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INTERFACES CIENTÍFICAS

DISPERSAL OF ARBUSCULAR MYCORRHIZAL FUNGI BY BATS IN AN URBAN TROPICAL FOREST FRAGMENT

DISPERSÃO DE FUNGOS MICORRÍZICOS ARBUSCULARES POR MORCEGOS EM UM FRAGMENTO URBANO DE FLORESTA TROPICAL

DISPERSIÓN DE HONGOS ARBUSCULARES MICORRIZALES POR MURCIÉLAGOS EN UN FRAGMENTO URBANO DE BOSQUE TROPICAL

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ABSTRACT

Associations of arbuscular mycorrhizal fungi (AMF) and plant species may occur in terrestrial and air environments and need an effective dispersal, which occurs mainly by biotic vectors. Studies on the influence of flying animals on this dispersal are scarce, thus, this study aimed to investigate the role of bats in AMF propagule dispersal and the most frequent means of transport. Analyses were performed in dorsoventral hairs and feces of bats in an urban tropical forest fragment in southern Brazil, from April to September 2018. Results were negative for the presence of spores in the hair, but spores of *Glomus invermaium* and *Acaulospora scrobiculata* were detected in feces of two bat species, evidencing that they can act as secondary spore dispersers. Efficient dispersal depends on the range extension of the dispersing species, so bats can be important dispersers of AMF, since they have the ability to fly and travel long distances.

KEYWORDS

Chiroptera; Ectozoochory; Endozoochory; Environmental Service; Glomeromycota; Mycorrhizal Colonization.

RESUMO

Associações de fungos micorrízicos arbusculares (FMA) e espécies de plantas podem ocorrer em ambientes terrestres e aéreos e precisam de uma dispersão efetiva, que ocorre principalmente por vetores bióticos. Estudos sobre a influência de animais voadores nessa dispersão são escassos, portanto, este estudo teve como objetivo investigar o papel dos morcegos na dispersão dos propágulos de FMA e o meio de transporte mais frequente. As análises foram realizadas em pelos dorsoventrais e fezes de morcegos em um fragmento de floresta tropical urbana no sul do Brasil, de abril a setembro de 2018. Os resultados foram negativos para a presença de esporos no pelo, mas esporos de *Glomus invermaium* e *Acaulospora scrobiculata* foram detectados em fezes de duas espécies de morcegos, evidenciando que podem atuar como dispersores secundários de esporos. A dispersão eficiente depende da extensão do alcance das espécies dispersantes, portanto, os morcegos podem ser importantes dispersores de FMA, uma vez que têm a capacidade de voar e viajar longas distâncias.

PALAVRAS-CHAVE

Chiroptera; Ectozoocoria; Endozoocoria; Serviço Ambiental; Glomeromycota; Colonização micorrízica.

RESUMEN

Las asociaciones de hongos micorrízicos arbusculares (HMA) y especies vegetales pueden ocurrir en ambientes terrestres y aéreos y requieren una dispersión efectiva, que ocurre principalmente por vectores bióticos. Los estudios sobre la influencia de los animales voladores en esta dispersión son escasos, por lo que este estudio tuvo como objetivo investigar el papel de los murciélagos en la dispersión de propágulos de HMA y los medios de transporte más frecuentes. Se realizaron análisis en pelo dorsoventral y heces de murciélago en un fragmento de bosque tropical urbano en el sur de Brasil, de abril a septiembre de 2018.Los resultados fueron negativos para la presencia de esporas en el pelaje, pero se detectaron esporas de *Glomus invermaium* y *Acaulospora scrobiculata* en heces de dos especies de murciélagos, lo que demuestra que pueden actuar como dispersores secundarios de esporas. La dispersión eficiente depende de la variedad de especies dispersantes, por lo que los murciélagos pueden ser importantes dispersores de HMA, ya que tienen la capacidad de volar y viajar largas distancias.

PALABRAS CLAVE

Chiroptera; Ectozoocoria; Endozoocoria; Servicio Ambiental; Glomeromycota; Colonización micorrízica.

1 INTRODUCTION

Several plant species have associations with different microorganisms that can represent symbiotic interactions (OLDROYD, 2013). Arbuscular mycorrhizal fungi (AMF), examples of these organisms, are part of the Phylum Glomeromycota, are cosmopolitan and occupy different continents and even remote oceanic islands (SAVARY *et al*., 2018).

