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Article

Food preference of *Phylloicus* sp. (Insecta: Trichoptera): experimental study with plant species from the Cerrado

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Abstract: The aquatic insects of the genus *Phylloicus* play a crucial role in aquatic ecosystems, shredding leaves and contributing to nutrient cycling in streams. Therefore, this genus is often used in laboratory experiments. However, in Cerrado regions such as Araguaia, these studies are impractical due to a lack of knowledge about basic aspects, such as their feeding preferences for local plants. Hence, our objective is to determine the native plant species in the Araguaia region preferred as food by *Phylloicus*. We conducted an experimental study comparing the consumption of three native Cerrado plant species: *Casearia sylvestris*, *Astronium fraxinifolium*, and *Ficus guaranítica* by *Phylloicus*. To assess differences in consumption, we performed an Analysis of Variance. The results revealed that *Phylloicus* larvae exhibited a feeding preference for *Casearia sylvestris* ($F = 9.71$; $p = 0.004$). This finding will contribute to the development of future experimental studies using *Phylloicus* in the Araguaia region, as understanding the feeding preferences of animals used in experiments is essential for their maintenance in the laboratory.
Keywords: *Decomposition; Aquatic detritus; Shredders; Aquatic invertebrates; Streams.*

Preferência alimentar de *Phylloicus* sp. (Insecta: Trichoptera): trabalho experimental com espécies vegetais do Cerrado

Resumo: Os insetos aquáticos do gênero *Phylloicus* desempenham um papel essencial nos ecossistemas aquáticos, fragmentando folhas e contribuindo para a ciclagem de nutrientes nos riachos. Por isso, esse gênero é frequentemente utilizado em experimentos de laboratório. No entanto, em regiões de Cerrado como o Araguaia, esses trabalhos são inviáveis por não se conhecer aspectos básicos como a sua preferência alimentar por plantas locais. Por isso, nosso objetivo é responder quais são as espécies vegetais nativas da região do Araguaia preferidas para a alimentação de *Phylloicus*. Fizemos um trabalho experimental comparando o consumo de três espécies vegetais nativas do Cerrado: *Casearia sylvestris*, *Astronium fraxinifolium* e *Ficus guaranítica* pelos *Phylloicus*. Para avaliar as diferenças no consumo, realizamos uma Análise de Variância. Os resultados obtidos revelaram que as larvas de *Phylloicus* demonstraram preferência alimentar por *Casearia sylvestris* ($F = 9.71$; $p = 0.004$). Esse achado ajudará no desenvolvimento de futuros trabalhos experimentais utilizando *Phylloicus* na região do Araguaia, uma vez que é essencial o conhecimento da preferência alimentar dos animais utilizados nos experimentos para sua manutenção em laboratório.
Palavras-chave: *Decomposição; Detritos aquáticos; Fragmentadores; Invertebrados aquáticos; Riachos.*

Introduction

Aquatic environments are systems characterized by intricate interspecific interactions, promoting a range of ecosystem services (Ferreira et al. 2023). In this context, shredder aquatic insects play a fundamental role in aquatic ecosystems by directly contributing to the organic matter decomposition process (Graça 2001). Shredder aquatic insects are responsible for breaking down and processing leaves and other organic debris into smaller sizes, facilitating the action of decomposing microorganisms and enabling nutrient cycling in the ecosystem more rapidly and efficiently (Wantzen et al. 2002). Consequently, these shredder invertebrates are key components in these ecosystems, as they consume organic matter and serve as a food source for other aquatic predator organisms (Carvalho & Uieda 2009).

The conversion of natural areas into agroecosystems has led to severe impacts on aquatic ecosystems (Malmqvist & Runde 2002), and one of the reasons is related to the alteration in the availability and quality of organic matter (allochthonous energy), which serves as a food source for many aquatic species (Graça 2001). Consequently, deforestation modifies the availability of allochthonous food resources in streams, resulting in a decline in groups of shredder aquatic insects. This phenomenon has been documented in both temperate climate streams (Fenoy et al. 2021) and tropical climate streams, such as in the Amazon (Lima et al. 2022) and the Cerrado (Brasil et al. 2013). This issue underscores the importance of studies to enhance our understanding of how different types of environmental impacts can affect the ecosystem service of decomposition provided by invertebrates (Prather et al. 2013).

