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# Reproductive biology of the morning glory *Merremia macrocalyx* (Ruiz & Pavon) O'Donnell (Convolvulaceae)[1]

**Elena Raimúndez-Urrutia[2,3], Lyz Avendaño, and Dilia Velázquez**

Departamento de Biología de Organismos, División de Ciencias Biológicas, Universidad Simón Bolívar, Caracas, Venezuela

Raimúndez-Urrutia, Elena, Lyz Avendaño, and Dilia Velázquez (Departamento de Biología de Organismos, División de Ciencias Biológicas, Universidad Simón Bolívar, Caracas, Venezuela). Reproductive biology of the morning glory *Merremia macrocalyx* (Ruiz & Pavon) O'Donnell (Convolvulaceae). J. Torrey Bot. Soc. 135: 000–000. 2008.—*Merremia macrocalyx* is a vine that grows over other plants or as a low creeper and is considered a weed of important crops. Study of its reproductive biology showed that its flowers comprise the typical features of the melittophilous pollination syndrome. Anthophoridae, Apidae, and Halictidae were its main floral visitors. *Merremia macrocalyx* showed a mixed mating system with features that promote outcrossing while allowing some autogamy. Floral biomass was allocated mainly to the corolla (attraction) which, together with the presence of herkogamy and protandry, favors out-crossing. Nevertheless, it is self-compatible and partially autogamous due to the position of anthers above the stigma. Foraging behavior of pollinators could also facilitate deposition of self pollen on the stigma. Ovule abortion was the main predispersal loss of potential seed production, closely followed by flower and fruit abortion. Predispersal seed predation and seed abortion were relatively low. Specimens of *Megacerus flabelliger* (Bruchidae) were found emerging from the seeds as well as some wasps belonging to families recognized as parasitoids. The mixed mating system showed by *Merremia macroclayx*, together with its pollination by generalist bees, provides advantages for its development as a weed.

Key words: facultative autogamy, *Megacerus flabelliger*, melittophily, *Merremia macrocalyx*, mixed mating system, self-compatibility, weed.

*Merremia* Dennst. ex Endl. (Convolvulaceae) is a pantropical genus with 11 species in Venezuela, and among them *Merremia macrocalyx* 1 (Ruiz & Pavon) O'Donell which grows naturally in savannas, thickets, and evergreen lowland to montane forests, from 50 to 1,100 m above sea level (asl) (Austin 1998). *Merremia macrocalyx* is an herbaceous vine with twining or trailing stems. Plants produce numerous few-flowered, axilar raceme-type inflorescences, with bisexual flowers exposed out from the foliage. The calyx is arranged in two series, with reddish-colored free sepals that overlap to form a tube which surrounds the base of the corolla. The latter is gamopetalous and infundibuliform with radial symmetry, white with a pale-yellow center, and with glandular trichomes and slits at its base. The sexual organs are placed at the center of the flower, included in the corolla tube. There are five heterodynamous stamens, arranged at two levels above and below the stigma, with extrorse yellow anthers. The bases of the filaments are expanded, encircling a nectar chamber. The ovary has four locules with an ovule each and the stigma is bilobed. A nectary disk is located at the base of the ovary. The fruit is a capsule with a very thin and membranous pericarp, longitudinally dehiscent by as many segments as locules (septifragal capsule). The seed coat is densely pubescent (Austin 1998).

In Convolvulaceae, floral features are fairly homogeneous and most of them show a melittophilous syndrome (Faegri and van der Pijl 1979, Austin 1997, 1998). Andrenidae, Anthophoridae, Apidae, Halictidae, and Megachilidae are frequent visitors of most species (Austin 1997, Piedade 1998, Kiill and Ranga 2003, Terada et al. 2005). Some are visited simultaneously by more than one member of these groups, and more sporadically by other insects such as lepidopterans and coleopterans (Piedade 1998, Kiill and Ranga 2003, Terada et al. 2005). Additionally, there are examples of pollination by sphingid moths, birds, and bats (Willmott and Búrquez 1996, Geiselman et al. 2002) and the activity of

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[3] Author for correspondence: E-mail: eraimundez@usb.ve

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nectar robbers has been reported (Piedade 1998, Kiill and Ranga 2000).