AMF assist in the uptake of soil nutrients, especially phosphorus and nitrogen, directly influencing plant growth (SMITH; SMITH, 2011) and in tolerance to biotic and abiotic stresses (SMITH; READ, 2008). They are able to colonize new habitats from extraradical mycelium hyphae, colonized roots and spores, single, multinucleated and asexual cells (SMITH; READ, 2008).

AMF sporulation depends on factors such as fungal and host plant identity, soil fertility, temperature, among others (SMITH; READ, 2008), and effective dispersal is essential to achieve a new suitable habitat, including a potential mycorrhizal partner (VAŠUTOVÁ *et al*., 2019).

Associations of AMF and plant species occur mainly in the terrestrial environment, including approximately 80% vascular plants (SMITH; READ, 2008), but may also occur in the air environment associated with epiphytes (CARRENHO *et al*., 2017).

The broad distribution of AMF demonstrates that they have an efficient dispersal strategy (SMITH; READ, 2008). In soil, its natural habitat, the formation of hyphae connections between plants (GORZELAK *et al*., 2015) facilitates the "displacement" of the fungus in the environment, but above ground the mechanisms involved in this process are poorly understood.

The presence of AMF and compatibility between symbionts may be key factors in the development of epiphyte plants (ALGUACIL *et al*., 2019), since in this environment conditions are harsh, with large differences in temperature, water and nutrient limitation (ZOTZ; HIETZ, 2001) and spores, as well as other mycorrhizal propagules, are dispersed from terrestrial to air environment, mostly by biotic vectors (TORRECILLAS *et al*., 2013).

Many mammalian species have terrestrial and arboreal habits and may act as important dispersers of AMF for epiphytes in the understory or canopy (EMMONS; FEER, 1997). Small animals as rodents are the main dispersers (MANGAN; ADLER, 1999), but other mammals, such as marsupials, may also perform this ecological service (VERNES *et al*., 2015).

This vertical transport of AMF can occur in two ways: ectozoochoric, where the propagules attach to the external parts of the animals, such as hair, feathers or legs, or endozoochoric, by accidental consumption of propagules followed by their release in feces (WARNER; FRENCH, 1970). Spores, even after passage through the gastrointestinal tract, may remain viable (TRAPPE; MASER 1976).

In the air environment, AMF are associated with epiphytic species of Piperaceae, Cactaceae and Bromeliaceae (CARRENHO *et al*., 2017), families known to produce fruit consumed by bats, mainly phyllostomids (MARQUES *et al*., 2012). AMF spores are found in these plants on the substrate near the roots and the amount may vary according to plant species (RABATIN *et al*., 1993) and may be transported by animals, wind, or gravity (EGAN *et al*., 2014). Studies on the possible role of flying animals on AMF spore dispersal are scarce and only wasps and birds have been evaluated (MCILVEEN; COLE, 1976; CORREIA *et al*., 2019).

Bats are the only flying mammals and act to maintain ecosystems because they perform different environmental services, such as pollination and seed dispersal (KUNZ *et al.*, 2011). They have the ability to fly and travel short- and long distances, which increase these services (AGUIAR *et al.*, 2014). These animals can also carry non-mycorrhizal, filamentous and yeast-like fungi, including dermatophytes (TENCATE *et al.*, 2012), which has been proven by the presence of spores and hyphae in feces, viscera and nasal fossae (TENCATE *et al.*, 2012; SHAPIRO *et al.*, 2015).

Considering the importance of propagule dispersal for the establishment of symbiosis in epiphyte plants and the diversification of habitats for AMF, the present study tested the following hypothesis: "Due to their high displacement capacity, bats act as vectors in the vertical dispersal of AMF either for carrying spores and hyphae on their dorsoventral hairs or via elimination through feces". Thus, this study aimed to investigate the performance of bats as AMF propagule dispersers and to evaluate the most frequent means of transport (feces/hair).

2 MATERIAL AND METHODS

The study was carried out at Parque do Ingá, located in the urban perimeter of the municipality of Maringá, State of Paraná, southern Brazil, between the parallels 23° 15´and 23° 34´ South latitude and the meridians 51°50´ and 52°06´ West longitude. The Conservation Unit is 47.3 ha area and represents a remnant of Seasonal Semideciduous Forest. The climate of the region is subtropical, with the coldest month average temperature below 18ºC and annual average temperature above 20ºC, with rainy summers and dry winters, and the average annual rainfall varies between 1,500 and 1,600 mm (MARINGÁ, 2011).

