**Centris aenea** (Hymenoptera, Apidae): a Ground-nesting Bee with High Pollination Efficiency in *Malpighia emarginata* DC (Malpighiaceae)

GA Oliveira¹, CML Aguiar¹, M Silva², M Gimenes¹

1 - Universidade Estadual de Feira de Santana - UEFS, Feira de Santana, Bahia, Brazil
2 - Faculdade de Tecnologia e Ciências, Salvador, Bahia, Brazil

**Abstract**

This study aimed to assess the efficiency of *Centris aenea* Lepeletier as pollinator of *Malpighia emarginata* DC. The pollination efficiency was determined according to three criteria: 1) the pollen deposition rate of *M. emarginata* and other plant species on the thorax ventral region of females, 2) the rate of *M. emarginata* fruit set after a single visit, and 3) the pollen deposition rate on the stigma after one single visit. We found that 59% of the pollen grains deposited on the ventral region of *C. aenea* came from flowers of *M. emarginata*. The fruit set after a single visit by *C. aenea* (21%) was higher than that reported previously for *Centris tarsata* Smith. We argue that pollination efficiency of *C. aenea* can be related to its body size, since that females of *C. aenea* can carry out larger amounts of pollen grains of *M. emarginata* than *C. tarsata*, as well females of *C. aenea* are able to touch a larger stigma area, resulting in higher fruit sets after a single visit. Our study suggests that only one visit by *C. aenea* ensures fruit set in *M. emarginata*.

**Introduction**

The production of West Indian cherry or acerola (*Malpighia emarginata* DC) is increasing in Brazil and a significant part of the fruit production has been exported mainly to the United States, Japan, and countries of the European Community. The fruit is not only consumed fresh but is also used as raw material for the food and pharmaceutical industries (Musser, 1995). Brazilian agro-business has had a growing interest in commercializing acerola but pollinator deficit, which results in low fruit set, is one of the factors hindering increases in productivity (Schlindwein et al., 2006; Guedes et al., 2011).

Interactions between *M. emarginata* and its pollinators are based primarily on the availability and collection of floral oils and pollen (Oliveira & Schlindwein, 2009; Vilhena et al., 2012; see Freitas et al., 1999, and Siqueira et al., 2011) for details on floral morphology). In *M. emarginata*, flower pollination is performed only by specialist floral visitors and its pollinators are bees (Apidae) belonging to tribe Centridini (Freitas et al., 1999; Siqueira et al. 2011; Guedes et al., 2011) (see Freitas et al.,1999 for scheme and details on pollinator’s behavior).

Some cavity-nesting *Centris* species have been suggested as promising candidates for pollination of *M. emarginata* (Buchmann, 2004; Oliveira & Schlindwein, 2009). The main advantage of these species lies in the easiness to obtain and relocate its nests because they undergo nidification in movable trap-nests (Jesus & Garófalo, 2000; Roubik & Villanueva-Gutiérez, 2009; Aguiar & Garófalo, 2004; Pina & Aguiar, 2011). On the other hand, other studies have found that ground-nesting Centridini species such as *Centris aenea* Lepeletier and *Centris varia* (Erichson), reach high abundance in some regions and visit a large number of *M. emarginata* flowers (Siqueira et al., 2011; Vilhena et al., 2012), and are important for their pollination services to the crop.
Comparative studies on the efficiency of several pollinators are needed to support the choice of the best species to be managed for increases in crop productivity. Although several cavity-nesting bees actually have favorable characteristics for management as crop pollinators, the role of each species in crop pollination has been scarcely studied. Studies on the nesting-cavity bees in crop areas have often focused on the bee high ability to occupy trap-nests (Oliveira & Schlindwein, 2009; Pina & Aguiar, 2011; Magalhães & Freitas, 2012) rather than on the abundance of bee species in crop flowers (e.g. Vilhena et al., 2012) or the efficiency of pollinators (Freitas et al., 1999; Freitas & Paxton, 1998; Freitas et al., 2002).

Preliminary samples obtained in the study area indicated that C. aenea, a ground-nesting bee, was the most abundant visitor on M. emarginata flowers whereas the cavity-nesting species Centris analis (Fabricius) and Centris tarsata Smith were much less frequent (unpublished data). Despite its low frequency in flowers, these two cavity-nesting species use the orchard as their nesting site (Pina & Aguiar, 2011), and females of C. analis provision their brood cells mainly with pollen of M. emarginata (unpublished data). C. aenea has been registered in other areas as the most frequent potential pollinator of M. emarginata flowers (Siqueira et al., 2011; Guedes et al. 2011; Vilhena et al., 2012) but its pollination efficiency has not yet been studied. For this reason, this study aimed to assess the efficiency of this species as pollinator of M. emarginata, and to support the choice of a potential species for the management to pollination of this crop.

