eISSN 2236-5257
10.46551/ruc.v26n2a4

Pollination ecology in *Centrosema brasilianum* (L.) Benth. (Fabaceae: Papilionoideae): Bee size influences pollen deposition

Ecologia da polinização de Centrosema Brasilianum (L.) Benth. (Fabaceae: Papilionodeae): O tamanho das abelhas influencia a deposição do pólen

Sara Sofia Medina Benavides[1]
Maria Rosângela Sigrist[2]
Gudryan Jackson Baronio[3]
Daniel Maximo Corrêa Alcantara[4]
Jeane Lima-Passos[5]
Bruno Henrique dos Santos Ferreira[6]
Camila Silveira Souza[7]

ABSTRACT

Objectives: to determine whether the quantity, and location of deposited pollen on the body of visiting bees differs according to the body size and behavior of bees. Methods: the morphological and behavioral attributes of species observed in the interaction were measured in 20 individuals of *C. brasilianum*, identifying the place of deposit and quantity of polinic charge in each bee. The relationship between the attributes of the interactants and of pollen deposited was determined using simple linear models. Results: Bee behavior and pollen deposition varied according to bee body size. Larger-sized bees with long tongues raised floral keels during visits and pollen deposition occurred on their heads and dorsal surfaces, showing greater deposition of pollen grains on the body surface. Smaller individuals with short tongues did not raise the keel. Conclusions: we assumed that larger bees may provide a more effective pollination service than smaller bees in *C. brasilianum*. Implications: a greater quantity of

---

[1] Doutoranda do Programa de Pós-Graduação em Biodiversidade e Uso dos Recursos Naturais. Universidade Estadual de Montes Claros (UNIMONTES). Montes Claros – MG – Brasil. medina.benavides.sara@gmail.com. https://orcid.org/0000-0002-6590-062X

[2] Docente do Programa de Pós-Graduação em Biologia Vegetal. Universidade Federal de Mato Grosso do Sul (UFMS). Campo Grande – MS – Brasil. sigristster@gmail.com. https://orcid.org/0000-0003-1971-3564

[3] Pós-Doutorando em Ciências Biológicas. Instituto de Biociências, Universidade de São Paulo (IB-USP). São Paulo – SP – Brasil. gudryan@gmail.com. https://orcid.org/0000-0003-2988-9168

[4] Pesquisador da FIOCRUZ Mato Grosso do Sul. Fundação Oswaldo Cruz (FIOCRUZ) Mato Grosso do Sul. Campo Grande – MS – Brasil. danielmxm@gmail.com. https://orcid.org/0000-0002-8333-377X

[5] Doutoranda do Programa de Pós-Graduação em Ecologia e Conservação. Universidade Federal de Mato Grosso do Sul (UFMS). Campo Grande – MS – Brasil. jeane.lpassos@gmail.com. https://orcid.org/0000-0002-3419-8735

[6] Pós-Doutorando em Ciências Biológicas. Universidade Federal de Mato Grosso do Sul (UFMS). Campo Grande – MS – Brasil. brubiologia19@hotmail.com. https://orcid.org/0000-0001-8494-9501

[7] Docente do Programa de Pós-graduação em Botânica Aplicada. Universidade Estadual de Montes Claros (UNIMONTES). Montes Claros – MG – Brasil. souza.camila.bio@gmail.com. https://orcid.org/0000-0003-0856-9392

Recebido em	Aceito em	Publicado em
14-05-2024	12-08-2024	10-09-2024

morphological and behavioral attributes in a larger sample number, would provide a more nuanced understanding of pollination efficiency and the consequences of differential pollen deposition on the reproductive success of *C. brasilianum*.

Key-words: Bees; Flower; Pollination; Pollen

RESUMO

Objetivo: determinar se a quantidade e lugar de deposição do pólen no corpo das abelhas visitantes difere de acordo com o tamanho corporal e comportamento das abelhas. Métodos: os atributos morfológicos e comportamentais das espécies observadas na interação, foram medidos em 20 indivíduos de *C. brasilianum*, identificando lugar de deposição e quantidade de carga polínica em cada abelha. A relação entre os atributos dos interatuantes e o pólen depositado foi determinado utilizando modelos lineares simples. Resultados: O comportamento das abelhas e deposição de pólen variaram de acordo com o tamanho corporal das abelhas. Abelhas maiores e com línguas longas levantaram as quilhas florais durante as visitas e a deposição de pólen ocorreu em suas cabeças e superfícies dorsais, mostrando maior deposição de grãos de pólen na superfície do corpo. Indivíduos menores e com língua curta não levantaram a quilha. Conclusões: Assumimos que abelhas maiores podem fornecer um serviço de polinização mais eficaz do que abelhas menores para *C. brasilianum*. Implicações: a medição de maior quantidade de atributos morfológicos e comportamentais em um número maior de amostras, forneceria uma compreensão mais matizada da eficiência da polinização e as consequências da deposição diferencial de pólen no sucesso reprodutivo de *C. brasilianum*.