Samples were taken fortnightly, from April to September 2018, beginning at dusk and lasting four hours/night. Six mist nets (9 m x 3 m) were set up along the park trails, and under each net, white non-woven fabric (TNT) was extended on the ground to facilitate visualization and collection of feces. The captured bats were carefully taken from the nets with the aid of leather gloves and, soon after, to investigate the presence of AMF propagules in their hair, Con-tact brand shelf liner was rubbed on the back and belly of these animals. Subsequently, the material was mounted on slides for microscopy, which were identified with the corresponding number of each animal. If spores were detected, these were transferred to other slide previously prepared with PVLG resin, using the end of a needle, under a dissecting microscope (MORTON *et al.*, 1993). Therefore, spores were evaluated with light microscopy for identification.

Feces samples were taken in two ways: from material collected in the TNT from defecation at the time of capture, and from material excreted by captured animals, which were kept in cotton bags for one hour. After that, the biometric data collection and taxonomic identification of the animals were performed.

Fecal samples were collected and packed in Eppendorf vials and, in the laboratory, were diluted in 50 mL distilled water and from the combination of wet sieving and sucrose centrifugation techniques (GERDEMANN; NICOLSON, 1963), to enable the extraction of spores of AMF.

After extraction, all supernatant was poured into a Petri dish and analyzed under a stereomicroscope. Upon finding the presence of hyphae and spore-like structures, these were separated and mounted on semi-permanent slides, in the mounting media, PVLG (MORTON *et al*., 1993). These were evaluated under light microscopy and identified to the species level based on Schenck and Pérez (1988), the original descriptions, and those provided by the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi website (INVAM, 2019).

Studies were conducted under permanent permit to collect zoological material from the Chico Mendes Institute for Biodiversity Conservation - ICMBio (number: 17869-3) and certification of the Ethics Committee for Animal Use (CEUA) of the State University of Maringá (number: 8700100516).

3 RESULTS

In total, 107 bat individuals belonging to six species were caught. Of these, five species are preferentially frugivorous belonging to the family Phyllostomidae and one insectivorous, of the family Vespertilionidae (Table 1). A total of 321 samples were taken and analyzed, 107 from ventral hair, 107 from dorsal hair and 107 from feces.

Table 1 – Bat species, number (N) and relative percentage (%) of catches in an urban tropical forest fragment

	N	%
Family Phyllostomidae		
Artibeus lituratus (Olfers, 1818)	64	59.8
Sturnira lilium (E. Geoffroy, 1810)	34	31.8
Platyrrhinus lineatus (E. Geoffroy, 1810)	04	3.7
Pygoderma bilabiatum (Wagner, 1843)	02	1.9
Carollia perspicillata (Linnaeus, 1758)	01	0.9
Family Vespertillionidae		
Myotis nigricans (Schinz, 1821)	02	1.9
Total	107	100

Source: Research data.

All results were negative for the presence of AMF propagules in bat hair, both belly and back hair. Only one spore was detected in feces of *A. lituratus*, belonging to *Glomus invermaium*, as well as in feces of *Myotis nigricans*, in which *Acaulospora scrobiculata* was verified.

Given the extremely rare occurrence of spores, no statistical analysis was applied in this study.

4 DISCUSSION

The presence of spores in feces of two bat species, *A. lituratus* and *M. nigricans,* shows that Chiroptera animals act as AMF spore dispersers, confirming the hypothesis of this study. However, our findings evidence bats play a limited role for this ecosystem service. Studies seeking to investigate the dispersal of mycorrhizal fungal spores by animals are rare, and so far, there are no studies addressing the bat/AMF interaction, although these mammals are important seed and pollen dispersers (KUNZ *et al*., 2011). Vašutová and collaborators (2019) analyzed 33 studies on the topic, which included invertebrates, vertebrates and ectomycorrhizal and endomycorrhizal fungi, and mostly addressed the interaction of Rodentia with ectomycorrhizal fungi. This is then the first report of chiropterans as AMF spore dispersers.

Most vertebrates, including other mammals such as marsupials, rodents, ungulates, carnivores, and primates (CLARIDGE; TRAPPE, 2005), may be classified as preferential, opportunistic or accidental fungivorous (CLARIDGE; TRAPPE, 2005) and play an important role in the dispersal of mycorrhizal fungi (VAŠUTOVÁ *et al*., 2019). Nevertheless, there is no record of any bat species that includes fungi in the diet, however, our results show that two species, *A. lituratus* and *M. nigricans*, had contact with AMF, since spores were found in their feces.

