianskya microsiphon*, (O. Kuntze) K. Schum. (Scrophulariaceae)] (Anderson & Johnson, 2008). In general, tubular flowers are expected to restrict entry into the floral tube only to visitors with adequate morphology. This trait is present on morning glory flowers (Convolvulaceae), therefore, it is expected that species of this family, including those of the genus *Ipomoea*, present a relation between the size of the floral tube and the size of the visitor resulting in the contact with reproductive structures, as a means of ensuring pollination (Paz *et al*., 2013).

Another factor affecting pollination efficiency is related to the temporal adjustment between visitors and flowers. Some plants, especially *Ipomoea* spp. (Convolvulaceae) with short-lived flowers and diurnal opening, early in the morning, are more visited by diurnal bees (Maimoni-Rodella & Yanagizawa, 2007; Paz *et al*., 2013). However, some species with diurnal flower opening are also pollinated by hummingbirds and butterflies (Machado & Sazima, 1987; Marais & Rausher, 2010) and species with nocturnal flower opening are usually pollinated by moth (Murcia, 1990; Paz *et al*., 2013). Pollination in *Ipomoea* species relates this group to a variety of possible pollinators, however, pollination efficiency of these visitors needs to be tested.

Among the floral visitors of diurnal species of *Ipomoea*, some bee species are generally associated with its flowers (Wcislo & Cane, 1996; Martins, 2002), such as the oligolectic bees of the genus *Melitoma* that are important pollinators of this plant genus (Terada *et al*., 2005; M.-Rodella & Yanagizawa, 2007; Pick & Schlindwein, 2011, Paz & Pigozzo, 2013; Paz *et al*., 2013). Oligolectic bees are generally more frequent and able to access floral resources easier than polylectic bees on flowers with restrictive morphology (Schlindwein, 2004). However, among the floral visitors of a plant species may occur specialist and generalist species and both can act as potential pollinators, but differing in quality of service provided (Ne'eman *et al.*, 2010).

Species of *Ipomoea* are commonly found on anthropogenic areas, so they can be an important source of pollen and nectar for the maintenance of populations of floral visitors of recognized importance in the literature (Maimoni-Rodella & Yanagizawa, 2007; Paz *et al.*, 2013). In this regard, this study aims to identify the most efficient pollinators of *Ipomoea bahiensis* Willd. Ex Roem. & Schult through analysis of the morphological and behavioural characteristics of the floral visitors. Particularly, the pollination efficiency is expected to be related to visitors with compatible size with the floral tube dimensions of *I. bahiensis* and behaviour that allows contact with reproductive structures.

MATERIAL AND METHODS

The study was carried out at the Campus of Universidade Estadual de Feira de Santana (Bahia, Brazil) (12°11'S, 38°58'W) that encompass a total area of approximately 1.2 km^2; the vegetation is predominantly herbaceous-shrub Caatinga, but is now covered by anthropized vegetation with non-native and invasive plants (Santana & Santos, 1999). The regional climate is classified as BSh or semiarid (Köppen & Geiger, 1928).

Ipomoea bahiensis is a ruderal and climbing plant species, with sympetalous corolla, funnel-shaped. The flowers are showy, with colour ranging from light to dark purple, the tube has more intense colour than the rest of the corolla and they are arranged in an inflorescence with 5-10 bisexual flowers. The species presents the reproductive organs inserted inside the floral tube, organised in the central region of this tube, being the five stamens located around the stigma (Pacheco-Filho *et al*., 2011).

The fieldwork was carried out between July and October 2014, which were the months with the greatest flowering of *I. bahiensis* in the study area. The data collection were made in four sample plots (20 x 20 m each) at least 200 m apart from each other.

Reproductive biology experiments were carried out during July 2014. The treatments on the experiment were: manual cross-pollination/geitonogamy (transfer of pollen among different flowers of a single individual of *I. bahiensis*), manual cross-pollination/xenogamy (transfer of pollen among flowers of different individuals), spontaneous self-pollination (flowers bagged without any intervention), manual self-pollination (transfer of anther pollen to the stigma of the same flower), apomixis/agamospermy (flowers emasculated and bagged to observe if fruit formation occur without fertilization), and control (flowers exposed to the action of visitors). For the tests, 40 flowers from the four sample plots studied (bagged before floral opening) were used for each treatment, in a total of 240 flowers.