According to Martin (1968), self-incompatibility characterizes the family. Within the genus *Merremia*, this breeding system has been described in a sphingophilous species (Willmott and Búrquez 1996) whereas self-compatibility has been attributed to melittophilous species which have been referred to as facultatively autogamous (Piedade 1998, Kiill and Ranga 2000).

Both pollination syndrome and breeding system are associated with other reproductive features, such as biomass allocation to floral functions, abortion of reproductive structures, and fruit and seed set (Cruden and Lyon 1985, Ramírez 1992). Besides, the numbers of seeds and fruits produced are related to factors such as predispersal seed predation which may significantly reduce the number of seeds (Crawley 1989, Jermy 1993). The Convolvulaceae show a close trophic relationship with bruchids of the genus *Megacerus* which feed only on the seeds of morning glories and cause considerable reduction of seed number (Center and Johnson 1974, Keeler 1980, Frey 1995, Austin 1997, Maes and Kingsolver 2003, Scherer and Romanowski 2005).

*Merremia macrocalyx* is widely used for the treatment of nervous dissorders but it is also regarded as a weed of important crops (Austin 1998, Groth 2001, Randall 2001). Nevertheless, knowledge about its reproductive biology is scant. The study of its floral features (morphology, anthesis, timing of anthers and stigma maturation, distance between them, and biomass allocation to male, female, attraction, and support functions), pollination syndrome, outcrossing promotion, presence of self-compatibility, predispersal losses (flower, fruit and seed abortion), and predispersal seed predation may help to develop programs for its agricultural production or its control as a weed.

**Materials and Methods.** STUDY SITE AND SPECIES. The field work was conducted in the "Bosque de Los Guayabitos", located at the Coastal Cordillera, southeast of Caracas (Estado Miranda, Venezuela, 10° 24′ N, 66° 52′ W, 1,200–1,430 m asl), where this plant grows on trees and shrubs or as a creeper. Formerly, the area was regarded as a cloud forest with evergreen trees and lush vegetation (Aristeguieta and Matos 1959), but today only fragments of the original forest remain, having been replaced by secondary vegetation. The climate is cool and humid, with temperatures ranging from 16 to 25 °C, and a mean relative humidity of 84%; the dry season is short, usually stretching from January to March (Macía et al. 1992). In the study area, *Merremia macrocalyx* flowers and fruits from August through March.

FLORAL AND POLLINATION BIOLOGY. Inflorescence type, perianth structure, flower colors, flower length (distance between the base of the flower and the apex of the tallest structure in the flower), inner and outer corolla diameters (at the opening of the corolla tube and at the corolla border, respectively), anthers to stigma distance (herkogamy), non-simultaneous maturation of anthers and stigma (dichogamy), number of stamens per flower, anther dehiscence type, and pistil features were measured in the field on haphazardly selected flowers. Anthesis time and duration of flower opening, flower longevity, orientation of the flowers with respect to the ground, and scent and nectar presence were also recorded by direct observation. Some flowers were collected and preserved in 70% isopropyl alcohol in order to describe the shape, grouping, and ornamentation of pollen grains.

The foraging behavior of flower visitors was described according to the methods of Faegri and van der Pijl (1979). The attraction unit to pollinators (individual flowers or whole inflorescence) was established depending on flower organization in the inflorescence, number of simultaneously opened flowers per inflorescence, and pollinator behavior while visiting flowers. Direct observation and capture of flower visitors were carried out five times a day: from 7:00 to 11:00 hours, from 11:00 to 14:00 hours, from 14:00 to 17:00 hours, from 17:00 to 19:00 hours, and from 19:00 to 22:00 hours. A total of 39 hours of observation was completed from mid-October through November. Visitors were noted and their visit behaviour was recorded. Captured insects were preserved in paper envelopes for examination with a stereoscopic microscope to verify the presence and location of *Merremia macrocalyx* pollen on their bodies. Morphologically different pollen grains on the insects were also determined. Probable pollinators were distinguished from flower visitors according to criteria of visit frequency, presence of pollen on the body, and effective contact between the body area with pollen and the stigma. Mann-Whitney *U* test was used to compare lengths of insect visits.

