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## Effect of nectar pillaging by native stingless bees (Hymenoptera: Apidae) in the abscission of flowers of *Bougainvillea spectabilis* Willd. (Nyctaginaceae)

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**ABSTRACT.** This study had as objective to evaluate whether the pillaging activity by native bees influences floral abscission. Samples were collected in ten individuals of *Bougainvillea spectabilis*. In the period between May 4 and June 1<sup>st</sup>, 2009, 2,874 flowers were collected on the ground and 2,895 from the plants, with three-day intervals between each collection and a total of 10 repetitions in each plant. We measured the total of closed flowers, open flowers, robbed flowers, normal flowers, open robbed flowers and non-robber open flowers, in both soil and plant. For the statistical analysis, the T-test was used to see whether there was a difference between the averages obtained from the evaluated characteristics between the soil flowers and plant flowers. Simple linear regression was used to see whether there was a relationship between the closed flowers and robbed closed flowers found on the ground and open flowers and non-robbed open flowers in the plant. There were significant differences regarding all variables measured between soil and plant. A correlation was found at both closed flowers and robbed closed flowers found on the ground and open flowers and non-robbed open flowers in the plant.

**Keywords:** insect-plant interaction, floral damage, neotropical bees, reproductive success, ornamental plant.

**RESUMO.** Efeito da pilhagem de néctar por abelhas nativas sem ferrão (Hymenoptera: Apidae) na abscisão floral de *Bougainvillea spectabilis* Willd. (Nyctaginaceae). O presente trabalho teve como objetivo avaliar a atividade de pilhagem de abelhas nativas influenciando a abscisão floral de *Bougainvillea spectabilis*. As coletas foram realizadas em dez indivíduos de *B. spectabilis*. Foram coletadas 2.874 flores no solo e 2.895 na planta no período de 4/5/2009 a 1/6/2009 com intervalo de três dias entre cada coleta, totalizando 10 repetições em cada indivíduo. Foram mensuradas as flores fechadas, flores abertas, flores fechadas pilhadas, flores fechadas não pilhadas, flores abertas pilhadas e flores abertas não pilhadas tanto no solo como na planta. Para as análises estatísticas foi utilizado o Teste-T para verificar se houve diferença entre as médias obtidas das características avaliadas entre as flores do solo e as flores da planta, além de regressão linear simples para verificar se houve relação entre as flores fechadas e flores fechadas pilhadas; flores abertas e flores abertas pilhadas, encontradas no solo e flores fechadas e flores fechadas pilhadas; flores abertas e flores abertas pilhadas na planta. Houve diferença significativa em relação a todas as variáveis entre o solo e a planta. Foi verificada correlação tanto nas flores fechadas e flores pilhadas fechadas encontradas no solo quanto nas flores abertas e flores abertas não pilhadas na planta.

**Palavras-chave:** interação inseto-plantas, danos florais, abelhas neotrópicas, sucesso reprodutivo, planta ornamental.

### Introduction

The hypothesis of specialization in the evolution of pollination prevailed for a long time, but recently it is believed that pollination systems vary from a generalist form to highly specialized ones, beginning with the idea that a plant tends to specialize for the most effective pollinator, inversely to what happens in the generalist trend –

the effectiveness is unexpected. In other words, the characteristics related with efficiency do not promote fidelity in pollination (BARRETO FREITAS, 2007).

In general, flowers are attractive, with petals fused to form a floral tube, threads fused with the corolla, making possible the access to the nectar produced in the base of the flower nectary disk, allowing the floral visitors that present mouth parts

with the length related to the size of the nectary chamber, to reach the nectar (CARVALHO et al., 2007b).

Many flowering plants need a pollinator agent to accomplish the exchange of pollen grains, guaranteeing genetic variability. They present available attractive resources, such as pollen, nectar and oils and important feeding sources for these agents (MALOOF; INOUE, 2000; ROUBIK, 1982). These resources might attract several species that have no pollination, stealing the resources offered – the robbers (MALOOF, 2001). The pillage causes significant damages in the flower, causing premature abscission, and influencing the reproductive success of the species (ROUBIK, 1982; MOTHERSHEAD; MARQUIS, 2000).

