

# Are the germination and vigor of *Ficus* and *Cecropia* seeds affected by the digestion process of a frugivorous bat?

## A germinação e o vigor de sementes de *Cecropia* e *Ficus* são afetadas pelo processo de digestão em morcego frugívoro?

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### Abstract

Taking into account the importance of the bats as seed dispersers, the aim of this study was to evaluate the seed consumption of two arboreal species (*Cecropia pachystachya* and *Ficus gomelleira*) by a species of frugivorous bat (*Sturnira lilium*). As hypothesis, seeds of *Cecropia* and *Ficus* ingested by *Sturnira* are favored in the germination process under laboratory conditions. Bats were captured with mist nets and their fecal excretion were collected and submitted to the following treatments: (1) digestive system *Cecropia* Group, (2) digestive system *Ficus* Group, (3) *Cecropia* control Group, and (4) *Ficus* control Group. The passage of seeds through *Sturnira lilium* digestive system reduced the percentage of seed germination (19% – *C. pachystachya* and 16% – *F. gomelleira*) and vigor in both plant species. This result indicates that loss of seed viability occurs during the process of ingestion and digestion of seeds, reducing the percentage of germination. However, most seeds cross the digestive system without damage, resulting in good germination rates, which may allow the dispersion and establishment of *Cecropia* and *Ficus* propagules in other areas.

**Keywords:** Bats, Chiroptera, endozoochory.

### Resumo

Considerando a importância dos morcegos como dispersores de sementes, o objetivo deste trabalho foi avaliar o consumo de sementes de duas espécies arbóreas (*Cecropia pachystachya* e *Ficus gomelleira*) por uma espécie de morcego frugívoro (*Sturnira lilium*). Como hipótese, sementes de *Cecropia* e *Ficus* ingeridas por *Sturnira* são favorecidas no processo de germinação, em condições de laboratório. Morcegos foram capturados com redes de neblina e suas fezes coletadas e submetidas aos seguintes tratamentos, (1) Grupo sistema digestório *Cecropia*, (2) Grupo sistema digestório *Ficus*, (3) Grupo controle *Cecropia* e (4) Grupo controle *Ficus*. A passagem das sementes através do sistema digestório reduziu a percentagem de sementes germinadas (19% – *C. pachystachya* e 16% – *F. gomelleira*) e seu vigor em ambas as espécies de plantas. Esses resultados indicam que, durante o processo de ingestão e digestão das sementes, ocorre perda de viabilidade das mesmas, diminuindo a percentagem de germinação. Porém, a maior parte das sementes atravessa o sistema digestório incólume, resultando em boas taxas de germinação, o que pode permitir a dispersão e o estabelecimento dos propágulos de *Cecropia* e *Ficus* em outras áreas.

**Palavras-chave:** Morcegos, Chiroptera, endozooecoria.

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## Introduction

Seed dispersal in tropical forests depends heavily on mammals and birds, a dynamic process that has great influence on the distribution patterns of plant individuals in the community. This process of transport and distribution of the seeds can provide an appropriate location for germination and seedling establishment (Fenner, 1985). Thus, seed dispersal is important for the maintenance of forests, as well as for recovering areas that have undergone human action (Galindo-González *et al.*, 2000; Garcia *et al.*, 2000). It is estimated that 50 to 90% of the species of fruiting trees found in tropical forests have their seeds dispersed by animals (Fleming, 1979; Howe and Smallwood, 1982).

Among the dispersion mechanisms, endozoochory, that is the dispersion after passing through the digestive system of vertebrates, is one of the most important, including the interaction known as dispersive mutualism, where the animals obtain nutrients from fruits that they consume dispersing the diaspores away from the parent plant, undergoing less intraspecific competition than when remaining close to the progenitor plant (Fenner, 2000; Galetti *et al.*, 2006), besides decreasing the predation (Janzen, 1970). This process can also colonize recently open habitats or deposit the seeds specifically in microhabitats more favorable to their germination and establishment (Howe and Smallwood, 1982).