Palavras chave: Abelhas, Flores; Polinização; Pólen.

INTRODUCTION

Flowers can be visited by a wide variety of visitors, mainly bee species that can vary in size, energy, and resource requirements, which present differences in their behavior and visiting efficiency, resulting in different pollination effectiveness [1]. Pollination effectiveness (PE) can be measured as the amount of pollen delivered during a single visit [2;3]. Such variation in pollen load has been positively associated with various pollinator morphological and behavioral traits, including body size [4; 5], tongue length [6; 7], hairiness [8; 9], and visit duration [8; 10].

Furthermore, flower handling behavior – how the pollinator lands on and moves on/into the flower – in general, may influence the contact between the pollinator body and the reproductive organs of the plant [11; 12]. These features have implications for flower-visitor adjustment in providing pollen transfer from anthers to stigmas, and therefore, the effectiveness of the animal as a pollinator [13; 14]. Thus, morphological coupling between the flower and floral visitor is key to understanding the pollination mechanism[15]. Bees are the main pollinators of angiosperms, and their morphological and behavioral diversity makes it possible to exploit resources from a large variety of flowers [16].

In most studies, floral traits such as size, color, architecture, arrangement of reproductive structures, and performance are considered important factors influencing the behavior of particular groups of visiting bees [17; 18; 19]. Some studies even suggest that variation in these flower characteristics (phenotypes) can cause changes in the behavior of the bees [20; 21]. On the other hand, considering that more striking floral traits can be attractive to more species of bees and induce a greater number and variety of visitors, especially generalist visitors [22], it may lead to think that, in addition to the increase in deposition of pollen, its loss can also be considerably increased due to: (i) pollen deposition at different sites in visitors' bodies, (ii) densities from different plant species, and (iii) heteroespecific pollen [23; 24].

A potential model system to understand the relationship between pollen deposition and visitor behavior is found in interactions between bees and *Centrosema brasilianum* (L.) Benth. (Leguminosae, Papilionoideae), species native to dry tropical areas. This is a herbaceous perennial vine bearing large, showy, and highly specialized nectar flowers, as seen in their resupinate form, which is adapted for pollination (nototribic or dorsal) by large insects, most commonly medium to large bees, such as tribe Xylocopini, Centridini, and Euglossini [25; 26; 27]. However, it may also present a low percentage of self-compatibility (<20%) [28].

Centrosema brasilianum specie have an ordinary pollination mechanism, in which the insects land on the banner, moving the keel downwards, allowing the stigma and anthers to be exposed at the top, contacting the back of the pollinator; thus, after the pollinator has left the flower, the keel returns to the initial position [25; 26]. This behavior is typically performed in flowers with standard morphology, where some of their petals developed a function of protecting reproductive structures and resources against environmental conditions and robbery (i.e., nectar/pollen stealers, pollen eaters and occasional visitors) [29; 30], allowing the development of specialized behaviors in some species of bees, such as the mechanical opening of the keel to reach nectar, or also called raising behavior [31], term that will be used in this article.

The flowers of *C. brasilianum*, unlike other species of the genus *Centrosema*, have a keel of medium to extended size, which possibly allows only bees with medium and long proboscis and high body weight to move the keel and reach the resource. [32; 33], indicating a pollination relationship that could involve, in addition to floral morphological attributes and pollinators, also behavioral traits necessary for the successful fitness of the species.

In *C. brasilianum*, the weight of most bees seems to be insufficient to trigger a mechanism that allows access to nectar, requiring additional effort by bees on the keel to access the resource [34; 35]. Here, according to our hypothesis that bee traits are associated with their specific visiting behavior, to answer the following question: Does the body morphology (body length and tongue length) of bee visitors to *C. brasilianum* can be related their intrafloral behavior, quantity and deposition site of pollen, and stigma contact during floral visits?

MATERIALS AND METHODS

Study area

The study was conducted in a *Centrosema brasilianum* native population with individuals separated by up to 200 meters with 60–200 flowers per plant located on the *campus* of the Federal University of Mato Grosso do Sul (UFMS) in Campo Grande, Mato Grosso do Sul, Brazil (20°27'S e 54°37'W, 530 m). The sampled area consists of *Cerrado* and *Mata de Galeria* vegetation (Riparian Forest) that surrounds the UFMS campus.

Interaction sampling

To identify the interactions, focal observations were carried out during ten non-consecutive days in April/2016 from 06:00 to 17:00 hours. Each session of focal observations lasted one hour, with an interval of five minutes per session, totaling 45 hours. For all visitors (bees), we recorded the frequency, intrafloral behavior (floral keel raise), location of pollen deposition and stigma contact with the bee body. Some of the bees were collected in vials and sacrified with ethyl acetate. The body and tongue length were measured using a digital caliper, while the presence and location of pollen deposition on the bee body were checked using a stereomicroscope. Afterwards, the insects were identified by specialists. The samples of the bees were deposited in the Zoological Collection of the Universidade Federal de Mato Grosso do Sul (ZUFMS). A voucher of *C. brasilianum* was collected and deposited in the CGMS Herbarium of the Universidade Federal de Mato Grosso do Sul, Campo Grande (CGMS: 4939).

