Buzz Pollination in Three Species of the Genus *Senna*: Bioacustics Characteristics of the Vibrations

Rachel Anne Alencar Martins[1], Arnaldo Alves Martins Júnior[2]

Abstract: ***Buzz pollination is a strategy for plants to transfer their gametes through a pollinator. Female bees vibrate the anthers in order to eject pollen found in anthers that open into apical pores. In this study, vibrations of three species of bees were analyzed, which are effective common pollinators of three species of plants of the genus Senna that occur in a region around Chapada do Araripe - CE, Brazil. Some properties of the vibrations were analyzed, such as: dominant frequency, wing beat frequency and duration. The goal was to analyze whether there are differences between the vibrations of the same species of bee vibrating different species of plants, as well as whether there are similar characteristics between different species of bees vibrating flowers of the same plant. Our results showed that there is plasticity in this behavior, bees of the same species modify their vibrations by foraging for pollen from different plant species and there is also a tendency for different species of bees to converge to higher or lower frequencies when visiting the same species of plant. Furthermore, the duration of vibrations seems to be related to indirect forms of pollen deposition in the pollinator body.***

Keywords: Pollen flowers, poricidal anthers, behavioral plasticity, pollen foraging

1. Introduction

About 20,000 plant species have flowers that release pollen through small apical pores in their anthers (Buhmann 1983). The pollination of these plants is only possible because of the ability of some female bees to vibrate these anthers in order to eject this pollen. These vibrations received, in English, the name of “buzzing”, an onomatopoeia for the sound made by this type of foraging behavior (Buchmann 1983), and is produced by the flight muscles and wings (Michener 1962). Buzz-pollinated species have arisen independently several times over the course of plant evolution, and occur in 65 families and 400 genera (Buchmann & Hurley 1978), including some of the most important agricultural crops such as tomatoes and potatoes. Bees use the vibration of their indirect flight musculature for various purposes, in addition to collecting pollen through buzzing, bees can also use them to compact soil in nests or defend themselves from predators (Buchmann 1983). Although flight muscle displacements have been measured during flight (King & Buchmann 2003), the production of vibrations associated with activities has not received the same attention. The natural frequency of Bombus terrestris L. body parts is similar to the vibration frequency in flowers, and the vibration frequency can be controlled by the natural frequencies of the sclerotized parts of the exoskeleton (King 1993). Thus, the natural frequency of the thorax (anatomically, mesosoma) can be close to the frequency at which the flowers vibrate, that is, their resonance frequency and the lower flight frequency, due to the dynamic inertia of the vibration of the wings (King & Buchmann 2003).

Bees can use buzzing to collect pollen from a large number of plants of different morphology such as Solanum, Papaver or Pedicularis, for example. This reveals that the ability to remove pollen by buzzing is not related to a single type of floral morphology, nor to a single phylogenetic group (De Luca & Vallejo-Marín 2013), although some convergent morphological features seem to be especially involved in the collection of pollen by vibrating bees (Buchmann 1983). Among the many plant species with adaptations to pollen collection by buzzing, the flowers of plants of the genus Senna have anthers with poricidal dehiscence, heteranthery, enantiostyly, absence of floral nectaries and floral asymmetry, characteristics related to buzz pollination (Barret et al. . 2000).

Only female bees perform buzz pollination, because these bees collect pollen to feed their larvae (Buchmann 1983). The typical behavior of bees during the visit occurs as soon as it lands on the flower, usually clinging to the anthers with its mandibles and bending its body over the rest, covering them with the ventral part of its body. The bee then flaps its wings and rapidly contracts its flight muscles (King 1993). The result is that these vibrations are transmitted to the anthers by the head, mandibles and ventral part of the abdomen (King 1993, King & Buchmann 2003). The buzzing resonates with the anthers causing the pollen grains inside them to gain energy and be expelled through the apical pores, when they adhere to the bee's body through electrostatic forces (Buchmann & Hurley 1978). Flowers such as those of Cassiinae that have heteranteria, different anthers (in size, position in the flower and amount of pollen) with different functions (collection and pollination), generally deposit pollen in different areas of the pollinator's body.