Materials and Methods

Study Site

The sample was collected at an acerola orchard (12°16’S, 38°58’W) located in the countryside of the municipality Feira de Santana, Bahia, Brazil. The climate is semi-arid with average annual precipitation of 802 mm and average annual temperature of 24°C (CEI, 1994). The orchard contains approximately 300 plants of M. emarginata, grown under natural conditions with no irrigation or pesticides.

Flower visitors

The visitors of M. emarginata flowers were sampled using an entomological net once a month, from July 2010 to June 2011, from 0700 to 1700 hours, for 30 minutes every hour. In each sample, two collectors walked throughout the orchard collecting bees in flowering plants.

We measured twenty-five females of the most abundant species (C. aenea). Their entire body length (between the median ocellus and the end of the abdomen) and intertegular width (the distance between wing bases) were measured with a digital paquimeter.

The Reproductive System

Reproductive biology tests (Dafni et al., 2005) were performed in November 2010 and April 2011, when more than 50% of the plants were in bloom. Two-hundred and forty flowers in pre-anthesis were previously bagged with voil bag and received the following treatments: 1- hand self-pollination (autogamy); 2- spontaneous self-pollination; 3- hand cross-pollination (xenogamy); 4- control 1, marked flowers kept under natural conditions and available to flower visitors (natural pollination). Flowers were kept bagged for approximately 15 days until fruit set, when bags were removed and the fruit set for each treatment was quantified.

Pollination Efficiency of Centris aenea

The pollination efficiency of C. aenea was determined according to three criteria. First, the pollen deposition rate of M. emarginata and other plant species on the thorax ventral region of female C. aenea collected while visiting acerola flowers. To reach this goal, all pollen grains from the females ventral region (n=12) were removed with glycerinated gelatin. Next, the pollen grain-glycerinated gelatin mixture was dissolved in warm water and centrifuged at 2500 rotations per 10 min; the pollen grains were transferred to assay tubes containing acetic acid for at least 24 h for dehydration and then the material was processed using the acetolysis technique (Erdtman, 1960). After this chemical treatment, the pollen grains were kept for at least one hour in a glycerin aqueous solution at 50% for rehydration.

For the analysis of the pollen types deposited on females ventral region, four slides were prepared using pollen sample obtained in each female. Slides were prepared with colorless gelatin, and one slide also for each sample was prepared with gelatin colored with safranin at 1%. The slides were examined under an optical microscope to identify and count the pollen of M. emarginata and other pollen types. At least 1,000 pollen grains per sample were counted.

The second criterion determined the rate of M. emarginata fruit set after a single visit by C. aenea. Fifty-two flowers in pre-anthesis were bagged for the experiment; twenty-five of them were emasculated. On the next day, flowers were exposed to a single visit by C. aenea and were rebagged to prevent further visitations until fruit set. Two procedures were conducted in this experiment: in the first, 77 flowers were kept bagged, insect visits forbidden; in the second, 78 flowers were exposed to unrestricted bee visits (natural pollination, control 2). Fruit set was determined 15 days later for both experiments.

The third criterion analyzed pollen deposition rate on the stigma. The use of this technique aimed to complement the fruit set rate experiment after a single visit by C. aenea and count the pollen grains of M. emarginata that females of C. aenea were able to deposit on the stigma of flowers after
one single visit. Fifty flowers in pre-anthesis were bagged and on the next day, 20 flowers were exposed to a single visit by C. aenea (10 of which were emasculated). After the first visit, the stigmas were removed from flowers and fixed on slides with glycerinated gelatin colored with safranin. Ten of the remaining 30 flowers were left bagged to prevent visits and 20 (10 of which were emasculated) were exposed to unrestricted bee visits from 0700 to 1700 h. After this period, the stigmas were also removed and fixed in slides with glycerinated gelatin colored with safranin. The deposited pollen grains were counted in the laboratory.

The Kruskal-Wallis test was used to compare the number of pollen grains deposited on the stigma in each treatment. Differences in fruit set rates among treatments of the reproductive system of M. emarginata and after the single visit on C. aenea were analyzed using the Chi-square test ($\chi^2$).