After, to determine possible variation between plants, the width and length of the flower, and the height and length of the keel in 20 flower structures/plant, for five individual plants (n = 100 flowers) was measured.

Statistical analysis

To include the body size of the bees in the analysis, they were separated into two groups: Bee species were categorized as large (body length ≥ 10 mm) or small (< 10 mm), as modified from Frankie et al.[36] and Danieli-Silva et al.[37]. These functional groups of bees delimited here have a systematic delimitation marked at the order level, but, phylogenetically, are not true groups (e.g., big bees, small bees) because they are not monophyletic. In the present study, we used this classification of functional groups because bees are morphologically similar and tend to forage in the same way [37;38], in addition to exerting similar selective pressure on the plants visited [39]. In addition, the floral measurements (width and length of the flower, and the height and length of the keel) of the five individuals were compared using simple linear models (average measurement of the 20 flowers per individual – five individuals).

The relationship between raising behavior probability (response variable) and body size of bees which visited *C. brasilianum* flowers was modeled using a binomial generalized linear model (binomial distribution: behaviour ~ body size). We also modeled the relationship among pollen quantity (response variable) on bee bodies and bees' body size and behavior during floral visit using a linear model (pollen quantity ~ body size*behaviour). We checked the model's residuals using *DHARMa* package and performed analysis of variance using *car* package [40; 41], in R Core Team [42]. All models met the assumptions of normality and heteroscedasticity.

RESULTS

Among the sampled individuals of *C. brasilianum* there was no significant difference in the length (31.1 ± 1.8 mm; $f = 2.15$, $t = 7.66$, $p > 0.05$) and the width (34.6 ± 2.0 mm; $f = 3.47$, $t = 8.57$, $p > 0.05$) of the flower, or in the length (21.7 ±1.0 mm; $f = 2.87$, $t = 7.46$, $p > 0.05$) and the height (12.0 ± 0.7 mm; $f = 3.47$, $t = 6.54$, $p > 0.05$) of the keel of flowers. Flowers were visited by nine species, whose genus *Xylocopa* was the richest (n = 2 spp.) and most abundant (n = 6 individuals) (Table 1).

Bees species	Body size (mm)	Proboscis length (mm)	Abundance	Behavior	Pollen deposition	Quantity (pollen grains)
Smaller bees						
Megachilinae						
Anthidiini	17.95	6.18	1	Not raise	Dorse/on the wings	~120
Apinae						
Bombus sp.	14.56	1.29	1	Not raise	Dorse/on the wings	~145
Eulaema (Apeulaema) nigrita (Lepeletier, 1841)	12.72±1.59	1.54±0.26	2	Not raise	Dorse/on the wings	~89±16
Mesoplia sp.	14.57±0.32	1.55±0.15	3	Not raise	Dorse/on the wings	~114±28
Andreninae						
Oxaea sp.	16.69±0.27	6.14±1.64	4	Not raise	Dorse/on the wings	~138±35
Larger bees						
Apinae						
Eulaema sp.	20.02±1.37	7.68±1.88	2	Raise	Head	~235±015
Epicharis (Epicharis) flava (Friese, 1900)	19.23±1.98	5.67±0.82	2	Raise	Head	~285±28
Xylocopa (Neoxylocopa) suspecta Moure & Camargo, 1988	18.35±1.17	6.55±3.64	2	Raise	Head and beginning of the dorse	~290±36
Xylocopa sp.1	20.75±1.68	9.26±0.64	4	Raise	Head	~196±24

Table 1. Body length and size of the tongue, abundance, intrafloral behavior (raises or does not raises keel), local of pollen deposition/contact with stigma and quantity of pollen grains deposited of bees in the flowers of *Centrosema brasilianum* on the campus of the Universidade Federal de Mato Grosso do Sul, Campo Grande/MS, Brazil. Bees classified within the Apidae family and their subfamilies according to Melo and Gonçalves 2005.

When visiting the flowers, all species triggered the keel to collect nectar, contacting anthers and stigma (Fig. 1) and thereby serving as pollinators.