Biomass Allocation to Floral Whorls. The biomass, expressed as dry weight, allocated to floral whorls was determined in 24 flowers preserved in alcohol. Dry weight was used as an estimate of resources allocated to the different functions in the flower because it is a good indicator of energy (calories) distribution (Ramírez 1992). Floral whorls were dried for 10 days at 40°C until constant weight was attained. These measurements were used to estimate the relative allocation to androecium (stamens), gynoecium (pistil), attraction (corolla + nectary), and physical support (calyx). The following ratios were calculated: androecium biomass/gynoecium biomass (relationship between male and female functions), androecium biomass/total flower biomass (male function), gynoecium biomass/total flower biomass (female function), attraction biomass/total flower biomass (attraction function), and calyx biomass/total biomass (support function).

Breeding and Mating System. Four hand-pollination treatments were performed following the methods described by Ruiz-Zapata and Arroyo (1978), to determine the presence of self-incompatibility, autogamy, or agamospermy. Ten individuals were used for each treatment. Plants were chosen to have distances greater than 20 m among them to avoid using the same individual. Flowers were bagged in the bud stage to prevent external, non-controlled, pollinations: 1) 37 flowers were emasculated in the bud stage and were cross-pollinated (CP) by placing pollen of conspecific individuals located as far away as possible on receptive stigmas, 2) 44 bagged flowers were pollinated with pollen from genetically identical flowers (induced self-pollination, ISP), 3) 26 bagged flowers were left untreated until fruit development to test for autogamy (autonomous self-pollination, ASP), and 4) agamospermy (AG) was investigated using 31 bagged flowers emasculated in the bud stage and left untreated until fruit development. A fifth group of 49 flowers was marked and left undisturbed on the plants in order to determine fruit and seed set as a result of natural pollination (NP). Fruit and seed set were evaluated in all treatments, and the seeds were opened and checked for the presence of mature embryos.

The index of self-incompatibility (ISI) for the species was assessed by the ratio between the number of seeds produced by ISP and the number of seeds produced by CP (Ruiz-Zapata and Arroyo 1978, Suárez et al. 2004). A value of 1.0 indicates that the plant is self-compatible, less than 1.0 means that self-compatibility is incomplete, and 0 indicates self-incompatibility (Ruiz-Zapata and Arroyo 1978, Suárez et al. 2004). If the species is self-compatible, autogamy can be assessed using the ratio between the percentage of fruits formed by autonomous self-pollination and that of the fruits formed by induced self-pollination, thus setting an index of automatic self-pollination (IAS) (Ruiz-Zapata and Arroyo 1978, Suárez et al. 2004). A value approaching 1.0 indicates complete autogamy, lower than 1.0 means partial autogamy and approaching 0 means that the plant mechanically prevents self-pollination (Ruiz-Zapata and Arroyo 1978, Suárez et al. 2004).

*G*-tests of independence were used for statistical comparisons of treatments. This method is useful to compare results obtained from treatments because number of flowers per treatment varied (Jaimes and Ramírez 1999). Significance level used was $P < 0.001$.

Predispersal Seed Predation. Predispersal seed predators were reared from a group of 411 mature fruits collected haphazardly from all the plants. These fruits were carefully checked to detect the presence of eggs outside the pericarp. All the seeds were taken out of the fruits and placed in a plastic basket covered with a fine-mesh bag at room temperature in the laboratory. It was periodically monitored to verify the emergence of adult insects. When an insect emerged from a seed, both the insect and the seed were removed from the basket. The number of seeds not fed upon, the number of seeds fed upon, and the number of seeds with eggs on them but no insects emerging (after observation for several days) were recorded.