Pollen is the recipient of male gametes in plants, and is essential for their reproduction. It is also important for the reproduction of bees, as it is a fundamental food for their offspring (Westerkamp 2004). This dynamic of conflicting interests between bees and pollen flowers offers many paths for investigating the relationships between vibrations and their different functions, and the relationships between vibrations and pollen ejection in different plant species. Thus, it is expected that the characteristics of the vibrations used by bees to remove pollen depend not only on the morphology of the bees, but also on the morphology of the plant, and thus are the result of an intricate process of natural selection based on gains in terms of aptitude, caused by the efficiency gain in the reproductive success of plants and bees. (De Luca & Vallejo-Marín 2013).

Plant species of the genus Senna (Leguminosae-Caesalpinioideae) produce flowers in which pollen is the only attractant and resource available to visitors (Vogel,

Licensed Under Creative Commons Attribution CC BY

1978). These plants are pollinated exclusively by bees, and their flowers have anthers with a division of labor between pollination and collection (Muller, H. 1881, 1882, Muller, F. 1883). These characteristics present in pollen flowers and especially in the genus Senna led us to investigate the possible differences between the characteristics of the vibrations of bees while foraging for pollen from plants of this genus. Is there any difference in buzzing properties when the same bee species buzzes flowers of different plant species? And on the other hand, can different species of bees converge at similar frequencies of vibration when buzzing flowers of the same plant species? Studies investigating these properties of buzz pollination had only been carried out for plants of the Solanum genus, but the species of the Senna genus have morphological patterns different from those of Solanum.

2. Methods

Study area

The observations, recordings and collections were made in an area around Chapada do Araripe (CE), which comprises the municipalities of Crato (7°13'57.90"S; 39°24'52, 79"W) and Juazeiro North (7°14'13.97"S; 39°19'19.81"W). The average annual precipitation is around 760 mm, concentrated between the months of January and April (66.3%), and the average annual temperature is 24.1C (FUNCEME). This region has different types of vegetation, such as: humid forest, dry forest, cerradão, cerrado and carrasco (Fernandes 1990, Campelo et al. 2000). **The study area has many representatives of the Cassiinae subtribe, and the species studied are easily found on the slopes of the Chapada, mainly in clearings and forest edges or in flatter areas, below the plateau, in swamp locations close to rivers or creeks. The three species studied are native plants widely distributed throughout Brazil (Irwin &Barneby 1982, Randel 1988, 1989, 1990).**

Plant species

Senna spectabilis, S. acuruensis and S. alata have poricidal anthers, differentiated stamens in terms of size, position and function in the flower and are enantiostylic species, common characteristics among pollen flowers. And like all flowering plants that have poricidal anthers, they are pollinated exclusively by female bees capable of collecting pollen through buzzing.

Bee species

For the study, we used three species of effective pollinating bees common to plant species of the genus Senna: Xylocopa frontalis, Xylocopa grisescens and Centrisobsoleta. The collected Xylocopa frontalis measured 3.6 cm and weighed 2.2 g on average. X. grisescens measured 2.9 cm and weighed 1.57 g on average. Centris obsolete was the smallest species present in this study, measuring 2.3 cm and weighing 0.7 g on average.

Data analysis

The recordings were divided between flight and buzzing, and the sound files were equalized with a high-pass filter to improve the signal-to-noise ratio of the recordings, using the Audacity 1.2.6 software (Audacity, GNU General Public License). The files generated in Audacity were analyzed in the software Raven Pro 64 1.4, where measurements of dominant frequency, duration, wing beat frequency (basic frequency, which is 1 divided by the period) were made with an FFT of 155.

3. Results

Sixty-three recordings were performed in S. acuruensis; 59 recordings for S. spectabilis and 47 for S. alata. In figure 1 it is possible to observe the frequencies of flight and buzzing of bees in each plant species.