The feeding preference of shredder aquatic insects is closely associated with leaf quality, which is measured by physical characteristics such as hardness and chemical properties like nutritional content (Gessner & Chauvet 1994, Hladyz et al. 2009). In the Brazilian Cerrado, a significant portion of streams is surrounded by Gallery Forest, crucial for biodiversity conservation, supplying 60% to 80% of the energy demand in these lotic systems (Bambi et al. 2017). In the Gallery Forest vegetation of the Cerrado, some species have low-quality leaves with high concentrations of lignin, cellulose, tannins, and polyphenols (De Sousa-Neto, Lins & Martins 2017, Rezende, Leite, & Ramos 2018), making consumption by shredders challenging (Rezende et al. 2021). To date, only seven articles have conducted experimental studies using shredder aquatic insects from Cerrado streams in laboratories (Ferreira et al. 2015, Rezende, Leite, & Ramos 2018, Reis et al. 2018, Moretti & Santos 2019, Sena et al. 2020, Rezende 2021). It is known that the genera *Phylloicus* (Mueller 1880), *Tipula* (Linnaeus 1758), *Simulium* (Latreille 1802), and *Chironomus* (Meigen 1803) can be used for such purposes. Additionally, plant species used in these studies with greater success were *Maprounea guianensis* (Candolle, A. P 1807), *Inga laurina* (Willdenow, C. L 1806), *Miconia leucopaca* DC. (Candolle A. P 1828), and *Richeria grandis* (Martius 1832). However, all these studies were conducted in the central plateau region of Brazil, and it is known that due to variations in soil and altitude, the woody vegetation in this region differs from that found in other Cerrado regions, such as the Araguaia region located further north, closer to the transition with the Amazon Biome.

Given the variations in vegetation across the Cerrado and recognizing that the few articles conducting laboratory experiments with shredder aquatic insects in the Cerrado were situated in the central plateau region, we aim to investigate native plant species from the eastern region of the Brazilian Cerrado (Araguaia region). Our objective is to identify the preferred food sources of *Phylloicus* and understand how these preferences may differ compared to findings from more central regions. This study seeks to fill knowledge gaps regarding the feeding ecology of these invertebrates in the Araguaia region, contributing to a comprehensive understanding of dietary patterns of these organisms throughout the Cerrado.

Material and Methods

1. Sample, transportation, and acclimatization of Phylloicus

The *Phylloicus* specimens were collected from first and second-order streams located in the Córrego Fundo watershed, a tributary on the left bank of the Garças River (latitude coordinates –15.782 and longitude –52.311), situated in the municipality of Barra do Garças, in the eastern region of the state of Mato Grosso. The stream is located within the Cerrado Biome, characterized by a tropical climate with two distinct seasons: a rainy season from October to April and a dry season from May to September.

The *Phylloicus* larvae were manually collected through active search and with the aid of an aquatic entomological net. Subsequently, the larvae were placed in a styrofoam box containing a battery-operated aquarium aerator for transportation from the field to the laboratory. Within the box, leaves, a portion of sand, and stream water were added to help acclimate the larvae during transportation. In the laboratory, the samples were transferred to a container with fresh, clean, and oxygenated water, maintained under conditions similar to the natural environment, allowing the larvae to adjust to the new conditions. This period, known as the laboratory acclimatization phase, lasted for seven days. The biological material (*Phylloicus sp.*) used in the study was identified according to Prather (2003).

2. Leaf selection and incubation

During the reconnaissance period of the stream where the *Phylloicus* specimens were collected, the field team identified three plant species abundant in the gallery forest that were undergoing leaf loss. The three selected species, based on the aforementioned criteria, were identified by the curator of the Herbarium at the Federal University of Mato Grosso, Araguaia campus.

Thus, the chosen species were *Casearia sylvestris* Swartz, *Astronium fraxinifolium* Schott, and *Ficus guaranitica* Schodat (Figure 1). *Casearia sylvestris* Swartz belongs to the Salicaceae family and is abundant in the tropical and subtropical regions of the Americas. It is found from Mexico to the Antilles and extends southwards to Paraguay and Argentina (Carvalho 2007). In Brazil, this species is present in nearly all biomes and in various vegetation formations, including riverine or riparian environments (Carvalho 2007). The crude protein content of the leaves of this plant is 13%, and the lignin content with ash is 25.61% (Carvalho 2007).

Astronium fraxinifolium Schott, belonging to the Anacardiaceae family, is a tree species found in the Cerrado, Atlantic Forest, Amazon Rainforest, Caatinga, and Pantanal. This species is not endemic to Brazil, as it is also present in other South American countries with the Cerrado, Amazon, and Atlantic Forest biomes (Silva-Luz et al. 2023). Specimens of this family are rich in secondary compounds, with phenolic lipids, flavonoids, and triterpenes being typical metabolites (Correia, David, & David 2006).