Among mammals, fruit-eating bats (Chiroptera) have been considered as efficient seed dispersers due their foraging behavior (Neuweiler, 2000; Preciado-Benítez *et al.*, 2015), such as frequent exchange of feeding sites (Morrison, 1980), flights of several kilometers per night (Williams and Williams, 1970), consumption and rapid digestion of food during the pursuit trajectory (Fleming, 1982). In addition, the emptying of the intestines during the flight certainly increases the chance of adequate dispersion and the establishment of diaspores (Neuweiler, 2000), making them excellent dispersers of many species of plants, including pioneer and secondary plants (Galindo-González *et al.*, 2000; Galletti *et al.*, 2006; Munin *et al.*, 2012). On the other hand, some bat species can also act as seed predators chewing and killing the embryo to obtain nutrients of seeds, as bat genus *Chiroderma* (Nogueira and Peracchi, 2003). However, little information is available regarding the negative effects of frugivorous bats on seeds of consumed plants taking into account the diversity of species in the tropics and their interactions.

The fruit-eating bat species *Sturnira lilium* (É. GEOFFROY, 1810) (Phyllostomidae) occurs from the Antilles and Mexico to the northeast Argentina, Paraguay and Uruguay and throughout the Brazil. It is a medium-sized species (forearm: 42.0 mm; body mass: 21 g), with gray to orange coloring. It is considered an excellent seed disperser of several species of plants, especially pioneers, in the Neotropics (Simmons, 2005; Reis *et al.*, 2007).

*Sturnira lilium* has been considered as a specialist in fruits of various species of Solanaceae (Marinho-Filho, 1991; Passos *et al.*, 2003; Mello *et al.*, 2008). Andrade *et al.* (2013) corroborate the hypothesis that *Sturnira* bats do not forage opportunistically, separating their diet hierarchically and selected fruits of their preferred plant genera first, even when other fruits are offered in abundance. This preference seems to be related to the fact that solanaceous fruits present a longer fruiting time (8 to 10 months), in relation to other species (Marinho-Filho, 1991). Fruits from other families, such as Cecropiaceae, Melastomataceae, Moraceae, Myrtaceae and Piperaceae, and nectar and pollen are also consumed by this bat species (Garcia *et al.*, 2000; Passos *et al.*, 2003; Munin *et al.*, 2012). Among others, *Sturnira* bats visit *Cecropia pachystachya* TRÉCUL (Cecropiaceae) and *Ficus gomelleira* KUTH & C. D. BOUCHÉ (Moraceae). *Cecropia pachystachya* is a pioneer and evergreen species, which is characteristic of moist soil on the edge of woods and glades (Lorenzi, 2008). *Ficus gomelleira* is a large evergreen species that belongs to the early secondary ecological group (Rolim *et al.*, 1999).

Thus, this study was carried out taking into account the importance of *Sturnira lilium* as a seed disperser and the lack of information on whether there are some negative effects on seed dispersal and germination processes by this species. It aimed to evaluate the effect of digestion of the seeds of *Cecropia pachystachya* and *Ficus gomelleira* by *S. lilium* in the Rio Negro region of the Pantanal in Mato Grosso do Sul, Brazil.

## Material and methods

### Study area

The study was carried out in the Pantanal Research Institute (IPAN) that has an area of 2,618 ha (19°33'20.9" south latitude; 55°36'47.19" west longitude) and is located in the Farm Santa Emília, Rio Negro Pantanal, municipality of Aquidauana, state of Mato Grosso do Sul, Brazil. It took place along the edges of vegetation areas, which presented *Ficus* and *Cecropia* trees, with the presence of ripe infructescences at the time. The site is located in Pantanal wetlands, Aquidauana sub-region, which has approximately 70% of its surface occupied by the Cerrado biome, characterized by the predominant presence of woody vegetation (Pott *et al.*, 2011).

According to Alvares *et al.* (2014), the region is localized in the Tropical zone and characterized as Aw – with dry winter, presenting average annual temperature between 24 and 26°C and rainfall that ranges from 1300 to 1600 mm per year. From September to November, the maximum temperature exceeds 40°C and, in May, June and July, when there are cold fronts, the minimum average may drop below 20°C. The winter is dry (May to Septem-

ber) with the driest months in July and August; the summer is rainy, with 80% of rainfall concentrated between November and March (Bononi *et al.*, 2008).

The study area has well preserved vegetation, despite the selective extraction of wood in various locations, providing successional processes. However, most of the area still has native vegetation, consisting of grasses and herbs in floodplains, surrounding small elevations, regionally known as “cordilheiras” and “capões”. These sites have tree species which occurs in the Cerrado biome and Deciduous or Semideciduous Forest, such as *Andira cuyabensis* BENTH., *Astronium fraxinifolium* SCHOTT, *Bowdichia virgilioides* KUNTH, *Cecropia pachystachya*, *Ficus gomelleira*, *Handroanthus aureus* MATTOS, *Hymenaea stigonocarpa* (MART.) HAYNE, *Vatairea macrocarpa* (BENTH.) DUCKE, and other. These areas are surrounded by fields, with a predominance of grasses such as *Acroceras zizanioides* (H.B.K.) DANDY, *Leersia hexandra* Sw., *Oriza latifolia* DESV., and *Panicum laxum* Sw. (Oliveira *et al.*, 2013).