Predispersal Seed Losses and Relative Reproductive Success. Inflorescences and infructescences were haphazardly collected in the field and preserved in 70% isopropyl

Table 1. Floral measurements of *Merremia macrocalyx* (means ± SD) ($N$ = 31 for all measurements).

| Measurements | Mean ± SD (cm) |
|---|---|
| Flower length | 3.73 ± 0.33 |
| Corolla tube diameter (inner) | 1.36 ± 0.21 |
| Corolla tube diameter (outer) | 5.10 ± 0.55 |
| Pistil length | 2.42 ± 0.29 |
| Stamen length (shortest) | 2.19 ± 0.24 |
| Stamen length (longest) | 2.84 ± 0.21 |

alcohol. The number of flowers per inflorescence was counted in 62 inflorescences, the number of ovules per ovary was counted in 96 flowers in anthesis or in well-developed floral buds, and the number of fruits was counted in 60 mature infructescences; barren infructescences (infructescences in which no fruit was formed) were also considered to obtain the mean number of fruits per infructescence. All mature fruits were opened and examined to assess the total number of seeds per fruit. This total number included normal (well-formed) and aborted (not well-formed) seeds. The number of aborted seeds was counted directly in each fruit. These values were translated into abortions of flowers or fruits, ovules, and seeds. Ovule abortion could be considered a prezygotic loss because these ovules may not have been fertilized, while seed abortion could be considered a postzygotic loss because it includes seeds that begin development but do not reach maturity. Predispersal seed predation was calculated as the percentage of damaged seeds divided by total number of seeds in the rearing basket. Relative reproductive success was estimated as the proportion of ovules maturing into viable seeds in an inflorescence (Wiens 1984). This proportion may be regarded as the realized fecundity, and in this case also considers losses by predispersal predation of the seeds. In this measurement, the inflorescence (infructescence) is considered as the reproductive unit.

**Results.** FLORAL AND POLLINATION BIOLOGY. Floral dimensions are summarized in Table 1. The difference in height between stamens and stigma (0.42 cm with longest stamens and 0.23 cm with shortest stamens) places *Merremia macrocalyx* among herkogamous plants according to the criteria of Ramírez (1993).

Anthesis took place between 5:30 and 8:00 with anthers already opened; they twisted spirally with complete dehiscence. The stigma became receptive at about 10:00, when the style ceased to grow. The different maturation time of anthers and stigma indicates protandry. Small quantities of nectar were observed throughout the day and a sweet scent was noted on the flowers. The pale-yellow color of the corolla tube contrasted it against the flatter white part and could act as a nectar guide. Flower orientation in relation to the ground varied from vertical to horizontal. The closure of flowers was progressive until the corolla fell off roughly 24 hours after opening, so that flowers were partially open at night. The pollen is oblate and tricolpate.

The pollination unit is the flower, as only a single one opened per inflorescence per day. Twelve species of visiting insects were captured during the observation period (Table 2). Of these, a halictid bee (Hymenoptera: Halictidae), a carpenter bee of the genus *Ceratina* (Hymenoptera: Anthophoridae), and a stingless bee of the genus *Trigona*, the bumble bee *Bombus atratus* and the honey bee *Apis mellifera* (all these last three insects Hymenoptera: Apidae) were regarded as effective pollinators according to their behavior on the flowers, the distribution of pollen carried on their bodies, and contact with the stigmas. All these insects had *Merremia macrocalyx* pollen on their hind legs and abdomens. The halictid bee also had pollen on its mouthparts and on the wings. None of the bees carried pollen from *M. macrocalyx* alone, as pollen grains from other plant species were noticed on each of them.

The behavior of all insects on the flowers was similar, entering along the corolla tube into its depth and touching both the anthers and the stigma. While at a plant, they visited several flowers and *Apis mellifera* even repeated flowers already visited. Diverse bee groups were observed in the flowers throughout diurnal hours, albeit more taxa were observed during morning hours (Table 2). Visits by halictid bees in the morning or in the afternoon were all of about the same length ($z = 1.30$, $P > 0.05$). Visits by *Ceratina* sp. in hours around the noon were shorter than visits late in the afternoon ($z = 2.32$, $P < 0.05$). The only two observed visits of *Trigona* sp. in morning hours were shorter than the later one, which was the longest visit done by any of the observed insects to a flower, although in this case statistical significance was not established. In the case of *A. mellifera*, morning

Table 2. Insect visitors to flowers of *Merremia macrocalyx*.