Floral damages caused by opportunist species that do not accomplish pollination, among them native stingless bees, have been considered harmful in commercial species and are studied due to their economical importance by presenting a considerable decrease in productivity (BOIÇA JUNIOR et al., 2004). Pillaging rates by bees on native species such as *Bignoniaceae* Juss. and *Rubiaceae* Juss. can be considerable, and result in lower attractiveness to pollinating species (CARVALHO et al., 2007a e b).

*Nyctaginaceae* Juss. has three pollinator types: 1 – Hymenoptera, visitors of *Abronia* Juss and *Boerhavia* L. flowers. The latter have typically melittophilous flowers, with coloration going from white to purple, and with nectaries four millimeters from the reproductive organs (AGUIAR; SANTOS, 2007; GONZÁLEZ; LÓPEZ, 2004); 2 – Lepidoptera – family Hesperidae that pollinates *Bougainvillea* Choisy. This plant has psychophile flowers, with red coloration, going through yellow and purple (the individuals of Hesperidae also act as pollination agents in *Allionia* L. and *Abronia* Juss). Still belonging to the order Lepidoptera, Sphingidae is the second group of pollinator agents in importance for *Mirabilis* L. (LEAL et al., 2001; GONZÁLEZ; LÓPEZ, 2004); and 3 – Trochilidae (hummingbirds) in *Mirabilis froebelii* (Behr) Greene that exhibits a combined system of pollination, in which the hummingbirds visit their flowers until the evening, when moths start visiting (LEAL et al., 2001; GONZÁLEZ; LÓPEZ, 2004). The involucre of *Mirabilis* sp. is an effective barrier of nectar protection against bees (GONZÁLEZ; LÓPEZ, 2004).

*Bougainvillea* (Nyctaginaceae), have 14 species designated usually as “bougainvilleas”, which are native to South America and known as “primavera” or “flores de papel”. The flowers are surrounded by bracts, which have various functions, such as insect

attraction. When the fruits mature, the bracts lose their color (KOBAYASHI et al., 2007).

*Bougainvillea spectabilis* Willd. is a woody bush, native to the Northeast of Brazil, cultivated as an ornamental plant worldwide (LORENZI; SOUZA, 2001), presents small flowers of cream color surrounded by bracts, can reach, when adult, 5 to 10 meters in length, has a reproductive cycle at the end of the dry station and is pollinated by night moths, but the availability of nectar in its nectar ducts during the day propitiates a rich alimentary source for other insects (KOBAYASHI et al., 2007).

Carvalho et al. (2007a) recorded a great percentage of flowers that suffered pillage in *Tocoyena formosa* (Cham. & Schltdl.) K. Schum. (“jenipapo-bravo”), but in other native species these relationships are still not well understood, especially in *B. spectabilis*, which presents a lack of phenological, reproduction and floral development studies. The present work has as objective to evaluate the floral bee visitors and analyze whether the activity of nectar pillage by native bees influences the floral abscission of *B. spectabilis*.

## Material and methods

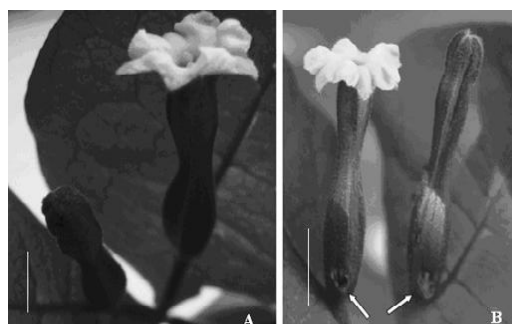
The collections were performed in ten individuals of *B. spectabilis* in the city of Campo Grande, center-west Brazil. Four of the ten studied plants were located at the campus of the Federal University Mato Grosso do Sul (UFMS), which presents a remainder of savannah and riparian forest (20°27' S and 54°37' W, altitude of 530 m). The two areas belong to the Natural Reservation Private Patrimony of UFMS (RPPN/UFMS), a total of 36.5 ha, and the vegetation consists of Savannah (EITEN, 1979). The other six plants were located in the vicinity of the UFMS campus, in residential neighborhoods. The climate of the area is of Rainy Tropical Savannah (subtype Aw) (KÖPPEN, 1948) with dry winters (May to August) and rainy summers (December to March). The annual mean precipitation is 1,532 mm and annual mean temperatures are between 20 and 22°C (EMBRAPA, 1985).