### Bat sampling

In June 2011, the capture activities were carried out with the capture effort corresponding to two sampling nights, each one with the aid of two nets of 7 x 3 m close to the ground (capture effort was calculated in accordance with Straube and Bianconi, 2002). Nets were set at 6h pm and remained open during six hours. The nets were nearby the trees with infructescences and were inspected every hour when necessary. The specimens captured were removed from the nets and transported to the laboratory of the IPPAN, where they rested for about 30 minutes until the excretion of feces. This time is sufficient for processing the recently ingested food and evacuation of the intestines (Mikich, 2002). Feces were then collected and placed in paper bags.

At the end of the sampling, the specimens were released in the same sampling area, following the guidelines of the American Society of Mammalogists (Sikes and Gannon, 2011). Identification of captured specimens of *S. lilium* was confirmed based on Gannon *et al.* (1989). This species was already captured in the study region by Fischer *et al.* (2015). Analysis of the Ethics Committee for Animal was not necessary because the research was inserted inside the Laboratory of Chiroptera as standard procedures. Bat captures were allowed by license of capture - IBAMA License nº. 14/2005.

### Seed collection and germination test

Initially, *F. gomelleira* and *C. pachystachya* trees were identified through direct observation, evaluating the family and genera level, confirmed through identification keys of Carauta (1989) for *Ficus* and Romaniuc Neto *et al.*

(2009), for *Cecropia*. The ripe infructescences were taken from different trees on the site and stored in paper bags. In relation to captured bats, 10 fecal units were obtained and inspected in order to find seeds of *C. pachystachya* and *F. gomelleira*, which were removed and identified. The collected seeds were transported to the Interdisciplinary Laboratory for Research in Environmental and Biodiversity Systems, University Anhanguera-Uniderp, where the experiment was conducted.

The seeds were placed in Petri dishes lined with two sheets of moistened germitest paper and an aqueous solution of 0.1% Ranol fungicide was used to control the infestation of pathogenic fungi in the seeds. The volume of the fungicide solution was equal to 2.5 times the weight of the substrate. These dishes were maintained in a growth chamber at 27°C, with periods of twelve hours of artificial lighting (fluorescent lamps). Four treatments were assembled, each one consisting of four replicates of 50 seeds each:

- (1) *Cecropia* Digestive System Group, with *Cecropia* seeds that passed through the bat digestive system;
- (2) *Ficus* Digestive System Group, with *Ficus* seeds that passed through the bat digestive system;
- (3) *Cecropia* Control Group, with *Cecropia* seeds extracted directly from plants; and,
- (4) *Ficus* Control Group, with *Ficus* seeds extracted directly from plants.

The counting of germinated seeds was daily (experimental time of 36 days), with seeds being considered as germinated when there was a protrusion of 2.0 mm primary root. At the end, the non-germinated seeds were subjected to a tetrazolium viability test (1% in aqueous solution) for assessing the viability of seeds after the test (Brasil, 2009).

### Statistical treatment

Experimental design was completely randomized with four replications for each treatment. The percentage of germination was estimated according to the formula  $\%G = (\sum ni \cdot N^{-1}) \cdot 100$ , where  $\sum ni$  corresponds to the total number of seeds germinated in relation to the number of seeds placed to germinate ( $N^{-1}$ ) (Borghetti and Ferreira, 2004). The relative frequency of germination was calculated by the formula  $Fr = 100 \cdot (ni / \sum ni)$ , where  $ni$  is the number of germinated seeds per day and  $\sum ni$ , the sum of the total number of germinated seeds (Labouriau, 1983).

The seed vigor was also estimated, being indirectly measured by the germination mean time (GMT) estimated in days. The germination was quantified from the kinetic point of view,  $t = \sum ni \cdot ti / \sum ni$ , where  $t$  indicates mean germination time,  $\sum ni$  = number of germinated seeds in a time interval and  $\sum ti$  = time interval in days (Laboriau and Agudo, 1987). The germination speed index (GSI) was calculated by the formula  $GSI = G1/N1 + G2/N2 + \dots Gn/Nn$ ,

where G1 is equal to the number of seeds that had germinated at the first count, N1 is equal to the number of days elapsed for the first count, G2 equals the number of seeds germinated at the second count, N2 equals the number of days elapsed for the second count, and n, the final count (Maguire, 1962).