| Order | Family | Species | Location of pollen load (n) | Number of flowers visited per plant (n) | Visit time (n) | Visit length (sec/flower) Mean ± SD (n) | Number of pollen types* |
|---|---|---|---|---|---|---|---|
| Diptera | | Species 1 | Not observed (1) | – | 07:00–11:00 (1) | – | – |
| | | Species 2 | Not observed (3) | – | 07:00–11:00 (3) | – | – |
| Coleoptera | Scarabaeidae | *Strigoderma* sp. | Over all body, especially on hind legs and mouth parts (18) | – | All day long (18) | – | 2 |
| Hymenoptera | Anthophoridae | *Ceratina* sp. | Hind legs and abdomen (9) | 1–4 (9) | 11:00–14:00 (6)<br>17:00–19:00 (3)<br>07:00–11:00 (6) | 5.33 ± 1.86 (6)<br>15.67 ± 5.13 (3)<br>6.67 ± 1.03 (6) | 3 |
| | Apidae | *Apis mellifera* | Hind legs and abdomen (14) | Numerous (14) | 17:00–19:00 (8) | 3.25 ± 0.71 (8) | 3 |
| | | *Bombus atratus* | Hind legs and abdomen (4) | 1–4 (4) | 07:00–14:00 (4)<br>07:00–11:00 (2) | 3.50 ± 1.29 (4)<br>9.00 ± 1.41 (2) | 3 |
| | | *Trigona* sp. | Hind legs and abdomen (3) | 1 (3) | 11:00–14:00 (1) | 45 (1) | 2 |
| | Halictidae | Species 1 | Mouth parts, medial and hind legs, abdomen and wings base (16) | 2–3 (16) | 07:00–11:00 (10)<br>14:00–17:00 (6) | 6.6 ± 2.12 (10)<br>8.5 ± 2.43 (6) | 3 |
| | Formicidae | *Conomyrma* sp. | Not observed (6) | – | 07:00–11:00 (6) | – | – |
| | Vespidae | Species 1 | Not observed (2) | – | 07:00–11:00 (2) | – | – |
| | | Species 2 | Not observed (3) | – | 07:00–11:00 (3) | – | – |
| Lepidoptera | Pieridae | *Leodonta dysoni dysoni* | Not observed (1) | – | 07:00–11:00 (1) | – | – |

* In each insect with pollen, one of the pollen types is *Merremia macrocalyx*.

Table 3. Dry biomass allocation to floral whorls and functions in *Merremia macrocalyx*. Floral functions are expressed as percentages of total floral biomass (mean ± SD; $N = 24$ for all measurements).

| Parameter | Mean ± SD |
|---|---|
| Dry biomass (mg) | |
| Flower | 43.8 ± 5.36 |
| Gynoecium | 0.84 ± 0.18 |
| Androecium | 2.65 ± 0.40 |
| Nectary | 0.47 ± 0.22 |
| Corolla | 21.2 ± 4.86 |
| Calyx | 18.6 ± 2.28 |
| Floral functions (%) | |
| Male function/female function | 3.38 ± 1.22 |
| Female function | 1.94 ± 0.47 |
| Male function | 6.11 ± 1.09 |
| Attraction function | 49.1 ± 6.41 |
| Support function | 42.9 ± 5.97 |

visits were longer than afternoon visits ($z = 3.10$, $P < 0.05$). Observation at night hours did not reveal visits by any group of animals.

The beetle *Strigoderma* sp. (Coleoptera: Scarabaeidae) and two species of flies (Diptera) were only occasional visitors; the beetle presented few pollen grains irregularly distributed over its body. The ant *Conomyrma* sp. (Hymenoptera: Formicidae) was occasionally found on flowers of *Merremia macrocalyx*. Finally, two wasps (Hymenoptera: Vespidae) and the butterfly *Leodonta dysoni dysoni* (Lepidoptera: Pieridae) visited flowers to collect nectar but did not touch pollen or stigma (Table 2) and may thus be regarded as nectar robbers.

Biomass Allocation to Floral Whorls. Dry weights of floral whorls used to estimate the biomass (= energy) allocated to different functions in the flower are shown in Table 3. Biomass was allocated mainly to the corolla, which functions as an attractant. Weight of the nectary may be added to this function, as it also acts to attract and reward pollinators. Allocations to each whorl in Table 3 are calculated as proportions related to the total weight of the flower. Attraction biomass showed the highest proportion value, whereas female function showed the lowest biomass allocation. The ratio between male function and female function was clearly biased towards the former (Table 3).