In order to compare the different pollination treatments mentioned above, a generalized linear model with binomial distribution was constructed (Zuur *et al*., 2009), using the treatment as a predictor variable and fruit formation (binary) as a response variable. Afterwards, a planned comparisons was performed comparing pollination treatments with the control using the *glht* function of the *multcomp* package (Hothorn *et al*., 2008). All analysis were performed on R environment unless stated otherwise (R Core Team, 2017)

Morphology of the flowers. In order to evaluate if the floral visitor sizes matches with flower traits, anther and stigma lengths, corolla length and diameter, and width of the internal area of the floral tube available for passage of visitors through the flowers to the nectary (n=20) were measured using a digital calliper. The flowers were collected in the four sample plots selected. The voucher plant specimens of this study are deposited at the Universidade Estadual de Feira de Santana Herbarium (HUEFS-212109).

Morphology and behaviour of floral visitors. Morphological and behavioural aspects served as a baseline to determine the potential pollinators. First, the size of floral visitors was measured to compare with the diameter of the floral tube. These measures of body length (from mid-ocellus to apex of the abdomen) and inter-tegular width (distance between the bases of the tegulas) were made using a digital calliper. The size classification for floral visitors was modified from the categories of Roubik (1989) and Frankie *et al.* (1983), in which visitors were classified as large (length> 14.0 mm, width> 4.0 mm), robust medium (length: 10.0 to 14.0 mm, width: 4.0 to 6.0 mm), intermediate medium (length: 10.0 to 14.0 mm; width: 3.0 to 4.0 mm), small medium (length: 7.0 to 10.0 mm; width: 2.0 to 4.0 mm), and small (length: <7.0 mm, width: <2.0 mm).

The observations of visitor behaviour were made from 04:00 to 15:00 h, during two days in July, August, September, and October/2014 and the following was observed: type of resource collected, parts of the body that contacted the reproductive structures of flowers, and visit duration. The floral visitor specimens collected were deposited in the entomological collection Prof Johann Becker at the Museu de Zoologia da Universidade Estadual de Feira de Santana (MZFS).

Pollination efficiency. The experiments to verify the pollination efficiency in *I. bahiensis* were carried out with floral visitors considered as potential pollinators according to the following criteria: 1) high frequency of visits, 2) body size matching floral tube width, 3) behaviour of touching the reproductive structures of flowers during the collection of resources.

In order to test the pollination efficiency of the most frequent visitors to flowers, preliminary experiments with a visit to flower were carried out, but no fruit formation was verified, therefore this experiment was carried out with three visits to flower. Bagged flowers in bud stage were used in the experiments and were un-bagged after the floral opening, in which only selected visitors were allowed to make three visits per flower. This procedure was performed on 30 flowers for each potential pollinator and the flowers were marked and bagged again to follow the fruit production.

RESULTS

In the reproductive biology experiments, only the treatments of geitonogamy and xenogamy were similar to the results obtained in natural conditions (Control) with 60% of probability of forming fruits, also there was 12.5% of fruit formation in the manual self-pollination test (χ^2=96.81, d.f.= 5, p<0.001) (Fig. 1).

According to morphometric data of the flowers, *I. bahiensis* was classified as a large flower, due to the corolla size (53.02 ± 7.38 mm). The floral tube was 9.23 ± 0.99 mm wide, but the distance between the tube and reproductive structures, through which the visitor could cross to collect the resources, presented a narrow width (3.51 ± 0.70 mm). The anthers presented different lengths (12.32 ± 1.40 mm, 16.70 ± 2.49 mm, 21.12 ± 1.86 mm), and the stigma presented length compatible with small and medium anthers (15.02 ± 1.74 mm).