To know whether there is difference in the values of the seed germination parameters between Digestive System Group and Control Group, the non-parametric Mann-Whitney tests (U tests) ( $p < 0.05$ ) were used. U tests were conducted using the Assistat statistical program, with independent tests for each plant species.

## Results

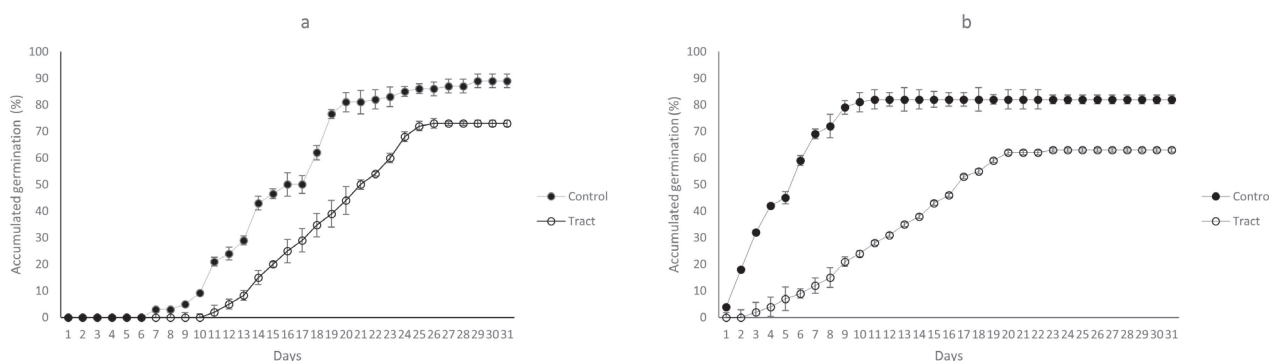
Ten *Sturnira lilium* bats (four males and six females) were captured by a capture effort of 504 m<sup>2</sup>.h. *Cecropia* Control Group began the process of primary root protrusion on the first day of testing and Digestive System *Cecropia* Group on the third day (Figure 1) and presented significant differences in percentage of germinated seeds ( $U = 0, p = 0.014$ ) and vigor (GMT and GSI:  $U = 0, p = 0.014$ ) when compared to *Cecropia* Digestive System Group. *Ficus* Control Group started the process on the seventh day, while Digestive System *Ficus* Group began on the 11<sup>th</sup> day

(Figure 2) and similarly, presented significant differences in percentage of germinated seeds ( $U = 0, p = 0.014$ ) and vigor (GSI:  $U = 0, p = 0.014$  and GMT:  $U = 1, p = 0.029$ ) when compared to *Ficus* Digestive System Group.

There was a lower percentage of germination of the seeds after their passage through the digestive system and slower germination over a longer period of time (decreased vigor) (Table 1). Both plant species had a polymodal distribution of germination type with multiple germination peaks (Figure 2). After the end of the test, the tetrazolium test indicated that the embryos were dead.

## Discussion

The presented data suggest that the seed passage through the digestive system of *Sturnira lilium* leads to a reduction in germination percentage and a decrease in the seed vigor for both *Cecropia pachystachya* and *Ficus gomelleira*. Moreover, the dead embryos observed in the end of the germination tests indicate a deleterious effect in germination of *F. gomelleira* and *C. pachystachya* through their seed consumption by *S. lilium*. Similar results were obtained by Oliveira *et al.* (2013), with ingested seeds of *Cecropia* and *Ficus* by *Platyrrhinus lineatus* (É. GEOFFROY, 1810) in Rio Negro Panta-