Myotis nigricans is an insectivorous bat consuming insects of the orders: Ephemeroptera (REIS; PERACCHI, 1987), Diptera (REIS; PERACCHI, 1987; REIS *et al*., 1999), Coleoptera (REIS; PERACCHI, 1987; REIS *et al*., 1999), Lepidoptera (REIS *et al*., 1999) and arthropods of the order Araneae (NORA; CHAVES, 2006), and found in both well-preserved and disturbed environments (REIS; PERACCHI, 1987).

With the ingestion of different species of insects, *M. nigricans* can eventually ingest spores of ectomycorrhizal and endomycorrhizal fungi, as several Diptera species (ŠEVČÍK, 2010), for example, use the sporocarps as food and place for the development of their larvae and studies prove the presence of viable spores in the intestines of these insects (LILLESKOV; BRUNS, 2005) and their larvae (KITABAYASHI *et al*., 2016). Houston and Bogher (2010) observed viable spores in the feces of ten Coleoptera species of the genera *Blackbolbus* and *Bolborhachium*, and Rabatin and Stinner (1988) found spores in the intestines of beetles of the families Carabidae and Scarabaeidae.

Lilleskov and Bruns, (2005) suggest that mycorrhizal fungi spores run through food webs and remain able to originate new individuals, as they found viable spores in the intestine of insects and also their predators, such as centipedes and salamanders. The same was observed by Trappe and Claridge (2005), who considered that *Strix occidentalis caurina* owls can disperse fungal spores over long distances as they feed on fungus-eating squirrels. Thus, it is possible that, in the present study, *M. nigricans* consumed an insect that had previously ingested AMF spores, including *Acaulospora scrobiculata*, and thus acted as a secondary disperser.

Artibeus lituratus also presented AMF spores in its feces. It is a frugivorous bat consuming fruits of a large number of species and is present in varied environments, both preserved and under strong human influence, including urban environments (REIS *et al*., 2017). There are also data demonstrating that the species is a seed disperser of most of the plants it uses (REIS; PERACCHI, 1987; FÁBIÁN *et al*., 2008). Approximately 80% vascular plants associate with mycorrhizal fungi (SMITH; READ, 2008), so many of the plants used by *A. lituratus* are included in this interaction. Correia and collaborators (2019) reported for the first time that forest birds can co-disperse viable seeds and AMF spores, showing a mechanism not yet considered for the establishment of mycorrhizal associations. Nevertheless, studies addressing this theme are required to confirm the co-dispersion by bats.

The dispersal distance is very important for the survival of AMF and it depends on the home range and displacement of the dispersing species. Small animals make this transport over relatively short distances, but larger animals, especially those generalists in habitat use, can carry spores over long distances (VAŠUTOVÁ *et al*., 2019). Chiropterans, because of their ability to fly, can travel long distances. Large phylostomids such as *A. lituratus, Artibeus fimbriatus* (Gray, 1838), *Artibeus obscurus* (Schinz, 1821) and *Phyllostomus hastatus* (Pallas, 1767) can travel up to about 12 km/month (ARNONE *et al*., 2016), a capacity with great importance to the dispersal of fruits and seeds and also to that of AMF, as the present study indicates.

The low number of AMF spores found in feces can be explained once direct detection is difficult, since the number of spores accidentally ingested by bats is probably low and AMF spores rarely appear in large groups and in many cases, they are heterogeneously distributed in the environment (SMITH; READ, 2008).

Bats can use natural shelters such as caves, large leaves or tree hollows, as well as artificial, abandoned buildings, basements, attics and bridges (MORATELLI; CALISHER, 2015), where they may have contact with AMF spores and these attach to their hair, however, despite the capture effort, it was not possible to detect the presence of spores in the dorsoventral hair of the studied bats. Correia and collaborators (2019) also found no spores in feet and feathers of birds and suggested that AMF species that form ornamented spores might be more susceptible to attachment to feathers and feet of animals. For the present study, depending on the location and degree of attachment of spores in the hair, their removal with the adhesive tape may not have been adequate, thus generating a false negative result.

Further, the study site comprised an urban park, in which there are no studies on mycorrhizal composition, and which presents modified soil, characterized by decreased mycorrhizal and fungal diversity (NEWBOUND *et al*., 2010). Such information corroborates Cousins and collaborators (2003), who observed this characteristic in soil of non-vegetated urban plots and Bainard and collaborators (2011), who compared mycorrhizal colonization of urban and forest trees and noticed a reduction in the former. Thus, AMF spore dispersal by bats in an anthropized area becomes significant for the establishment of these fungi. Nonetheless, because it is the first report and the small number of spores found, it is necessary to conduct more samplings in order to determine the real relevance of these animals and their interaction with the AMF.

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