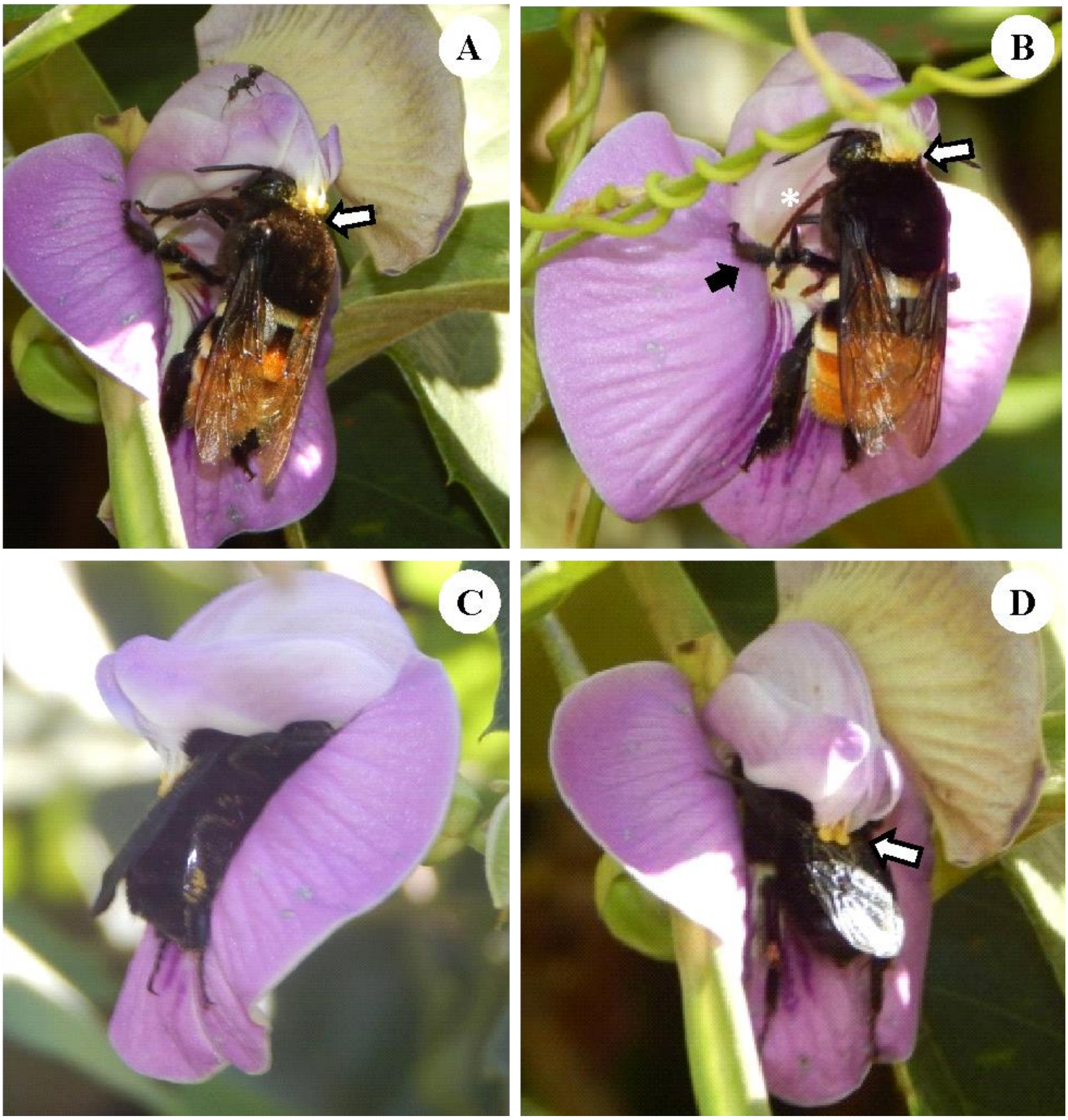

Figure 1. Large bee *Eulaema* sp. (A, B) collecting nectar in *Centrosema brasilianum* flower: Places the second pair of legs on the sides of vexillum (black arrow) and raises the keel when contacting stigma, receiving pollen on the occipital part of the head (white arrow in A and in B the * shows the tongue). The smaller bee *Bombus* sp. (C, D) does not raise the keel and in this case, the sexual organs of the flower contact the dorse and wings (white arrow in D).

However, the larger bees (19.86 ± 1.64 mm), with longer tongues (7.45 ± 2.19 mm) (n = 4 spp.), raising the keel, getting pollen and contacting stigmas mainly by the head, on the vertex and occiput, but also by the dorsal surface of the pro and mesothorax (Figure 1A-B, 2). For this, the bees supported the first and second pairs of legs on the sides of vexillum (flag or lower petal) and raised the keel (Fig. 1A-B, 2). Smaller bees (15.14 ± 2.03 mm), with short tongues (3.63 ± 2.57 mm), only pushed the keel, without raising it to receive the pollen, thus contacting the stigma with the dorsal surface of the pro and mesothorax and across the wings (Figure 1C-D, 2). Overall, larger bees were more likely to exhibit keel-raising behavior when visiting the flowers ($F_{1,\ 18}$=89.93; R^2=0.81; $p<0.0001$; Fig. 2).