Breeding and Mating System. *Merremia macrocalyx* sets fruits by autonomous self-pollination, induced self-pollination, and cross-pollination (Table 4). None of the emasculated flowers from the agamospermy treatment set fruits. The index of self-incompatibility (ISI = 0.85) indicates that *M. macrocalyx* is a partially self-compatible species, and the autogamy index (IAS = 0.44) renders it as a species capable of producing some autogamous progeny.

The largest proportion of fruits was formed by cross-pollen (CP vs. ISP $G = 28.0$, $P < 0.001$; CP vs. ASP $G = 31.3$, $P < 0.001$) while no significant difference was found between the treatments of induced and autonomous self-pollination ($G = 1.51$, $P > 0.001$). Also, the number of seeds was significantly higher in the cross-pollination treatment in comparison with autonomous and induced self-pollination treatments (Table 4), with no statistical difference between the latter (CP vs. ASP $G = 100$, $P < 0.001$; CP vs. ISP $G = 82.8$, $P < 0.001$; ISP vs. ASP $G = 7.76$, $P > 0.001$). Neither was there any significant difference between the natural production of fruits and seeds (NP treatment) compared to the cross-pollination (CP) treatment ($G = 10.5$, $P > 0.001$ for fruits; $G = 0.56$, $P > 0.001$ for seeds).

Predispersal Seed Predation. Of the 411 fruits examined, only four (0.97%) had eggs attached to the pericarp, 72 (17.5%) had egg(s) attached to the surface of the seed(s), and the remainder lacked eggs on either fruit or seeds.

Table 4. Number of flowers, fruits, seeds, and fruit/flower and seed/ovule ratios for *Merremia macrocalyx* as affected by pollination treatments.

| Treatment | Number | | | | |
|---|---|---|---|---|---|
| | Flowers | Fruits | Fruits/Flower | Total seeds | Seeds/Ovule |
| Natural pollination | 49 | 48 | 0.98 | 123 | 0.63 |
| Cross pollination | 37 | 28 | 0.76 | 87 | 0.59 |
| Induced self-pollination | 44 | 8 | 0.18 | 21 | 0.12 |
| Autonomous self-pollination | 26 | 2 | 0.08 | 3 | 0.03 |
| Agamospermy | 31 | 0 | 0 | 0 | 0 |

Of the 1,893 seeds examined, 1,534 (81%) were not fed upon nor showed any egg attached to their surface. Bruchids emerged from 164 seeds (8.7%), and 144 seeds (7.6%) had eggs attached to their surface but no bruchids emerged from them. A few diverse hymenopterans emerged from 51 seeds (2.7%).

Adult bruchids were identified as males and females of *Megacerus flabelliger* Fåhraeus (Coleoptera: Bruchidae) (C. D. Johnson, pers. comm.). Emergence of those insects was through circular exit holes in the seed coat. Only one adult emerged from each seed, even in the few cases with more than one egg per seed. The bruchid larvae destroyed almost the entire embryo, leaving the seed hollow.

Wasps were identified as belonging to the families Braconidae, Eulophidae, Eupelmidae, and Eurytomidae (M. El Souki, pers. comm.). The damage noticed in the seeds from which these wasps emerged was less than that seen in the seeds with bruchids, as only the cotyledons were partially damaged, although embryo viability was not verified. Wasps emerged through little exit holes in the seed coat. A single small exit hole could be seen on each seed. No wasps emerged from seeds from which bruchids emerged, yet the former could emerge from seeds with eggs attached to their outer surface.

Predispersal Seed Losses and Relative Reproductive Success. Reproductive values of *Merremia macrocalyx* used to calculate reproductive losses are summarized in Table 5. Ovule abortion (30%) represented the highest predispersal loss, followed by flower and fruit abortion (25.5%), while seed abortion (5.71%) and seed predation (8.66%) were the lowest. After all losses, on average 6.19 of the 13.8 ovules in an inflorescence set normal seeds, which is equivalent to a relative reproductive success of 45%.