Each individual of *B. spectabilis* was considered as a sampled unit. Ten inflorescences were collected (Figure 1) on the ground and 10 in the plant, between May 4<sup>th</sup> and June 1<sup>st</sup>, 2009, with three-day intervals between each collection, totaling 10 repetitions. The total of closed flowers was evaluated (floral buttons) (FF), open flowers (FA), robbed closed flowers (FFP), flowers closed not robbed (FFNP), robbed open flowers (FAP) and opened flowers not robbed (FANP) on the ground and in

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the plant. For the statistical analyses, Student's t-test was used (ZAR, 1996) to verify whether there was a significant difference among the characteristics and among the flowers on the ground and the flowers of the plant. A simple linear regression was used to verify whether there was a relationship between the closed flowers and robbed closed flowers; open flowers and robbed open flowers, found on the ground and closed flowers and robbed closed flowers; open flowers and robbed open flowers, found in the plant. The statistical program STATISTICA for Windows was used (PETRERE-JÚNIOR, 1993).

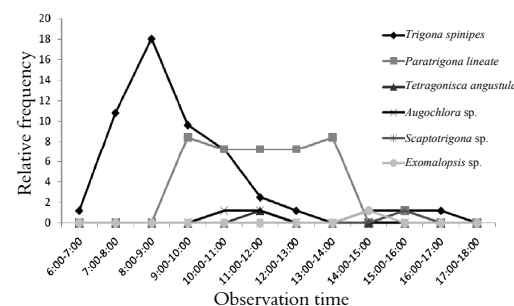
To verify the floral visitors and pillage activity, two individuals of *B. spectabilis* located in the UFMS campus were chosen. The activity and frequency of bee visitors was recorded during 12 hours of focal observation, and in each hour, 20 minutes were used to collect the bees with an entomological net (puçá) and 40 minutes for observation of the visitors' behavior (robber or pollinator). The captured visitors were killed by ethyl acetate, set in entomological pins, and later identified through specialized bibliographical reference (SILVEIRA et al., 2002). The collected specimens were deposited in the Zoological Collection at UFMS, Campo Grande, Mato Grosso do Sul State, Brazil.



**Figure 1.** Inflorescence of *Bougainvillea spectabilis* containing closed and open flower not robbed (A) and open and closed flower damaged by robber (B) (arrows indicating the damages); bar corresponds to 1 cm.

## Results and discussion

Six species of bees were recorded visiting flowers of *B. spectabilis*, on June 6<sup>th</sup>, 2009 at the UFMS campus. The mean temperature was 20°C ( $\pm$  2°C), relative humidity of the air 70%, and mean wind speed 1.9 m s<sup>-1</sup>. *Trigona spinipes* (54.25%) and *Paratrigona lineata* (39.75%) were the most frequent species, and the least frequent were *Augochlora* sp. (2.4%), *Tetragonisca angustula*, *Scaptotrigona* sp. and *Exomalopsis* sp. (1.2% each) (Figure 2).



**Figure 2.** Relative frequency and hours of activity of bee species recorded in *Bougainvillea spectabilis* on June 6<sup>th</sup>, 2009 at the UFMS campus.

Prevalence of pillaging activity was observed for the more frequent species of bees, *Trigona spinipes* and *Paratrigona lineata* (Table 1). These species of native bees were recorded stealing the nectar by the floral base, opening holes or using the opened holes (Figure 3A, C and D). *Augochlora* sp. was recorded collecting pollen from open flowers, but not necessarily accomplishing pollination.