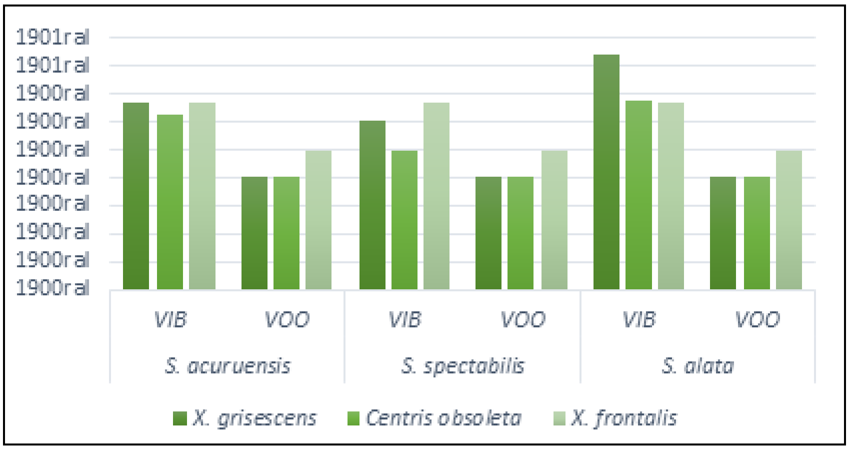


Figure 1: Wing beat frequencies by the three bee species, during buzzing and flight, in the three plant species

Based on the wing beat frequencies, it can be observed that there was an increase between flight and buzzing frequencies in all species. The flight frequency remains constant regardless of the visited plant. The highest frequencies were those of X. grisescens vibrating S. alata flowers. The vibration variations were smaller in X. frontalis, which vibrated S. acuruensis and S. alata flowers at very similar wing beat frequency. In general, one can observe a trend towards an increase in the wing beat frequency of X. grisescens and Centris obsolete in S. alata, followed by lower frequencies in S. acuruensis and S. spectabilis. There were greater buzzing in the frequencies of wing beats when buzzing flowers, ranging from 250 to 333 Hz. As for differences in flight frequency, X. grisescens had greater variations ranging from 200 Hz to 420 Hz on average during flight.

Licensed Under Creative Commons Attribution CC BY

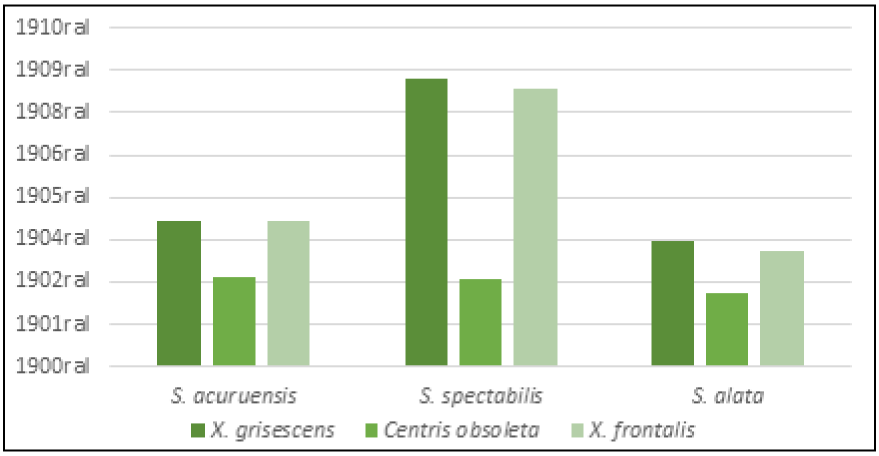


Figure 2: Dominant frequencies of bees buzzing flowers of S. acuruensis, S. spectabilis and S. alata

Bees presented dominant frequencies (Figure 2) of different intensities in each of the plant species, as well as in the wing beat frequencies, the two species of the genus Xylocopa buzzed at very similar intensities in the three plant species. However, the dominant frequencies of greater intensity were those of X. grisescens. X. frontalis maintained very similar dominant frequencies when buzzing S. spectabilis flowers, and Centrisobsoleta maintained lower dominant frequencies in all plant species.

Figure 3: Wing beat rates of bee species when buzzing the flowers of the three plant species.

Wing beat frequencies maintained a basic pattern for the three bee species, with higher rates in S. acuruensis, medium in S. spectabilis and lower in S. alata. X. frontalis buzzed the last two at the same frequency, around 200 Hz (Figure 3).