**Discussion.** Based on floral morphological features, the pollination syndrome of *Merremia macrocalyx* corresponds to melittophily (Faegri and van der Pijl 1979). The corolla acting as a landing platform for visitors, the production of small amounts of nectar, the sweet and weak scent of the flowers, dawn anthesis, and the presence of nectar guides are all characteristic of flowers pollinated by bees. The wide base of the stamens adnate to the corolla might be regarded as a structural nectar guide (Piedade 1998, Kiill et al. 2000, Kiill and Ranga 2004), while the pale-yellow color of the corolla tube contrasting with the white open part appears to be a visual nectar guide. Mixed nectar guides have already been described in other morning glories (Kiill and Ranga 2000, 2004).

Table 5. Reproductive values, predispersal losses due to abortion and relative reproductive success in *Merremia macrocalyx*. Values are mean ± SD (*N*).

| Reproductive values | Mean ± SD (*N*) |
|---|---|
| Flowers per inflorescence | 3.45 ± 2.42 (62) |
| Fruits per infructescence | 2.57 ± 2.05 (60) |
| Ovules per flower | 4.00 ± 0.00 (96) |
| Seeds per fruit | 2.80 ± 1.11 (100) |
| Aborted seeds per fruit | 0.16 ± 0.44 (100) |

Melittophily characterizes Convolvulaceae and involves many bee groups (Devall and Thien 1989, Piedade 1998, Kiill et al. 2000, Kiill and Ranga 2000, 2003, 2004, Terada et al. 2005, Maimoni-Rodella and Yanagizawa 2007), including some of those captured by us. In particular, *Apis mellifera* and some species of the genera *Trigona* and *Bombus* have been identified as pollinators of *Merremia* species (Piedade 1998, Kiill and Ranga 2000, Kiill et al. 2000, Cortopassi-Laurino et al. 2003). In *M. macrocalyx*, bees were the most effective pollinators because they touched the anthers during their visit to flowers and their pollen-carrying body areas made contact with the stigma. Nevertheless, these visitors seem to be generalists in view of the fact that pollen from other plants was found on all. In agreement with previous reports (Piedade 1998, Kiill and Ranga 2000, Kiill et al. 2000), most bees visited morning glories seeking for both pollen and nectar, although behavior of *Trigona* appears to be solely for pollen foraging because their mouthparts do not allow access to the nectary.

Even though scarab beetles were observed on flowers, no damage was seen. This observation contrasts with previous studies in which presence of coleopterans on flowers of Convolvulaceae has been attributed to floral herbivory, causing major damage to reproductive structures (Frey 1995, Piedade 1998). In some other morning glories the harmless presence of adult specimens of *Megacerus* has been recorded but it has been proposed that these beetles oviposit in the ovaries (Keeler 1980, Devall and Thien 1989).

Our results from hand pollination treatments place *Merremia macrocalyx* among self-compatible Convolvulaceae, able to produce a proportion of offspring via autogamy. Compatibility and facultative autogamy have been reported for other three out of four *Merremia* species, all of which are pollinated by bees (Piedade 1998 and references therein). Nevertheless, it may be suspected that an inbreeding-depression mechanism might be acting on *M. macrocalyx* that favors out-crossing because fruit and seed production by induced self-pollination was less than fruit and seed production by cross-pollination. An alternative view is that this species may have recently lost its self-incompatibility and still keeps features of its xenogamous past.

In *Merremia macrocalyx*, floral biology, pollinator behavior, and low production of fruit and seed by autogamy indicate a reproductive strategy that promotes out-crossing. The highest allocation of floral biomass to the corolla also promotes out-crossing by shifting more biomass to the purpose of attraction. Plants that favor out-crossing allocate most energy (biomass) to pollinator attraction and pollen production (Lovett-Doust and Cavers 1982, Cruden and Lyon 1985). The higher allocation of biomass to male function instead of female function and the opening of few flowers per inflorescence (standing out from the foliage as is usual in *M. macrocalyx*) also promote pollen export (Cruden and Lyon 1985, Piedade 1998, Kiill and Ranga 2000, 2003, 2004).