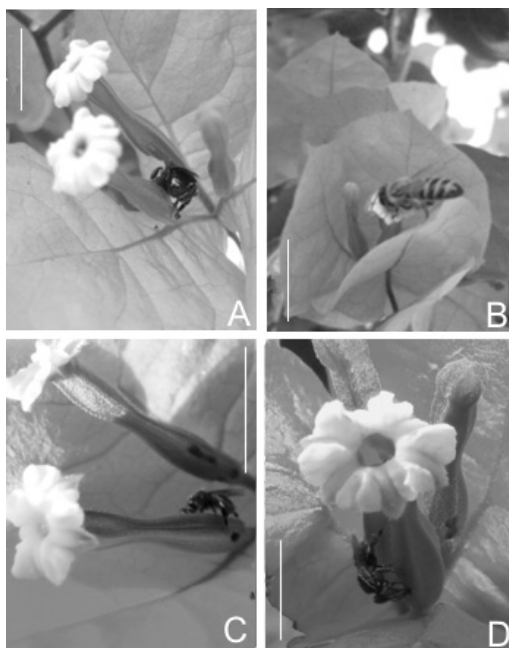
**Table 1.** Frequency of the predominant species of bees in *Bougainvillea spectabilis* in relation to the pillaging activity and pollination collected in the UFMS campus.

Bee species	Observed activity and frequencies			
	Robber	%	Pollination	%
<i>Trigona spinipes</i>	106	56.10	0	0
<i>Plebeia</i> sp.	78	41.27	1	0.52
<i>Augochlora</i> sp.	3	1.59	1	0.52

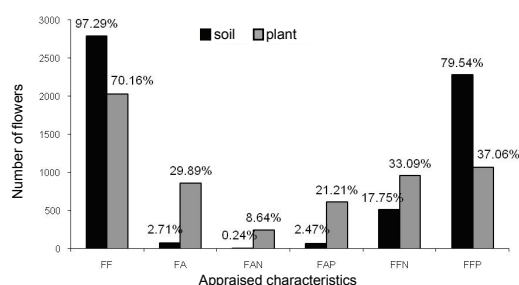
We recorded 2,874 flowers on the ground and 2,895 flowers in the plant, totaling 5,769 flowers. The robbed flowers showed damages in the floral base (Figure 1), where the nectary disks are.

There were significant differences relating to all the variables measured between the soil and the plant, being the closed flowers, or Floral Buttons (FF) ( $\chi^2 = 770$ , d. f. = 9,  $p = 0.001$ ), Open Flowers (FA) ( $\chi^2 = 824.25$ , d. f. = 9,  $p = 0.001$ ), Open Flowers Robbed (FAP) ( $\chi^2 = 490.25$ , d. f. = 9,  $p = 0.001$ ), Open Flowers Not Robbed (FANP) ( $\chi^2 = 59$ , d. f. = 9,  $p = 0.001$ ), Closed Flowers Robbed (FFP) ( $\chi^2 = 1831$ , d. f. = 9,  $p = 0.001$ ) and Closed Flowers Not Robbed (FFNP) ( $\chi^2 = 327$ , d. f. = 9,  $p = 0.001$ ).

In relation to the flowers on the ground, FF represented 97.29% and the FA 2.71%; FFP 79.54% and FFNP 17.75%; FAP 2.47% and FANP 0.24% of the total. In relation to the flowers of the plant, FF represented 70.16% and the FA 29.84%; FFP 37.06% and FFNP 33.09%; FAP 21.21% and FANP 8.64% (Figure 4). The great amount of closed flowers robbed in the period of the collections must have significantly reduced the reproductive success, reducing the rates of produced seeds.



**Figure 3.** Visiting or pillaging bee species on *Bougainvillea spectabilis*. (A) *Trigona spinipes* robbing floral base; (B) *Apis mellifera* collecting nectar by the floral opening; (C) *Tetragonisca angustula* robbing floral base; (D) *Paratrigona lineata* robbing; bar corresponds to 1 cm.



**Figure 4.** Characteristic of the Flowers (FF - Closed Flowers, FA - Open Flowers, FFP - Closed Flowers Robbed, FFNP - Closed Flowers Not Robbed, FAP - Open Flowers Robbed, FANP - Flowers Opened Not Robbed) sampled in soil and plant in the period between May 4<sup>th</sup> and June 1<sup>st</sup>, 2009, with their respective records and percentages.