Ipomoea bahiensis flowers were visited by 28 insect species. The most frequent visitors were bees (70%): *Melitoma* spp. [*Melitoma segmentaria* (Fabricius, 1804), *Melitoma* sp. 1, *Melitoma* sp. 2)] (Relative frequency = 25%), *Apis mellifera* Linnaeus, 1758 (23%), *Pseudaugochlora pandora* (Smith, 1853) (12%), and *Ancyloscelis apiformes* (Fabricius, 1793) (6%); and butterflies (30%): *Morys compta compta* (Butler, 1877) (10%) and *Phoebis sennae marcellina* (Cramer, 1777) (8%). The other floral visitors had a relative frequency lower than 5% (Tab. I). Among these visitors *Melitoma* spp., *Melitomella murihirta*, *Trigona spinipes* collected pollen and nectar from the flowers, the others visitors collected only nectar.

Medium-sized bees were the visitor species with nearest body size to the size to the floral tube (Fig. 2). The medium-sized bees most similar to the internal size of the tube were: *A. mellifera*, *Melitoma* spp., *Thygater* (*Thygater*) *analis* (Lepeletier, 1841) (intermediate-medium), *Gaesischia* (*Gaesischia*) **cf.** *similis* Urban, 1989, *Melipona quadrifasciata anthidioides* Lepeletier, 1836, *Melitomella murihirta* (Cockerell, 1912), *Ptilothrix* **cf.** *plumata* Smith, 1853 (small-medium), however only two bees were abundant (*A. mellifera* and *Melitoma* spp.).

Intermediate-medium-sized bees and also small-medium-sized *P. pandora* collected nectar from the flowers landing on a petal, heading towards the nectary through the tube wall, in a way that they contacted the dorsal part of the body on the reproductive structures of the flowers. On another hand, small bees, such as *A. apiformes*, also landed on the corolla and headed to the nectary, but always walking on the floral tube wall, both when they arrived and left the tube, contacting the anthers only occasionally. The robust-medium-sized bees were restricted due to the internal size of the floral tube and because of this could not enter the tube.

The behavior of *Melitoma* spp. while collecting nectar was similar to other intermediate medium-sized bees, resulting in contact of the dorsal part of the body with the reproductive structures of the flower. *Melitoma* spp. collected pollen by landing on a petals, entered the flower, and positioned themselves on the anthers, scraping them with the hind legs and storing the pollen in the scopa. The bees spun on the anthers to collect pollen, contacting the stigma with the abdomen.

Usually Lepidoptera species inserted the long proboscis in the corolla internal wall until reaching the nectary in order to collect nectar. They oriented themselves through the petal ribs and, with this behaviour, they did not touch the reproductive structures of flowers. *M. compta compta* was the only Lepidoptera that entered until half of floral tube. This butterfly insert the short proboscis in the corolla and to reach the nectary needs to force the entry into the floral tube, contacted the longer anthers, eventually adhering pollen

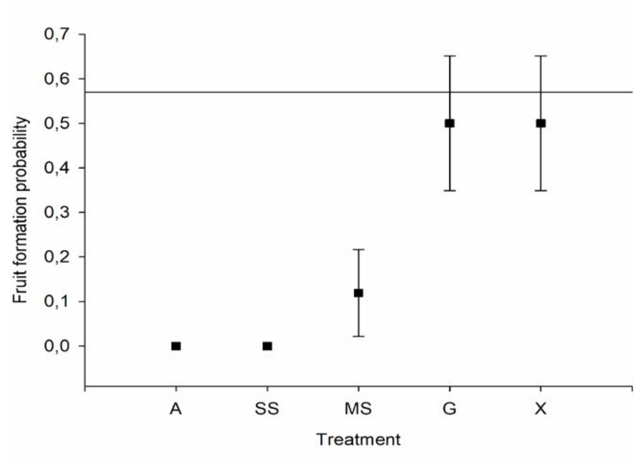

Fig. 1. Comparison between the probabilities of fruit formation in different treatments of reproductive biology test and the control in *Ipomoea bahiensis* (Convolvulaceae), in Feira de Santana (BA). Control in natural conditions in the horizontal line, points = other treatments: apomixis (A), spontaneous self-pollination (SS), manual self-pollination (MS), geitonogamy (G), and xenogamy (X).