Table 1: Results of the Kruskall-Wallis tests of wing beat rates of X. grisescens, X. frontalis and Centrisobsoleta in the three plant species. 1-3: S. spectabilis and S. alata; 1-2: S. spectabilis and S. acuruensis 3-2: S. alata and S. acuruensis

	1-2	1-3	2-3
X. grisescens	p<0,05	p<0,05	p<0,05
X. frontalis	p<0,05	p<0,05	NS
Centrisobsoleta	NS	NS	NS

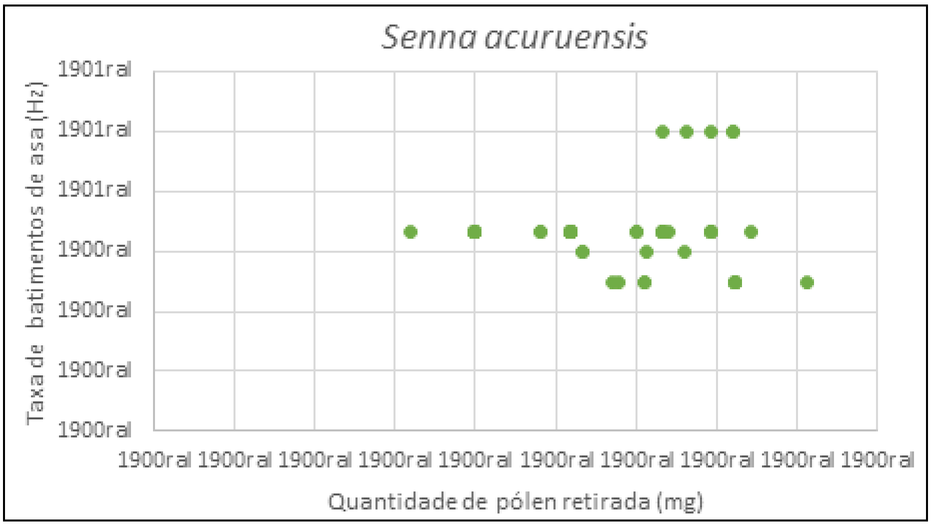
Wing beat rates of X. grisescens buzzing flowers of S. acuruensis and S. alata, S. acuruensis and S. spectabilis were significantly different, but between S. alata and S. spectabilis the vibrations were similar in intensity (Table 1). Likewise with respect to X. frontalis buzzing the same species. Centrisobsoleta showed no significant differences in the wing beat rate in the three plant species (Table 1).

Table 2: Maximum and minimum wing beat frequencies during buzzing, maximum and minimum wing beat frequencies during flight, bee weight and maximum and minimum duration of a vibration in all plant species.

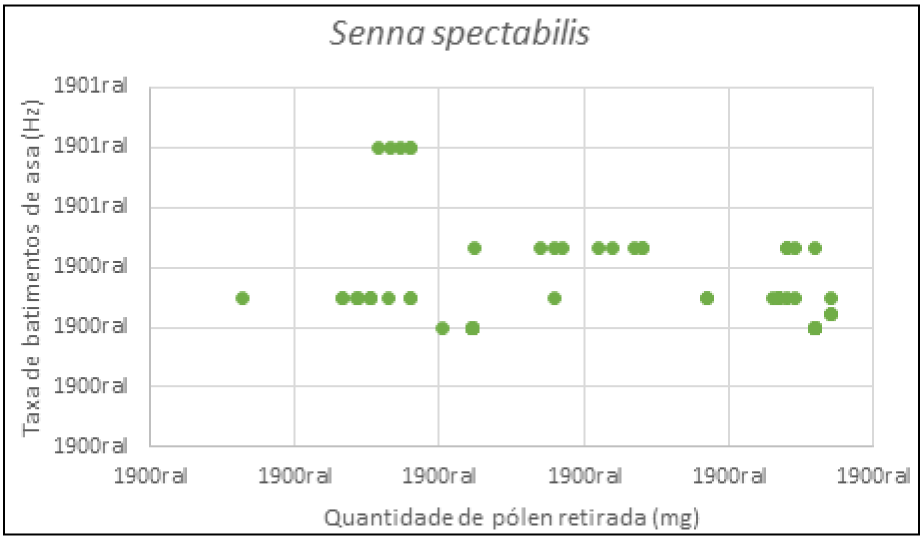
Bee	Buzzing Frequency (Hz)	Flight Frequency (Hz)	Weight (mg)	Buzzing duration (s)
X. grisescens	222±500	200±250	1.57±1.8	0.034±0.05
X. frontalis	250±333,33	200±166	2.044±2.118	0.036±0,045
Centrisobsoleta.	250±500	117±200	0.68±0,774	0.039±0,053