Verifying the relationship between the closed flowers and the closed flowers robbed found on the ground, a strong correlation was found ( $r = 0.68948$ ,  $n = 100$ ,  $p = 0.001$ ) (Figure 5A). The correlation between the open flowers and open flowers robbed found on the ground was also highly significant ( $r = 0.98472$ ,  $n = 100$ ,  $p = 0.001$ ) (Figure 5B). The relationship between open flowers and open flowers robbed in the plant showed high significance in the correlation test ( $r = 0.92404$ ,

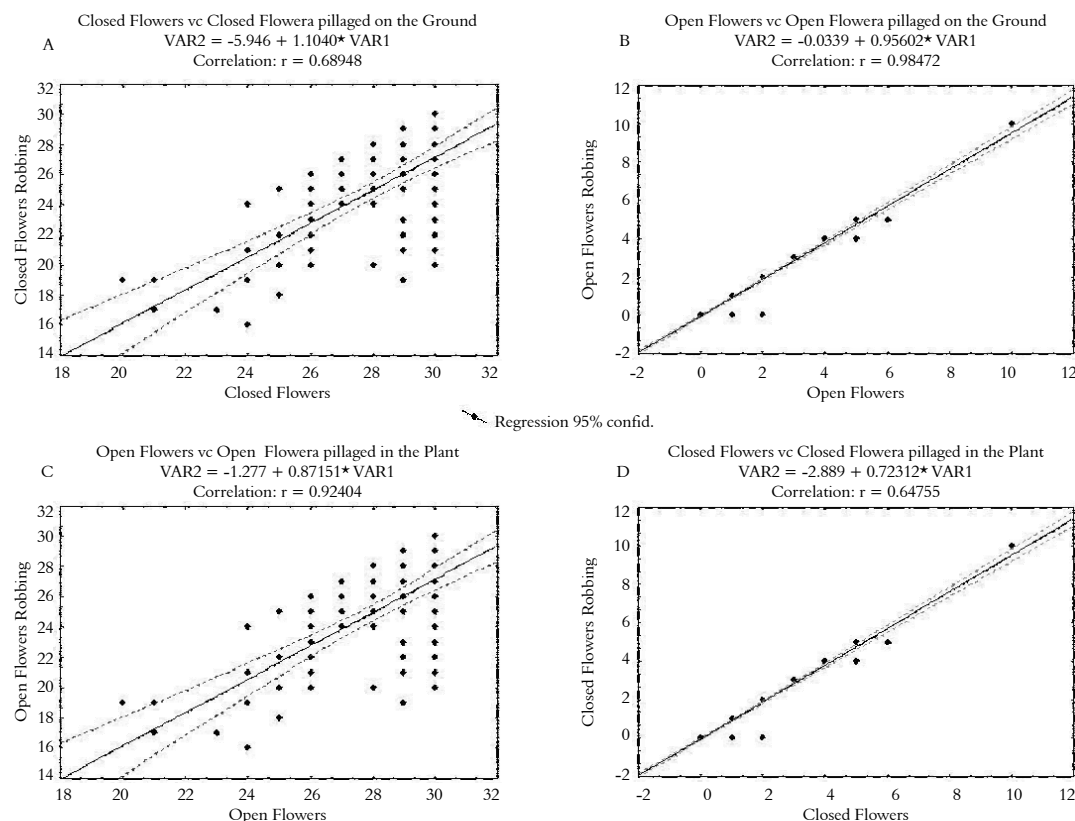
$n = 100$ ,  $p = 0.001$ ) (Figure 5C). The relationship between the closed flowers and closed flowers robbed found in the plant was also significant ( $r = 0.64755$ ,  $n = 100$ ,  $p = 0.001$ ) (Figure 5D).

The relationship between the closed flowers and the closed flowers robbed found on the ground indicates that the premature floral abscission happens before flower opening, due to the nectar pillaging by native bees. The correlation between the open flowers and open flowers robbed found on the ground shows that the flowers that have not been robbed as floral buttons (FF), suffered pillaging after anthesis, resulting either way in floral abscission.