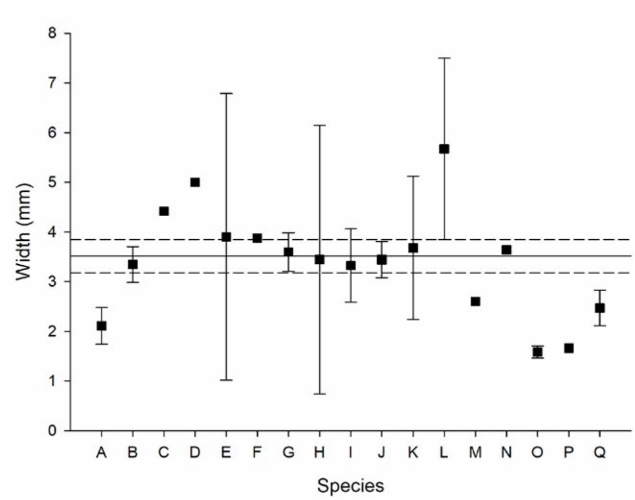

Fig. 2. Relation between the average width of bee species and the width of the internal area of the floral tube of *Ipomoea bahiensis*. Horizontal dashed lines = confidence interval of the internal area of the floral tube. Points = average visitor size, Bars = confidence interval. Species: (A) *Ancyloscelis apiformes*; (B) *Apis mellifera*; (C) *Centris* (*Hemisiela*) *tarsata*; (D) *Euglossa* (*Euglossa*) sp.; (E) *Gaesischia* (*Gaesischia*) **cf.** *similis*; (F) *Melipona quadrifasciata*; (G) *Melitoma segmentaria*; (H) *Melitoma* sp. 1; (I) *Melitoma* sp. 2; (J) *Melitomella murihirta*; (K) *Ptilithrix* **cf.** *plumata*; (L) *Ptiloglossa* sp.; (M) *Protomeliturga turnerae*; (N) *Thygater* (*Thygater*) *analis*; (O) *Trigona spinipes*; (P) *Augochlora* sp.; (Q) *Pseudaugochlora pandora*

to the front of the head. Probably the difference in nectar-gathering behavior among different species of butterflies can be related to the size of probocides.

The highest fruit production in the pollination efficiency test with three visits was recorded for *Melitoma* spp., followed by *A. mellifera* and *P. pandora*, and the genus *Melitoma* was considered the most efficient pollinator (Tab. II). Two species (*M. compta compta* and *A. apiformes*) were disregarded as efficient pollinators due to no fruit formation occurred in the efficiency test with three visits.

DISCUSSION

Fruit production by *I. bahiensis* was higher by cross-pollination than by self-pollination. Hence, pollen transfers between different flowers, from the same plant (geitonogamy) or from different plants (xenogamy), resulted in a higher fruit production, which gives support for the need of floral visitors for pollination to occur.

In several plant species, the diameter of the floral tube for passage of visitors until the resource is an important morphological trait for the determination of pollinators and their efficiency, restricting the access of visitors with a greater width than the tube. In *I. bahiensis* flowers, medium-sized bees showed to be potential pollinators (Tab. I), some species being more efficient than others. Among these bees, *Melitoma* presented a high number of visits, with behaviour and morphology that provided the contact with the reproductive structures of *I. bahiensis*, while collecting pollen and nectar. These bees also showed the highest result

Tab. I. Number of visits (V), floral visitor size (Le – Wi, Length – Width; S, Size; L, large; RM, robust medium; IM, intermediate medium; SM, small medium; small, SS) in *Ipomoea bahiensis* (Convolvulaceae), Feira de Santana (BA), from July to October 2014. Species marked with an asterisk collected both nectar and pollen while species without asterisk collected only nectar.