Licensed Under Creative Commons Attribution CC BY

Larger bees buzzed the flowers for less time, but at similar frequencies. The larger bees (X. frontalis) buzzed at higher dominant frequencies (3789.8±1234), followed by the medium bees (X. grisescens 3789.8±1033.6) and the smallest (Centrisobsoleta 1033.6±787). All species followed a trend towards higher frequencies in S. alata, and had very similar wing beat rate frequencies when visiting S. acuruensis. The bees vibrated the flowers of S. spectabilis for an average of 0.043 sec, S. acuruensis 0.086 s and S. alata for 0.027 sec.

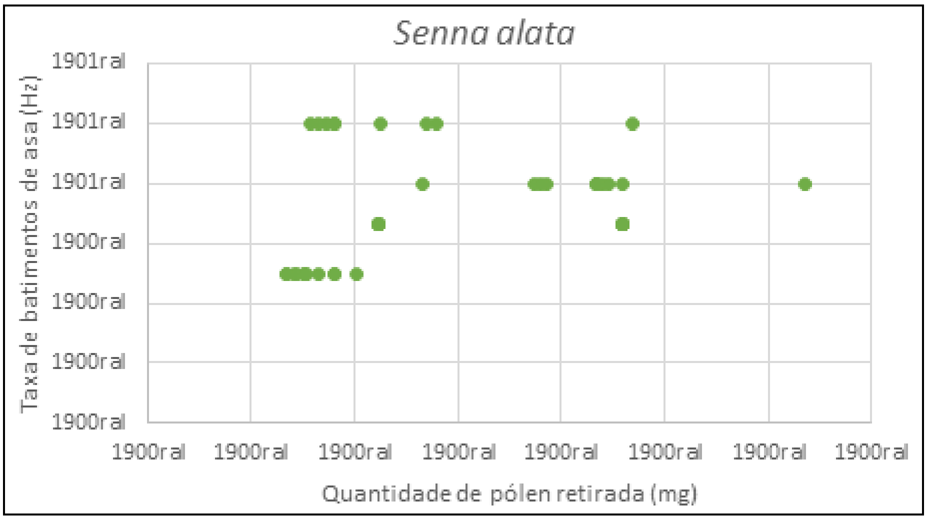


Wing beat rate (Hz)
Amount of pollen removed (mg)
Figure 4: Wing beat rate (Hz) related to the amount of pollen (mg) taken during a single visit in Senna acuruensis.



Wing beat rate (Hz)
Amount of pollen removed (mg)
Figure 5: Wing beat rate (Hz) related to the amount of pollen (mg) taken during a single visit in Senna spectabilis.

Licensed Under Creative Commons Attribution CC BY



Wing beat rate (Hz)
Amount of pollen removed (mg)

Figure 6: Wing beat rate (Hz) related to the amount of pollen (mg) taken during a single visit in Senna alata

The amount of pollen removed during the vibrations was almost constant between the wing beat frequencies of 250 to 500Hz. Hardly exceeding 0.010 mg per visit, even at higher wing beat rates (Graph 7). Most bees buzzed at frequencies between 300 and 400 Hz, and this frequency range was responsible for an ejection of 0.02 to 0.10 mg of pollen in flowers that received only one visit. With a tendency to less pollen ejected at frequencies greater than 500Hz. In Senna spectabilis the amount of pollen ejected with one visit did not show a significant correlation with the wing beat frequency (p=.08945)