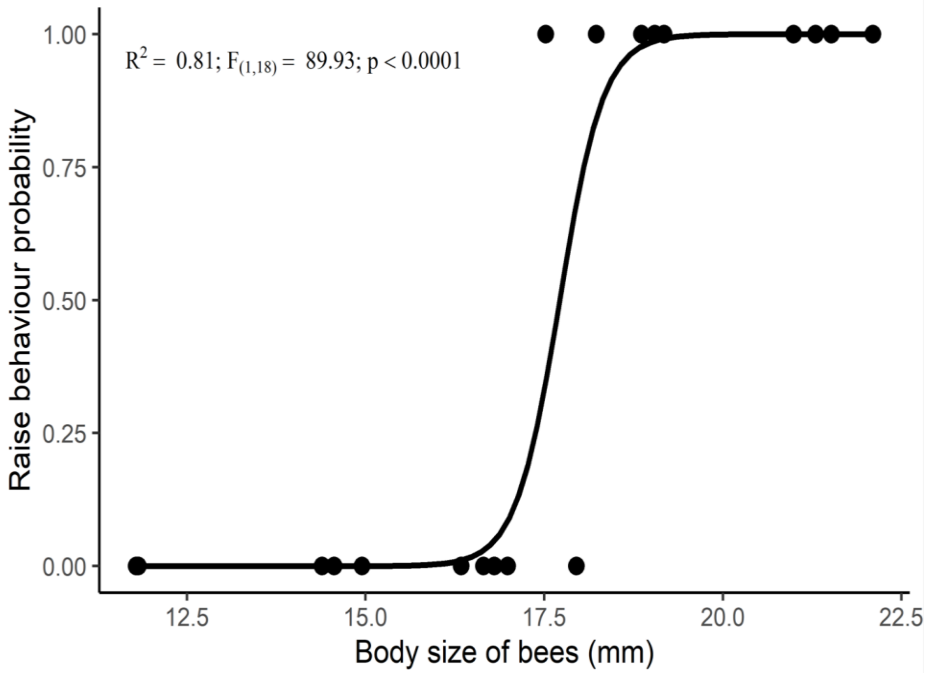

Figure 2. Positive relationship between raising behaviour probability and body size of bees which visited C. brasilianum flowers.

Furthermore, we observe a relationship between pollen quantity on the body of bees and their behavior (F3, 16=46.57; R^2=0.87; $p<0.0001$; Fig. 3). The bees which performed raising behavior showed had twice more pollen grains deposited on their bodies in average (260 ± 35 SD) than those that did not perform behavior (126 ± 17 SD).

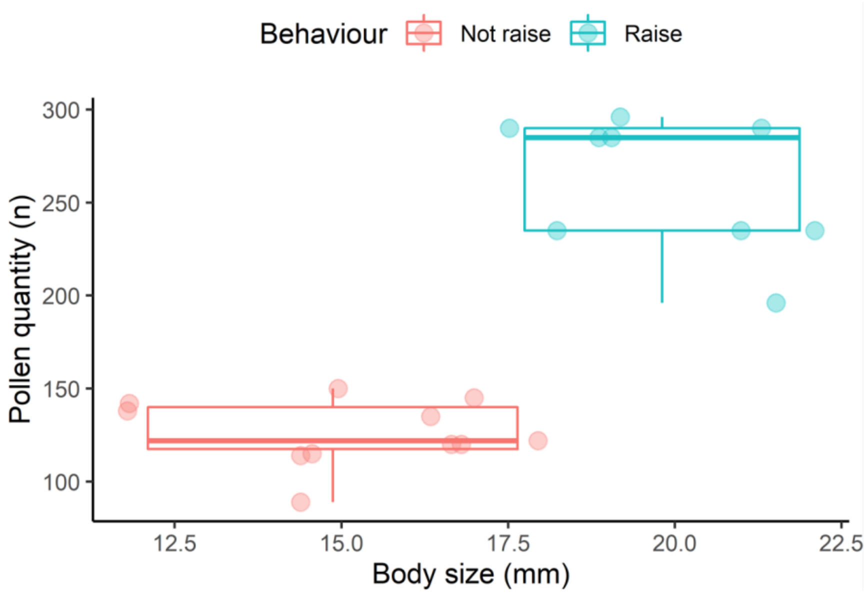

Figure 3. Difference in pollen quantity observed according to bees' behaviour during floral visits in *C. brasilianum*.

DISCUSSION

Most papilionate flowers with similar characteristics to *C. brasilianum* are pollinated by bees [25], mainly medium and large sized (8 to 12mm length) [43]. Accordingly, *Xylocopa* was the most common genus [32; 44]. However, the behavior of the bees changes according to its species producing different responses on pollen removal and deposition. The higher bees (>17mm) were 50% higher probable to raise behavior at the same time they have collected twice the pollen quantity in their bodies. Papilionate flowers have evolved to economize a large pollen quantity [45] and have developed specialized mechanisms for its gradual liberation [46; 47]. The pollination mechanism may vary depending on the species [48], but it usually requires a certain force exerted by the pollinator in order to move the keel petals and stamens, exposing the stigma [20; 49]. Therefore, variations in the size and morphological characteristics of pollinators affect their ability to collect resources, causing behavioral changes in order to obtain the rewards from flowers [18; 27].