The relationship between open flowers and open flowers robbed in the plant indicates that the flowers that were not robbed before the anthesis suffer pillaging from native bees afterwards, corroborating the correlation between open flowers and open flowers found robbed on the ground. The relationship between the closed flowers and closed flowers robbed corroborates the correlation between the closed flowers and closed flowers robbed found on the ground, showing that the closed flowers in the plant that suffer pillaging fall to the ground. *Trigona spinipes* and *Tetragonisca angustula* present a wide spectrum of floral resources that can be used (SIMIONI et al., 2007), often not being direct pollinators as the case of *B. spectabilis*. The frequency of visitation by *Apis mellifera* was not recorded in the day of study, but the presence of this exotic species was observed during the collections of the flowers, being recorded that this species exercises great influence on the use of resources for native species (NOGUEIRA-FERREIRA; AUGUSTO, 2007), thus being able to come to induce pillaging in native bees as a compensatory alternative for obtaining nectar.

Other orders of insects were observed benefiting from the openings made by the bees, such as as thumb-tacks (Hemipterida), ants of several species (Formicidae) and beetles of several species (Coleoptera).

The recorded floral damages should influence the reproductive success of *B. spectabilis* as well as recorded in other species (CARVALHO et al., 2007a e b). Irwin (2003) suggests that nectar pillaging results in a significant reduction in the visitation in *Ipomopsis aggregate* (Pursh) V.E. Grant, as well as the pollen dispersion in the population, not generating variability and reducing the production of seeds. Such reductions in the dispersion and pollination should affect in the same way the reproductive biology of *B. spectabilis*.



**Figure 5.** (A) Simple linear correlation between the closed flowers and closed flowers robbed of *Bougainvillea spectabilis* found on the ground; (B) Simple linear correlation between the open flowers and open flowers robbed of *Bougainvillea spectabilis* found on the ground; (C) Simple linear correlation between the open flowers and open flowers robbed of *Bougainvillea spectabilis* found in the plants; (D) Simple linear correlation between the closed flowers and closed flowers robbed of *Bougainvillea spectabilis* found in the plants in the period between May 4<sup>th</sup> and June 1<sup>st</sup>, 2009.

Evolutionary answers to minimize the action of pillaging in flowers are adopted, such as the concentration of alkaloid substances in the nectar that significantly reduce the number of pillaged flowers and the time of visitation (ADLER; IRWIN, 2005). In *B. spectabilis*, there are no records on the composition of the nectar and possible adaptations to minimize the effects of pillaging.

In *Mirabilis jalapa* L. (Nyctaginaceae), the pollen diversity, size and accomplish act directly in the induced abortion and performance of the seeds, and the decrease in the attraction of the pollinator caused by robbing influences negatively in the reproductive process (NIESENBAUM, 1999). Still in *M. jalapa*, the reproductive biology is associated to the self-fecundation of the flowers, in spite of being recorded for the south area of Brazil the presence of a type of moth that accomplishes pollination, besides floral robbers like *Xylocopa*, Halictidae (Hymenoptera), thrips (Thysanoptera) and

*Diabrotica* (Coleoptera), which obtain nectar through the perforation in the base of the chalice (LEAL et al., 2001).

In *B. spectabilis*, the reproductive alternatives are still uncertain; however, the great part of the reproduction is accomplished through stakes, as *B. spectabilis* is considered a commercial species with great ornamental interest, studies related to the floral biology, reproduction and their floral visitors should be motivated to maintain genetic variability, once the reproduction for stakes does allow such characteristics.

## Conclusion

The pillaging activity by native bees positively influences the precocious floral abscission in *Bougainvillea spectabilis*, presenting significant differences among the floral variables measured on the ground and in the plant, indicating a strong correlation between the pillaging activity for native

stingless bees and the premature fall of the inflorescences as floral buttons, developed buttons and flowers already open. Evaluations of the capacity and reproductive success in *B. specitabilis* are necessary for a better understanding of the pollination interactions and resources offered by the plant in its relationships with the floral insect visitors, pollinators, eventual visitors and robbing opportunists.

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