4. Discussion

The removal of pollen by buzzing is a mechanical activity that depends on several parameters that can be characteristic of bees, such as: force, acceleration and frequency. And parameters that can be characteristic of plants, such as: flower mass and pore size. (Buchmann & Hurley 1978). In this study, our perspective focused on bees and the characteristics of their vibrations, trying to analyze whether there are variations from species to species when visiting the same plant, and whether a species has the ability to modify its vibrations when visiting different plants. In previous works, studies were carried out on the vibrations and flight of bees that perform buzz pollination in Solanum rostratum using Laser Dopler Vibrometer (LDV), considering: amplitude, duration and frequency of vibrations. Frequency variations did not matter for the amount of pollen ejected in S. rostratum (De Luca et al. 2013). Another isolated study quantified the effects of frequency or amplitude on Actinidia deliciosa (kiwi) where frequencies from 100 to 500 Hz were equally effective for pollen removal from this species (Corbert et al. 1988). Again with the Solanum genus, another study, this time using a microphone coupled to a Smartphone, analyzed bee vibrations in S. paniculatum and S. stramoniifolium using the wing beat frequency (Burkart et al. 2011). This study showed that different species of bees have a particular buzzing pattern and that this pattern is related to the size of the bees. With the aid of a recorder, it was possible to record the sounds, in the field, produced by the bees during flight or buzzing flowers, and with that to obtain data on the frequency of the movements of the flight muscles accessed through the sound produced by the bee. The buzzing sound is complex and generated by different oscillations of the bee's body (King & Buchmann 2003). To capture these sounds, recordings made with a portable recorder proved to be efficient. In our study they showed good results, and easy handling and execution in the field. Our results support the observations of King & Buchmann (2003), as we found different spectra of buzzing and flight frequencies for different bee species, adding to this the fact that bees of the same species have plasticity in their behavior when visiting flowers of different plants. Explanations about why this behavior varies require further studies, because not only the behavior of bees is important in this process, we have to take into account that the anthers of flowers of different species have different sizes, humidity rates and amounts of pollen, so their natural frequencies are expected to vary (King 1993). As the bees are able to modify the intensity of their vibrations, this can happen in order to approach the natural frequency of the plants they visit, optimizing the collection of pollen or even their energy expenditure. Buzzing frequency appears to be linked to wing beat frequency (Burkart et al. 2011). When buzzing flowers of the three species of plants, the two species of bees of the genus Xylocopa presented very similar dominant frequencies, which may be related to their morphological similarities or even to their approximate size and weight. The tendency to have higher frequencies for some plant species may be related to the approximation of the bee's buzzing frequency to the optimal frequency for that flower, as in S. acuruensis, where there was a trend of increasing frequency for the three bee species.

Flowers with different sizes can also explain a higher frequency of wing beats in S. acuruensis and S. spectabilis, as both have an asymmetrical petal with curvature. Wing beat rates were similar for the three species. A possible explanation would be that the asymmetrical petal of these flowers can envelop the body of the three bee species during buzzing, which can cause the wings to end up not opening as much as they could and beating with a different frequency when compared to S. alata, which does not have the petal

Licensed Under Creative Commons Attribution CC BY

that surrounds the bees, and being a smaller flower, the bees have more than half of their body outside the flower, and the wings are free during the vibration.

When compared, there was a significant difference in the frequencies at which the same bee species buzz different plant species, showing that there is indeed plasticity in this behavior. Xylocopa frontalis presented a lower frequency variation of buzzing than the other bees, which may happen due to its buzzing already reaching higher frequencies than the others, in a maximum limit imposed by the morphological conditions of the bee.

Pollen removal was efficient between 250 and 500 Hz and there was no significant relationship between an increase in wing beat frequency and a greater amount of pollen removed. It is worth mentioning that all the bees collected were efficient in pollen foraging, but with the data obtained it is not possible to draw a parallel between the amount of pollen and variations in vibration frequency. Most vibrations were between 250 and 350 Hz and the amount of pollen ejected between 0.01 and 0.10 mg, only in point samples. Vibrations between 400 and 500 Hz removed more than 0.10 mg of pollen. There is a possibility that bees can use the plasticity of this behavior so as not to spend more energy, by not vibrating at frequencies greater than necessary to remove amounts of pollen that will not be significantly greater. However, there are physical characteristics of the environment or of the plants themselves, such as humidity and opening of the pores of the anthers, which can influence this process in order to release smaller or larger amounts of pollen depending on the time of visit or the ambient temperature, requiring further studies in this specific aspect that can clarify this relationship between frequency and ejection of pollen.