Two other features observed by us have been related to the enhancement of out-crossing in plants by reducing self-pollination: protandry and herkogamy (Lloyd and Webb 1986, Webb and Lloyd 1986). Protandry is more widespread than protogyny in angiosperms, as it follows the sequential development of the floral whorls (Lloyd and Webb 1986, Bertin and Newman 1993). Nonetheless, its effectiveness promoting out-crossing appears to be less than that of protogyny, because in protandry pollen grains may be placed on the stigma and remain viable until the onset of stigma receptivity (Bertin and Newman 1993). Such findings are further supported by Terada et al. (2005) who found that pollen-grain viability of *Ipomoea* species decreases only when the time for flower closure approaches. There are no previous reports of dichogamy in the available literature on Convolvulaceae, although pollen has been found exposed on the anthers just before anthesis, probably to ensure the availability of this resource to visitors when flowers open (Kiill and Ranga 2003, 2004). This may be interpreted as a way to promote pollen export. In *Merremia macrocalyx*, the visit of pollinators shortly after anthesis and earlier than the beginning of stigma receptivity could enhance pollen transport to other flowers, whereas pollinator activities later in the afternoon could result in more deposition of foreign pollen on the stigma.

Herkogamy has been described in other species of this family (Murcia 1990, Piedade 1998, Galetto et al. 2002, Terada et al. 2005). Terada et al. (2005) also pointed out that the presence of stamens longer than the style in various species of *Ipomoea* suggests that they possess at least a small capability for self-pollination, and that the presence of stamens shorter than the style denotes mechanisms that prevent self-pollination. The low yield of fruits recorded by us in the autonomous self-pollination treatment suggests that herkogamy in *Merremia macrocalyx* may be effectively preventing selfing, and only little pollen is deposited on the stigma by gravity. Piedade (1998) has already reported self-pollination due to anthers located above the stigma in *M. aegyptia*, and proposed that this is a way to ensure some reproduction. Also, the possibility that self-pollination may be facilitated by pollinator activities should not be disregarded (Lloyd and Schoen 1992, Suárez et al. 2004). The mixed reproductive strategy observed in *M. macrocalyx* has been pointed out as the most effective for plants that behave as weeds (Baker 1974) and it seems to be frequent in self-compatible morning glories (Stucky 1984, Kiill and Ranga 2000, Suárez et al. 2004, Terada et al. 2005).

Observed bee visitation patterns and the opening of several flowers on the same plant allowed geitonogamy because insects sequentially visited multiple flowers from a single individual. Geitonogamy is another form of autogamy in self-compatible plants, and is brought about by the same features that promote out-crossing, but may reduce xenogamy (Faegri and van der Pijl 1979).

The relative reproductive success found in this species was determined mainly by ovule abortion, which represented the main cause of predispersal seed losses. The most likely cause of ovule abortion could be some degree of self-incompatibility. Nevertheless, it has been claimed that ovule abortion may be a response to pollination limitation (Ramírez 1992), although it was not documented for *Merremia macrocalyx*.

Beetles of *Megacerus flabelliger* oviposit on seeds of already ripe fruits, affecting seeds but not fruit development. In other Convolvulaceae it has been noted that damage to fruits by insect larvae hinders their development by changing the rate of fruit formation (Kiill and Ranga 2004). In *Merremia macrocalyx*, the predispersal seed predation value was low in comparison with other studies of morning glories attacked by other *Megacerus* species (Keeler 1980, Devall and Thien 1989, Scherer and Romanowski 2005) or even by *M. flabelliger* (Frey 1995). Scherer and Romanowski (2005) found that the percentage of predation was related to the amount of fruit and seed produced, which in turn was dependent on the density of plants in a given locality. Presence of hymenopterans could also diminish damage seeds if they act as parasitoids of immature stages of bruchids. At least some of the hymenopteran species found in the seeds of *M. macrocalyx* belong to families regarded as parasitoids of *Megacerus* (Muruaga de L'Argentier and Terán 1980, Hetz and Johnson 1988).

To conclude, out-crossing is the main mean of seed production in *Merremia macrocalyx* and is encouraged by floral morphology, timing of sexual receptivity and generalist pollinators, but some selfing is possible. The mixed reproductive strategy appears to be appropriate for a weed, because the capability for self-pollination but favoring out-crossing adapt it to potential pollen limitation without sacrificing genetic variability.

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