In the *C. brasilianum* case, the larger bees with longer tongues cannot reach the flower resource, without raising the keel. This behavior allows pollinators' body adjustment during floral visits, according to the space between the keel and the vexillum being smaller in relation to the body morphology of these bees. By raising the keel, these large bees increase the space and are able to extend the long tongue and gain access to the nectar. Whether bees raised or did not raise the keel to access nectar, apparently did not affect the contact to the reproductive structures of *C. brasilianum*, only affecting the site of contact between the sexual organs of flowers and the bee's body, as well the amount of deposited pollen.

Bees with both visiting behaviors, raise or push the keel, can be considered as potential pollinators of *C. brasilianum*. However, the deposition of pollen destined for pollination should be located in areas of the bees' body which are not cleaned by them, called safe site [50; 51; 52]. Despite all bees being considered potential pollinators due to their potential of pollen transferring previously observed [26; 32]. Based on our results, is suggested that larger bees tend to provide a more effective pollination service than smaller bees for *C. brasilianum*, due to pollen deposition on the occipital part of the head, which certainly increases the chance of pollen transport to the next visited flower.

CONCLUSIONS

Our results demonstrate that smaller bees carry a reduced amount of pollen, and assuming that pollen deposition in smaller bees' bodies may occur in more cleanable sites, such as the lateral surface of the thorax and over the wings, this could result in significant loss of pollen to feed instead of to reproduction. We conclude that the size of visiting bees can directly affect their behavior, as well as access to the floral resource, thereby impacting the pollen deposition site and the quantity of pollen grains over the bee's body surface. In the future, other hypotheses – such as expecting that the morphology of *C. brasilianum* flowers may favor certain bee sizes, affecting pollination success due to location of pollen deposition – should be developed to test the implications of the differences in behavior between bees of different sizes, and the consequences of differential pollen deposition on the reproductive success of *C. brasilianum*, and finally, how it tends to affect fruit production in this species.

Data Availability Statement

The data that support this study will be shared upon reasonable request to the corresponding author.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Declaration of Funding

This research was supported by FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais) for the Postdoctoral scholarship to CSS (RED-00253–16; RED-00039-23); FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) for the doctorate scholarship to DMCA (process number 2016/20792-7); FUNDECT (Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul). GJB is grateful for the postdoctoral fellowship awarded FAPESP (process number 2021/09247-5 and 2023/04378-0), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil – process no. 88887.352134/2019-00) and CNPq (Conselho Nacional de Pesquisa - process number 165548/2020-7).

ACKNOWLEDGEMENTS

We thank Â. Sartori and F. O. de Freitas for the help with plant and bee species identifications respectively, and the support of the Programa de Pós-graduação em Biologia Vegetal from Universidade Federal do Mato Grosso do Sul.

REFERÊNCIAS

1. CORLETT, Richard T. Flower visitors and pollination in the Oriental (Indomalayan) Region. *Biological Reviews*, v. 79, n. 3, p. 497–532. 2004
2. NE’EMAN, Gigi et al. A framework for comparing pollinator performance: Effectiveness and efficiency. *Biological Reviews*, v. 85, n. 3, p. 435–451. 2010
3. KING, Caroline; BALLANTYNE, Gavin; WILLMER, Pat G. Why flower visitation is a poor proxy for pollination: measuring single-visit pollen deposition, with implications for pollination networks and conservation. *Methods in Ecology and Evolution*, v. 4, n. 9, p. 811-818, 2013.
4. WILLMER, Pat; FINLAYSON, Kathryn. Big bees do a better job: intraspecific size

variation influences pollination effectiveness. *Journal of Pollination Ecology*, v. 14, p. 244-254, 2014. DOI:10.26786/1920-7603(2014)22