Although studies carried out by means of recording have their limitations, they are an alternative that produces good results in studies of buzz pollination, proving to be an interesting alternative to works that use accelerometers or Laser Dopler Vibrometer (LDV), which often become unfeasible due to the high cost of the equipment or the impossibility of carrying out work in the field. Our results using this methodology corroborate previous results using a similar recording method as performed by Burkart et al. (2011). Our work showed that there is plasticity in the buzzing of bees visiting pollen flowers of three species of plants of the genus Senna, providing the first data that will allow further studies on buzz pollination in this group of plants.

The vibrations generated with the pollination function have not received much attention so far. Still, the few studies performed show a strong correlation between bee size and wing beat frequencies (Burkart et al. 2011). Buzz pollination is a complex subject that still has many basic questions to be clarified. As a first step to improve the understanding in this regard, our study showed that there is plasticity of buzzing behavior by Xylocopa frontalis, X. grisescens and Centris obsolete when visiting different plant species of the same genus. That is, despite being flowers that have similar basic characteristics (heteranthery, enantiostyly and available resources), other characteristics ended up influencing the foraging behavior of these bees. Even more information is needed about which plant characteristics are relevant in this process and how they are mediating the effects of vibrations and pollen ejection. Burkart et al. (2011) concluded that smaller bees were able to buzz at higher frequencies than larger bees such as Xylocopa. De Luca et al. (2012) found a positive correlation between bee mass and greater amplitudes in their vibrations. In our study, we obtained higher dominant frequencies in larger bees of the genus Xylocopa. On the other hand, the frequency of wing beats among smaller bees was higher, probably because in smaller bees the distance between the wings and the body is also smaller, making the cycle of this movement faster.

The mean flower buzzing time was 0.86 seconds in S. spectabilis, 0.043s in S. acuruensis and 0.027s in S. alata. This may be related to the size of the flowers, as the flowers of S. spectabilis and S. acuruensis are larger and more open, with a loss of pollen during the buzzing (a cloud of pollen can be observed leaving the flower during the process), which does not occur in S. alata, possibly because the flower is more closed, enclosing the pollen between the petals and the bee. Due to this characteristic, bees can buzz S. alata flowers for less time and obtain a greater amount of pollen, optimizing this buzzing time.

From the data obtained in this study, it is possible to see which variables we can study in the future, in order to make a clearer connection about the ability to adjust vibrations and the amount of pollen collected. A challenge from this study is to evaluate the consequences of these different patterns of buzzing and pollen removal on the fitness of plants and pollinators, allowing to test hypotheses that may clarify the role of loss or excessive collection of pollen, perhaps as an important selective pressure that could have favored the occurrence of buzz pollination in several groups of plants.

References

[1] BUCHMANN, S. L. & HURLEY, J. P. 1978. A biophysical modelo for buzz pollination in Angiosperms, Journal of Theoretical Biology, 72: 639-657.

[2] BUCHMANN, S. L. 1983. Buzz pollination in angiosperms. In: Handbook of experimental pollination biology (C. E. Jones & R. J. Little eds.) Scientifique and Academic Editions, New York, p. 73-114.

[3] CAMPELLO, F. C. B., LEAL-JUNIOR, G., SILVA, J. A. & CAMPELLO, R. C.B. 2000. Avaliação dos recursosflorestais da área de proteçãoambiental da Chapada do Araripe. ProjetoMMA- Ministério do MeioAmbiente, Secretaria da Biodiversidade e Floresta, Diretoria do Programa Nacional de Florestas, Crato-CE, 49.

[4] BARRET, S. C. H., JESSON, L. K. & BAKER, A. M. 2000. The evolution and function of stylar polymorphisms in flowering plants. Ann. Bot. 85: 253-265.