Species	V	Le – Wi (mm)	S
HYMENOPTERA			
Apidae			
Ancyloscelis apiformis (Fabricius, 1793)	226	6.60 - 1.99	SS
Apis mellifera (Lepeletier, 1836)	848	11.28 - 3.34	IM
Centris tarsata Smith, 1874	1	11.77 - 4.48	RM
Euglossa (*Euglossa*) sp.	9	10.82 - 5.00	RM
Gaesischia (*Gaesischia*) **cf.** *similis* Urban, 1989	7	9.57 - 3.66	SM
Melipona quadrifasciata Lepeletier, 1836	1	8.90 - 3.88	SM
Melitoma spp.* [*M. segmentaria* (Fabricius, 1804), *Melitoma* sp.1, *Melitoma* sp.2]	950	10.18 - 3.60	IM
*Melitomella murihirta** (Cockerell, 1912)	26	9.33 - 3.56	SM
Ptilothrix **cf.** *plumata* Smith, 1853	3	9.16 - 3.68	SM
Ptiloglossa sp.	26	16.32 - 5.67	L
Protomeliturga turnerae (Ducke, 1907)	1	6.7 - 2.0	SS
Thygater analis (Lepeletier, 1841)	26	10.3 - 3.64	IM
*Trigona spinipes** (Fabricius, 1793)	16	5.82 - 1.58	SS
Halictidae			
Augochlora sp.	8	7.18 - 2.01	SM
Pseudaugochlora pandora (Smith, 1853)	461	8.65 - 2.46	SM
LEPIDOPTERA			
Pieridae			
Phoebis sennae marcellina (Cramer, 1777)	295	18.13 - 4.01	L
Hesperiidae			
Cymaenes tripunctus theogenis (Capronnier, 1874)	107	14.13 - 4.53	L
Morys compta compta (Butler, 1877)	360	17.59 - 4.19	L
Nyctelius nyctelius nyctelius (Latreille, 1824	141	18.21 - 4.96	L
Perichares philetes adela (Hewitson, 1867)	10	22.71 - 5.86	L
Synale hylaspes (Stoll, 1781)	171	17.97 - 4.54	L
Sphingidae			
Xylophanes indistincta (Closs, 1915)	12	40.42 - 10.20	L

Tab. II. Pollination efficiency rate of the potentials pollinators of *Ipomoea bahiensis* (Convolvulaceae), in Feira de Santana, state of Bahia, Brazil.

Floral visitor	Flower (n)	Fruit (n)	Success (%)
Melitoma spp.	30	8	26.6
Apis mellifera scutellata (Lepeletier, 1836)	30	4	13.3
Pseudaugochlora pandora (Smith, 1853)	30	2	6.6
Morys compta compta (Butler, 1877)	30	0	0.0
Ancyloscelis apiformes	30	0	0.0

in the pollination efficiency tests, thereby confirming their role as an efficient pollinator. In contrast, small-sized visitors such as *A. apiformes,* although having access to the floral tube, did not contact the anthers and stigma since they have a smaller body size than the floral tube. Hence, the diameter of the tube and body size of the visitors are related to pollen removal and contact with stigma. Murcia (1990) also observed the relation between the size of visitors and flowers of *Ipomoea trichocarpa* Ell., in Florida (USA) for the pollination efficiency.

Other pollination studies with species of *Ipomoea* recognise individuals of *Melitoma* as very common visitors on these flowers, and these bees were considered oligolectic in pollen collection (Wcislo & Cane, 1996; Schlindwein, 1998; Martins, 2002). Generally, oligolectic bees visit many flowers from the same species, collecting resources faster than other visitors (Willmer & Stone, 2004). Schlindwein (2004) reports in studies performed in Brazil that oligolectic bees are nearly always efficient pollinators. However, generalist bees were also efficient in plant pollination. In the case of *I. bahiensis*, the flowers were much visited by the generalist honey bees (*A. mellifera*). This bee presented behaviour and size compatible with the flowers reproductive system and was the second species in the efficiency tests, contributing to increase the reproductive success of the plant, being considered a potential pollinator.

Association of morphological and behavioural traits of floral visitors is crucial to set apart efficient pollinators from those which are only floral visitors. Hence, the importance of visitor size and tube morphology for pollination in *I. bahiensis* is evident. In this sense, flowers with floral tubes can attract two groups of visitors (bees and butterflies) like observed in this study on *I. bahiensis* but only medium-sized bees acted as efficient pollinators, although the butterflies were also frequent, they played no role as flower pollinators.

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