Results

Pollination Biology and Flower Visitors

Results of the reproductive biology experiments show the occurrence of fruit set in treatments with hand cross-pollination (14.5%) and natural pollination (control 1) (24%); fruit sets did not occur in spontaneous or hand self-pollination experiments (Table I). No significant difference in fruit set rates between natural and hand cross-pollination ($\chi^2=1.5905, p=0.207$) were found. The number of fruits produced by natural pollination was different from the number of fruits obtained by hand self-pollination or spontaneous self-pollination ($\chi^2=14.524, p<0.001$). The number of fruits obtained by hand cross-pollination also differed from hand self-pollination and spontaneous self-pollination ($\chi^2=8.3258, p=0.004$). Fruits produced by natural pollination (N=96 fruits, control 1) had 2.95 ± 0.2 seeds per fruit.

Table 1. Fruit set of West Indian cherry (Malpighia emarginata) in a semi-arid area in Brazil.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of flowers</th>
<th>Number of fruits set</th>
<th>Flowers that set fruits (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous self-pollination</td>
<td>53</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hand self-pollination</td>
<td>53</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hand cross-pollination</td>
<td>55</td>
<td>8</td>
<td>14.5</td>
</tr>
<tr>
<td>Natural pollination (control 1)</td>
<td>54</td>
<td>13</td>
<td>24</td>
</tr>
</tbody>
</table>

Flowers of M. emarginata were visited only by bees belonging to tribe Centridini: Centris flavifrons (Fabricius), C. aenea Lepeletier, C. analis (Fabricius), C. fuscata Lepeletier, C. trigonoides Lepeletier, C. tarsata Smith, C. obsoleta Lepeletier, C. sponsa Smith, Epicharis flava (Friese) and three other non-identified species of Centris. Among these, the most abundant species was C. aenea (66% of all bees), with females measuring 15.3±0.9 mm in length and 0.59±0.23 mm in width.

Pollination efficiency of Centris aenea

Of the pollen grains (n=8,496) deposited on the ventral region of C. aenea, 59% came from flowers of M. emarginata. Other pollen types, such as Solanum paniculatum, Poincianella microphylla, Stigmaphyllon sp. and Salvia sp. were also found in the samples and comprised approximately 40% of all grains.

Table 2. Number and proportion of fruits set per Malpighia emarginata flower after a single visit by Centris aenea, in a West Indian cherry crop area in a semi-arid area in Brazil.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of flowers</th>
<th>Number of fruits set</th>
<th>Flowers that set fruits (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowers non-emasculated (exposed to one visit)</td>
<td>27</td>
<td>7</td>
<td>26</td>
</tr>
<tr>
<td>Flowers emasculated (exposed to one visit)</td>
<td>25</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>Flowers bagged (without visits)</td>
<td>77</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Natural pollination (control 2)</td>
<td>78</td>
<td>24</td>
<td>30.7</td>
</tr>
</tbody>
</table>

Fruit set after a single visit by C. aenea was 21% (Table II). There was no difference in the number of fruits produced after a single visit by C. aenea on emasculated (16%) and non-emasculated flowers (26%) ($\chi^2=0.76, p=0.38$). Similarly, results of these experiments did not differ from experiments obtained from fruit sets in flowers open to visitation (n = 78, 30.7%) (Control 2) ($\chi^2=1.46, p=0.22$) (Table II). Bagged flowers did not produce fruits.

After the single visit by C. aenea, pollen grains were deposited on stigmas of all the 20 flowers studied. The number of grains deposited on stigmas of emasculated flowers varied from 6 to 51 (31.7±12.9), whereas it varied from 13 to 67 (33±17.8) on non-emasculated flowers, and no difference in deposition rates among treatments was observed (Kruskal-Wallis H=10.7, p=0.9296). The number of pollen grains deposited on the stigmas of flowers with unrestricted visits were between 2 and 371 in emasculated flowers and between 6 and 844 in non-emasculated flowers. No significant difference was observed between these two treatments (Kruskal-Wallis H=10.7, p=0.18).

Discussion

Results of the reproductive biology experiments show that the population of M. emarginata we studied did not produce fruits in self-pollination experiments, only in cross-pollination experiments and is considered self-incompatible.
and dependent on biotic agents (Centridini bees) for pollination. However, the fruit set rate by cross-pollination was low in unrestricted visits experiments (natural pollination), as also observed in other Brazilian regions (Freitas et al., 1999; Guedes et al., 2011). The lack or low fruit set rates for the crop by self-pollination was also observed by other researchers (Schlindwein et al., 2006; Guedes et al., 2011, Siqueira et al., 2011). Self-pollination rates between 11% and 35% were observed for several varieties in irrigated M. emarginata orchards (Siqueira et al., 2011). As such, low fruit set per inflorescence and dependence on entomophilous pollination seem to be traits of this plant species (Freitas et al., 1999; Siqueira et al., 2011).