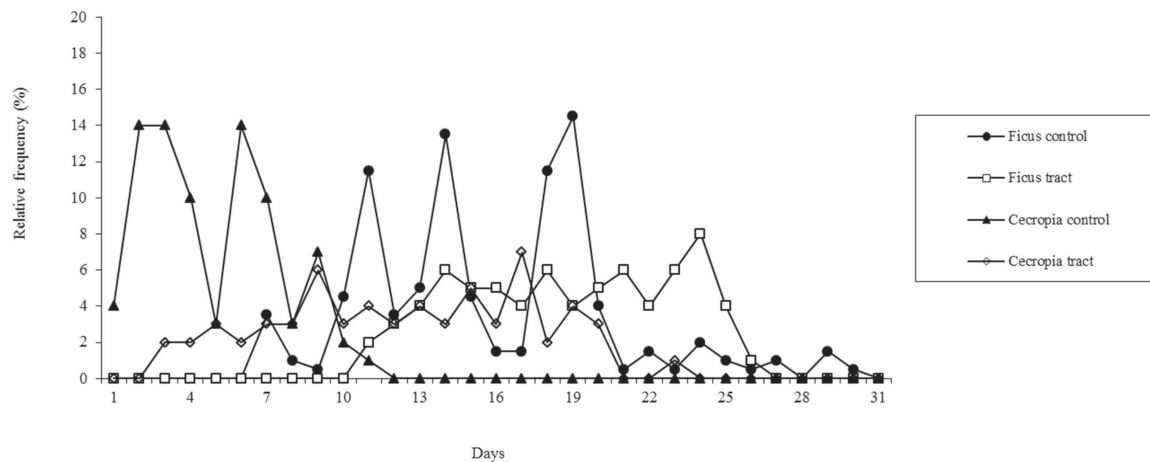


**Figure 1.** Accumulated germination (%) of (a) *Cecropia pachystachya* seeds (Groups - Control and Tract) and (b) *Ficus gomelleira* seeds (Groups - Control and Tract) before and after the passage through the digestive tract of the bat *Sturnira lilium*.

**Table 1.** Germination (%), germination speed index (GSI) and germination mean time (GMT) (in days) of *Cecropia pachystachya* and *Ficus gomelleira* before and after passage through the digestive system of *Sturnira lilium*. Means followed by the same letter in the column do not differ significantly by the Tukey test ( $p < 0.05$ ).

	<i>C. pachystachya</i>			<i>F. gomelleira</i>		
	Germination (%)	GSI	GMT (days)	Germination (%)	GSI	GMT (days)
Control	82 <sup>a</sup>	10.9 <sup>a</sup>	5.8 <sup>a</sup>	89 <sup>a</sup>	3.2 <sup>a</sup>	15.6 <sup>a</sup>
Digestive system	63 <sup>b</sup>	3.94 <sup>b</sup>	11.74 <sup>b</sup>	73 <sup>b</sup>	2.24 <sup>b</sup>	18 <sup>b</sup>





**Figure 2.** Relative frequency of germination of *Cecropia pachystachya* and *Ficus gomelleira* seeds for the four tested groups.

nal, demonstrating that this bat can alter the seed vigor in a negative way.

The decrease in seed vigor, however, is not always considered a negative factor in the germination process because the reduction in vigor may mean that germination is being distributed in space and time, situation related to the characteristics of the disperser animals (Fenner, 1985). For this reason, the increase in GMT and the decrease in GSI obtained in this study and also by Sato *et al.* (2008) may mean a change in the distribution of germination over time. This is not necessarily negative, since the passage of *Cecropia* and *Ficus* seeds through the digestive system could provide more favorable environmental conditions for their establishment than those found at the time they were dispersed (Lobova *et al.*, 2003). This hypothesis is confirmed by the study of Brancalion and Marcos Filho (2008), who observed that the distribution of the germination over long periods favors an increased seedling survival rate.

On the other hand, the decrease in the percentage of germination can be related to the possible damages that occur during the process of ingestion of the fruits. According to Barnea *et al.* (1992), damage may occur due to a great number of factors, such as the mastication of the seed and the pH of the digestive juice, characteristics which may influence the integrity of the ingested seeds. However, Tang *et al.* (2007), Oliveira and Lemes (2010) and Oliveira *et al.* (2013), working with consumed seeds of genus *Cecropia* and *Ficus*, demonstrated that most seeds normally pass through the bat digestive tract without damage and often did not lead to a change in the percentage of germination when compared with germination obtained from samples of fresh fruit seeds. Rossaneis *et al.* (2015) also demonstrated that the passage through the gastrointestinal tract of *S. lilium* produced no significant change in the rates of seed germination (*Cecropia glaziovii* and *Ficus guaranitica*).