5. FÖLDESI Rita et al. Larger pollinators deposit more pollen on stigmas across multiple plant species – A meta-analysis. *Journal of Applied Ecology*, v. 58 n.4, p. 699–707, 2021.
6. INOUYE, D. W. The ecology of nectar robbing. The Biology of Nectaries. In *The biology of nectaries*. Eds B Bentley, T Elias. New York: Columbia University Press, p. 153–173, 1983.
7. HEDERSTRÖM, Veronica et al. Do plant ploidy and pollinator tongue length interact to cause low seed yield in red clover? *Ecosphere*, v. 12, n. 3, p. e03416, 2021.
8. PHILLIPS, Benjamin B. et al. Shared traits make flies and bees effective pollinators of oilseed rape (*Brassica napus* L.). *Basic and Applied Ecology*, v. 32, p. 66-76, 2018. https://doi.org/10.1016/j.baae.2018.06.004
9. STAVERT, Jamie R. et al. Hairiness: the missing link between pollinators and pollination. *PeerJ*, v. 4, p. e2779, 2016.https://doi.org/10.7717/peerj.2779
10. CONNER, Jeffrey K.; DAVIS, Rachel; RUSH, Scott. The effect of wild radish floral morphology on pollination efficiency by four taxa of pollinators. *Oecologia*, v. 104, p. 234-245, 1995.https://doi.org/10.1007/BF00328588
11. PARK, Mia G. et al. Per-visit pollinator performance and regional importance of wild Bombus and Andrena (Melandrena) compared to the managed honey bee in New York apple orchards. *Apidologie*, v. 47 n. 2, p. 145–160, 2016. https://doi.org/10.1007/s13592-015-0383-9
12. THOMSON, James D.; GOODELL, Karen. Pollen removal and deposition by honeybee and bumblebee visitors to apple and almond flowers. *Journal of Applied ecology*, v. 38 n. 5, p. 1032-1044, 2001.
13. MORRIS, William F.; VÁZQUEZ, Diego P.; CHACOFF, Natacha P. Benefit and cost curves for typical pollination mutualisms. *Ecology*, v. 91, n. 5, p. 1276-1285, 2010.
14. NATTERO, Julieta; COCUCCI, Andrea Aristides; MEDEL, R. Pollinator-mediated selection in a specialized pollination system: matches and mismatches across populations. *Journal of Evolutionary Biology*, v. 23, n. 9, p. 1957-1968, 2010.
15. SOLÍS-MONTERO, Lislie; VALLEJO-MARÍN, Mario. Does the morphological fit between flowers and pollinators affect pollen deposition? An experimental test in a buzz-pollinated species with anther dimorphism. *Ecology and Evolution*, v. 7, n. 8, p. 2706-2715, 2017.
16. OLLERTON, Jeff; WINFREE, Rachael; TARRANT, Sam. How many flowering plants are pollinated by animals?. *Oikos*, v. 120, n. 3, p. 321-326, 2011.
17. DAFNI, Amots; KEVAN, Peter G. Flower size and shape: implications in pollination. *Israel Journal of Plant Sciences*, v. 45, n. 2-3, p. 201-211, 1997.
18. BRUNET, Johanne et al. The effects of flower, floral display, and reward sizes on bumblebee foraging behavior when pollen is the reward and plants are dichogamous. *International Journal of Plant Sciences*, v. 176, n. 9, p. 811-819, 2015.
19. E-VOJTKÓ, Anna et al. The neglected importance of floral traits in trait-based plant community assembly. *Journal of Vegetation Science*, v. 31, n. 4, p. 529-539, 2020.DOI: 10.1111/jvs.12877
20. STOUT, Jane Catherine. Does size matter? Bumblebee behaviour and the pollination of *Cytisus scoparius* L.(Fabaceae). *Apidologie*, v. 31, n. 1, p. 129-139, 2000.

21. PEAT, James; TUCKER, James; GOULSON, Dave. Does intraspecific size variation in bumblebees allow colonies to efficiently exploit different flowers?. *Ecological Entomology*, v. 30, n. 2, p. 176-181, 2005.
22. LÁZARO, Amparo; HEGLAND, Stein Joar; TOTLAND, Ørjan. The relationships between floral traits and specificity of pollination systems in three Scandinavian plant communities. *Oecologia*, v. 157, p. 249-257, 2008.
23. ARMBRUSTER, W. Scott; MUCHHALA, Nathan. Associations between floral specialization and species diversity: cause, effect, or correlation?. *Evolutionary Ecology*, v. 23, p. 159-179, 2009.
24. ARMBRUSTER, W. Scott; MUCHHALA, Nathan. Associations between floral specialization and species diversity: cause, effect, or correlation?. *Evolutionary Ecology*, v. 23, p. 159-179, 2009.
25. ENDRESS, Peter K.; FRIIS, Else M. *Early Evolution of Flowers*. Vienna: Springer Science & Business Media, 2012.
26. CARDEL, Yuria J.; KOPTUR, Suzanne. Effects of florivory on the pollination of flowers: an experimental field study with a perennial plant. *International Journal of Plant Sciences*, v. 171, n. 3, p. 283-292, 2010.
27. AMARAL-NETO, Laércio P.; WESTERKAMP, Christian; MELO, Gabriel AR. From keel to inverted keel flowers: functional morphology of “upside down” papilionoid flowers and the behavior of their bee visitors. *Plant Systematics and Evolution*, v. 301, p. 2161-2178, 2015.
28. MAASS, Brigitte L.; TORRES GONZÁLEZ, Alba Marina. Outcrossing in the tropical forage legume *Centrosema brasilianum* (L.) Benth. Proc. XIIIth Eucarpia Congress. Angers, France, 1992.
29. WESTERKAMP, Christian; CLASSEN-BOCKHOFF, Regine. Bilabiate flowers: the ultimate response to bees?. *Annals of botany*, v. 100, n. 2, p. 361-374, 2007. https://doi.org/10.1093/aob/mcm123.
30. ETCHEVERRY, Angela Virginia; VOGEL, Stefan. Interactions between the asymmetrical flower of *Cochliasanthus caracalla* (Fabaceae: Papilionoideae) with its visitors. *Flora*, v. 239, p. 141-150, 2018.https://doi.org/10.1016/j.flora.2017.10.006
31. ULUER, Deniz. A review for the pollinators of Papilionaceous flowers. Turkish Journal of Biodiversity, v. 4, n. 1, p. 36-52, 2021.
32. RAMALHO, Mauro; SILVA, Maise; CARVALHO, Gilson. Pollinator sharing in specialized bee pollination systems: a test with the synchronopatric lip flowers of Centrosema Benth (Fabaceae). *Sociobiology*, v. 61, n. 2, p. 189-197, 2014.
33. APONTE, Yannely; JÁUREGUI, Damelis. Morfoanatomía floral y algunos aspectos reproductivos de cinco especies de Centrosema (dc.) benth. (Fabaceae). *Ernstia*, v. 29 n. 2, p. 1-40, 2019.
34. FAEGRI, Knut; VAN DER PIJL, Leendert. *The principles of pollination ecology*. Pergamon Press. 1979.
35. CÓRDOBA, Silvina A.; COCUCCI, Andrea A. Flower power: its association with bee power and floral functional morphology in papilionate legumes. *Annals of Botany*, v. 108, n. 5, p. 919–931, 2011.
36. FRANKIE, G. W. et al. Characterstics and organization of the large bee pollination system in the Costa Rican dry forest. *CABI Digital databases*, p. 411-447, 1983. 19840215560