A plausible hypothesis to explain the low fruit set observed in M. emarginata is that this reproductive factor is directly related to the frequent ovule abortion in this plant species (Miyashita et al., 1964; Freitas et al., 1999), whereby few fruits are produced in spite of flower abundance. On the other hand, the low M. emarginata fruit set rate has also been explained by climatic conditions such as temperature, rainfall and luminosity, by genetic factors, and the low abundance of orchard pollinators (Yamane & Nakasone, 1961; Schlindwein et al., 2006, Carpentieri-Pipolo et al., 2008; Siqueira et al., 2011; Guedes et al., 2011). Guedes et al. (2011) recently registered pollination deficit in M. emarginata in a Brazilian semiarid region during the dry season, when pollinator abundance was much lower than during the rainy season. Those authors obtained a significantly higher fruit set after applying complementary hand cross-pollination, after natural pollination performed by bees during the dry season. In our study, pollination experiments were conducted whenever pollinator abundance was high, and therefore the low fruit set rate after natural pollination might not be related to the lack of pollinators.

Centris aenea was the most important of all species of Centris visiting flowers of M. emarginata, not only due to its abundance but also for its high floral fidelity, noticeable primarily by the frequent presence (more than 50% of all pollen grains) on the thorax ventral region of M. emarginata. C. aenea was also the most abundant species on acerola flowers at our study site, particularly during blooming peaks (unpublished data). An even higher abundance (above 80%) of C. aenea on acerola flowers was reported in a study conducted in the semiarid region of Bahia State (Siqueira et al., 2011). These data show that C. aenea prefers foraging for pollen on M. emarginata flower in acerola orchards, thus a positive assessment of the bee efficiency as effective crop pollinator.

The average number of pollen grains observed on the stigma after the single visit by C. aenea was enough to fertilize the ovules and produce fruits because only three pollen grains are required for fruit formation in M. emarginata. Cruden (2000) argued that the number of pollen grains deposited on the stigma indicates pollinator efficiency and depends on several factors such as size of the stigma area and duration of stigmatic receptivity. Stigmatic receptivity in M. emarginata lasts more than six hours (Freitas et al., 1999; Schlindwein et al., 2006), a trait that can increase the exposure time of flowers to pollinators as well as the chances for the effective pollination to occur because a larger number of co-specific pollen grains can be deposited on the stigmas, as already known (Dafni et al., 2005). As a consequence, there would be fewer chances for stigma contamination with hetero-specific pollen, avoiding clogging and leading to higher fruit sets and more effective pollinator visitations (Slaa & Biesmeijer, 2005).

We observed that M. emarginata fruit set after a single visit by C. aenea (21%) was much higher than that found for C. tarsata (7%) (Freitas et al., 1999). They reported that more than one visit per flower by C. tarsata is necessary for a high fruit set to be achieved, whereas our study suggests that only one visit by C. aenea ensures fruit set in M. emarginata. The different efficiencies in pollination after a single visit between the two bee species might not be due to behavior because both species have the same behavior pattern during floral oil collection on these flowers (Freitas et al., 1999; Schlindwein et al., 2006; Siqueira et al., 2011). However, pollination efficiency can be related to body size because C. aenea is approximately 1.5 times bigger than C. tarsata (n= 9, length = 10.4 ± 0.5 mm; width = 3.5 ± 0.25 mm; personal observation). Body size can be related to the amount of pollen remaining on the pollinator body and by consequence, the amount of pollen to be deposited on the stigma, as also observed by Cruden and Miller-Ward (1981) and by Cruden (2000). Due to its size, females of C. aenea can be more efficient in transporting larger amounts of pollen grains of M. emarginata than C. tarsata, as well females of C. aenea are able to touch a larger stigma area, resulting in higher fruit sets after a single visit.

Pollination of the M. emarginata population studied was conducted primarily by a ground-nesting bee, C. aenea. This bee was a highly efficient pollinator very abundant in flowers, whereas other species of Centridini, including the cavity-nesting species were much less frequent. Maintaining areas with adequate soil conditions for nesting of C. aenea, and also surrounding areas with vegetation that supplements the supply of nectar and even pollen when M. emarginata blooming is low are recommended strategies to keep the local populations of this pollinator.

Acknowledgments

We thank to the National Council for Scientific and Technological Development, Brazil (CNPq) (Proc. #475715/2008-0; #562518/2010-0) and to the Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB) (TO APP0042/2009) for financial support for this project. C.M.L. Aguiar received a research fellowship from CNPq (Proc. 306403/2012-9), G.A. Oliveira received a M.Sc. scholarship
from CAPES and M. Silva received a postdoctoral fellowship from FAPESB. We are grateful to the Universidade Estadual de Feira de Santana (UEFS) for logistic support.

References