Germination rates of the genus *Cecropia* are usually high, not needing for specialized dispersers so that their seeds can germinate in great quantity (Holthuijzen and Boerboom, 1982). However, Lobova *et al.* (2003) noticed that the passage of *Cecropia* seeds through the bat digestive system can increase the germination process because some part of fruit, as mesocarp and mucilage of seed, are removed during the passage. If there is no removal of these parts, the seed could fester in soil together with the fruit precluding the germination. Moreover, when the seeds are separated from the pulp by passing through the intestine of bats, this can increase their longevity by reducing the risk of predation, e.g., by ants or others insects. Tang *et al.* (2008) mention this situation with infructescences of *Morus macroura* Miq. (Moraceae) consumed by fruit-eating bats. Furthermore, the mature fruits that fall to the ground may deteriorate, and their seeds did not germinate (Tang *et al.*, 2008). The loss of moisture, thereby reducing the attack by fungi, also improves germination (Heer *et al.*, 2010).

Usually, the seeds of pioneer or early secondary plant species germinate quickly after being dispersed by the parent tree. However, some species of genus *Cecropia* do not present this pattern, because the soil seed bank, in certain regions, is largely composed of its seeds (Charles-Dominique, 1986; Vázquez-Yanes *et al.*, 1996). According to Salomão *et al.* (2003), *Cecropia* seeds have tolerance to desiccation and cold, which could allow their maintenance in the seed bank over a period of time in order to wait for favorable period to germinate and establish the seedlings. These seeds can germinate up to nine years after dispersion (Lobova *et al.*, 2003).

Regarding the genus *Ficus*, the germination rates of several species are usually low (Lorenzi, 2009), although the opposite has been observed in the present study, a fac-

tor probably related to the region of origin of the seeds (Baskin and Baskin, 2001). In species with explosive fruiting, such as *Ficus*, some of the propagules have very little prospect of being effectively dispersed by animals because many seeds will not be removed from the parent plant (Fleming, 1982). In these cases, the disperser may contribute to the maintenance of a high percentage of diaspores germination, and thus may increase the chances of survival and establishment of the seedlings. According to Janzen (1970) and Connell (1971), the dispersion can promote the reduction of intraspecific competition by modifying the amount of dispersed seeds in a given location or if recruitment sites are far from the parent plant. This increase the probability of survival of seedlings (Janzen–Connell hypothesis). Even if the germination rate is decreased almost by half, as in the case of *Platyrrhinus lineatus* and *Cecropia pachystachya* (Sato *et al.*, 2008), the bat can be considered an efficient disperser moving the seeds to large distance from their parents. For these reasons and according to the results obtained in this study, *Sturnira lilium* seems to present an appropriate behavior for an efficient disperser of *Ficus gomelleira*, transporting their seeds away from the parent plant.

Although frugivorous medium-sized bats, such as *Sturnira lilium*, tend to have a smaller home range than the large frugivorous bats (e.g., *Artibeus lituratus*) (Fleming *et al.*, 1972), they probably do not always follow the same path in searching for food. Instead, they may increase their flight time searching for new sources of food, especially when the availability is spatially unpredictable, as is the case of *Ficus* (Fleming, 1982). Considering that bats that find a fig tree with ripe infructescences may return for several consecutive nights for foraging (e.g., *Artibeus jamaicensis*) (Morrison, 1978), the mobility of the diaspores also increases because the bats usually return to their shelters every day (Fleming *et al.*, 1972; Morrison, 1978; Fleming, 1982).

Plants can respond in markedly different ways to the process of ingestion of their fruits and the ingestion and digestion strategies used by dispersers (Fenner, 2000; Robertson *et al.*, 2006). However, the potential for dispersal by bats is confirmed in many studies (Galindo-González *et al.*, 2000; Garcia *et al.*, 2000; Lobova *et al.*, 2003; Lopez and Vaughan, 2004; Henry and Jouard, 2008; Heer *et al.*, 2010; Oliveira and Lemes, 2010; Oliveira *et al.*, 2013). These studies have also demonstrated that ingestion by fruit bats may be an important factor in regeneration processes and succession of forest areas.

Results indicate that loss of seed viability occurs during the process of ingestion and digestion of the seeds by *Sturnira lilium*, reducing the percentage of germination. However, most seeds cross the digestive system without damage, resulting in goods germination rates, which may allow the dispersion and establishment of *Cecropia* and

*Ficus* propagules in other areas. Thus, this bat would be acting as a predator, but taking into consideration their ability to disperse the seeds, the bat could also be considered an efficient seed disperser. As *S. lilium* is a specialist in consuming fruits of the *Solanum* genus, it is possible that it acts as a predator of other plant species that are not Solanaceae - beyond those studied here.

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