[5] BYRNE, D. L., BUCHMANN, S. L. & SPANGLER, H. G. 1988, Relationship between wing loading, wing beat frequency and body mass in homopterous insects. Journal of Experimental Biology 135:9-23.

Licensed Under Creative Commons Attribution CC BY

[6] BURKART, A., LUNAU, K. & SCHLINDWEIN, C. 2011. Comparative bioacoustical studies on flight and buzzing of neotropical bees. Journal of pollination ecology 6(16): 118-124.

[7] CANE, J. H. & BUCHMANN, S. L. 1989. Novel pollen-harvesting behavior by the bee *Protrandrenamexicanorum* (Hymenoptera: Adrenidae). Journal of Insect Behavior 2: 431-436.

[8] CORBET, S. A., CHAPMAN, H. & SAVILLE, N. 1988. Vibratory pollen collection and flower form: Bumble bees on *Actinidia*, *Symphytum*, *Borago* and *Polygonatum*. Functional Ecology, 2: 147-155.

[9] DE LUCA, P. A. & VALLEJO-MARÍN, P. 2013. What´s the "buzz" about¿ The ecology and evolutionary significance of buzz pollination. Current Opinion in Plant Biology 16: 429-435.

[10] FERNANDES, A.1990. TemasFitogeográficos. Fortaleza: Stylus Comunicações.

[11] FUNCEME, FundaçãoCearense de Meteorologia e RecursosHídricos. http://www.funceme.br/

[12] IRWIN, H. S. & BARNEBY, R. C. 1982. The American Cassiinae. A synoptical revision of Leguminosae, Tribe Cassiieae, Subtribe Cassiineae in New World. Memories of the New York Botanical Garden 12: 1-114.

[13] KING, M. J. & LENGOC, L. 1993. Vibratory pollen collection dinamics. Transactions American Society of Agricultural Engineers 36: 135-140.

[14] KING, M. J. & BUCHMANN, S. L. 2003. Floral sonication by bees: mesosomal vibration by *Bombus* and *Xylocopa* but not *Apis* (Hymenoptera: Apidae) ejects pollen from the poricidal anthers. Journal of Kansas Entomological Society 76: 295-305.

[15] MICHENER, C. D. 1962. Na interesting method of pollen collecting by bees from flowers with tubular anthers. Revista de Biologia Tropical, 10, 167-175.

[16] MULLER, H. 1881. Two kinds of stamens with diferente functions in the same flower. Nature, 24: 307-308.

[17] MULLER, F. 1883. Two kinds of stamens with diferente functions in the same flower. Nature, 27: 364-365.

[18] RANDELL, B. R. 1988. Revision of the Cassiinae in Australia. 1. *Senna* Miller setc. *Chamaefistula* (Colladon) Irwin &Barneby. Journal of Adelaide Botanic Garden 11: 19-49.

[19] RANDELL, B. R. 1989. Revision of the Cassiinae in Australia. 2. *Senna* Miller setc. *Psilorhegma* (J. Vogel) Irwin &Barneby. Journal of Adeleide Botanical Garden 12: 165-272.

[20] RANDELL, B. R. 1990. Revision of the Cassiinae in Australia. 2. *Senna* Miller setc. *Senna*. Journal of Adeleide Botanical Garden 16: 1-16.

[21] RANDELL, B. R. & BARLOW, B. A. 1998. *Senna*. Flora of Australia 12: 89-138.

[22] SOUZA, B. C. & BORTOLUZZI, R. L. C. 2013. *Senna* in Lista de espécies da flora doBrasil. Jardim Botânico do Rio de Janeiro (http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB23149).

[23] WESTERKAMP, C. 1996. Pollen in bee-flower relations. Botanica Acta 109: 325-332.

[24] WESTERKAMP, C. 2004. Ricochet pollination in Cassis- and how bees explain enantiostily. In: Magalhães FB, Pereira JO. Eds. Solitary bees: Conservation, rearing and manegement for pollination. Fortaleza: Universidade Federal do Ceará,p. 255-230.

Licensed Under Creative Commons Attribution CC BY