37. DANIELI-SILVA, Aline et al. Do pollination syndromes cause modularity and predict interactions in a pollination network in tropical high-altitude grasslands?. *Oikos*, v. 121, n. 1, p. 35-43, 2012.
38. FAEGRI, Knut; VAN DER PIJL, Leendert. *Principles of pollination ecology*. 2013.
39. FENSTER, Charles B. et al. Pollination syndromes and floral specialization. Annu. Rev. Ecol. Evol. Syst., v. 35, p. 375-403, 2004.
40. FOX, John; WEISBERG, Sanford. *An R Companion to Applied Regression*, Third edition. Sage publications, Thousand Oaks CA. 2019 https://socialsciences.mcmaster.ca/jfox/Books/Companion/.
41. HARTIG, Florian. DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. *R Packag version 020*, 2018.
42. TEAM, R. Core. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2021. URL https://www.R-project.org/.
43. FRANKIE G. W. et al. Characteristics and organization of the large bee pollination system in the Costa Rican dry forest. In: *Handbook of experimental pollination biology*. Eds C Jones, R Little. New York: Van Nostrand Reinhold Company, p 411–447.
44. BORGES, Hélida Bruno Nogueira. Biologia reprodutiva de *Centrosema pubescens* Benth: (Fabaceae). *Boletim do Museu Paraense Emilio Goeldi2*, v. 1, n. 1, p. 31–38, 2006.
45. LEPPIK, Elmar Emil. Floral evolution and pollination in the Leguminosae. In: *Annales Botanici Fennici.* Societas Zoologica Botanica Fennica Vanamo, v. 3, p. 299–308, 1966.
46. VALLEJO-MARÍN, Mario et al. Division of labour within flowers: Heteranthery, a floral strategy to reconcile contrasting pollen fates. *Journal of Evolutionary Biology*, v. 22, n. 4, p. 828–839, 2009. DOI: 10.1111/j.1420-9101.2009.01693.x
47. LUNAU, Klaus et al. Just spines—mechanical defense of malvaceous pollen against collection by corbiculate bees. *Apidologie*, v. 46, p. 144-149, 2015. DOI: 10.1007/s13592-014-0310-5
48. WESTERKAMP, Christian. Keel blossoms: bee flowers with adaptations against bees. *Flora*, v. 192, n. 2, p. 125-132, 1997.
49. PARKER, Ingrid M. et al. Pollination of *Cytisus scoparius* (Fabaceae) and *Genista monspessulana* (Fabaceae), two invasive shrubs in California. *Madroño*, v. 49, p. 25–32. 2002
50. BUCHMANN, Stephen L. et al. Buzz pollination in angiosperms. In: *Handbook of experimental pollination biology*. Eds CE Jones, RJ Little. New York; Scientific and Academic Editions, Van Reinhold, p. 73–113, 1983.
51. KOCH, Laura; LUNAU, Klaus; WESTER, Petra. To be on the safe site – Ungroomed spots on the bee's body and their importance for pollination (Ed. RM Borges). PLOS ONE, v. 12, n. 9, p. e0182522. 2017.
52. DAFNI, A.; LEHRER, M.; KEVAN, P. G. Spatial flower parameters and insect spatial vision. *Biological Reviews*, v. 72, n. 2, p. 239-282, 1997.