Ficus guaranitica Schodat belongs to the Moraceae family and is considered by Berg & Villavicencio (2004) to be synonymous with *F. citrifolia*. Its geographical distribution is confirmed for the North region (Acre, Amazonas, Amapá, Pará, Rondônia, Roraima), Midwest (Goiás, Mato Grosso do Sul, Mato Grosso), Southeast (Minas Gerais, Rio de Janeiro, São Paulo), and South (Paraná, Rio Grande do Sul, Santa

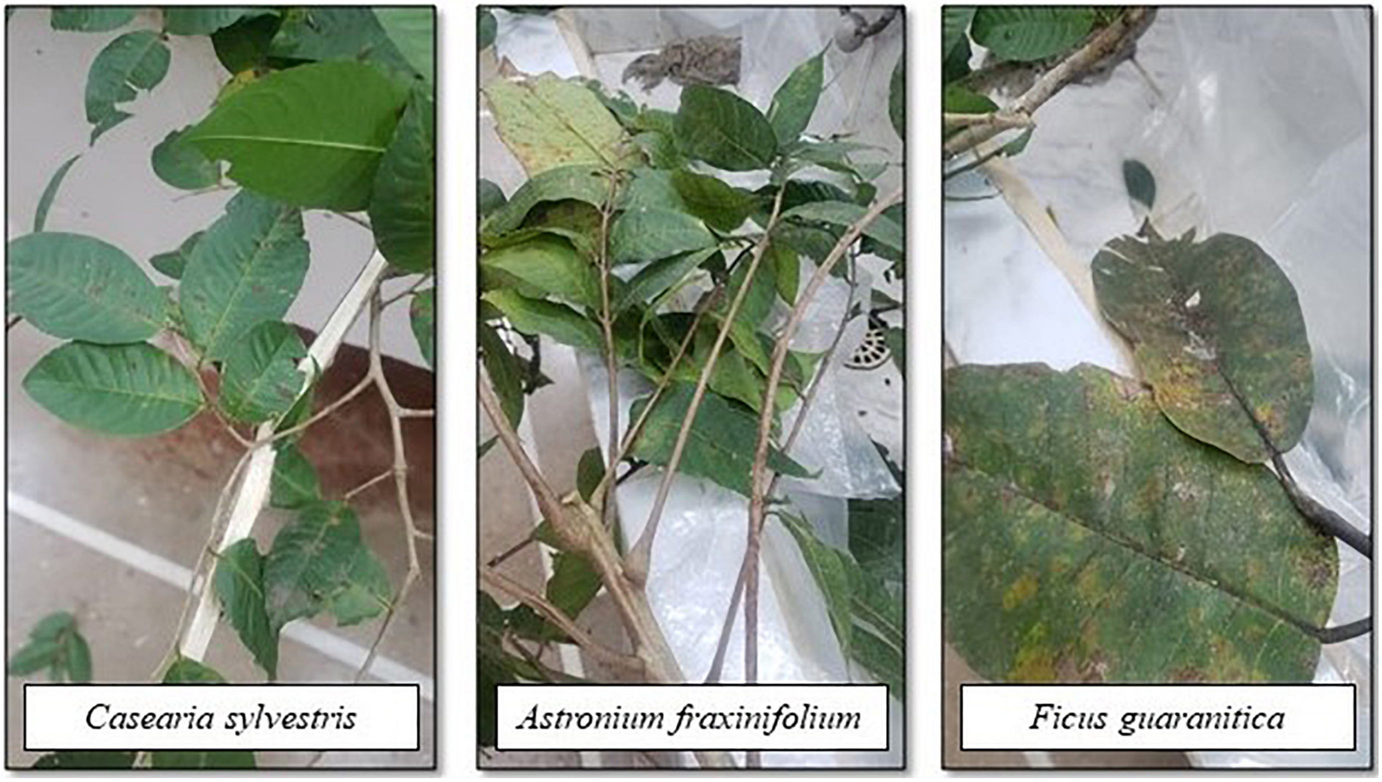

Figure 1. Cerrado plant species tested in the experiment of leaf consumption by *Phylloicus* sp.: *Casearia sylvestris*, *Astronium fraxinifolium* and *Ficus guaranitica*.

Catarina) of Brazil. Similarly, it is not endemic to Brazil, as it is also found in other South American countries with the Cerrado, Amazon, and Atlantic Forest biomes (Perdeneiras, Machado & Santos 2023).

Following this preliminary identification, leaves from the three species were collected in the gallery forest of the same region where the *Phylloicus* specimens were collected. Senescent leaves, free from apparent damage, were selected. Prior to the start of the experiment, the leaves were placed in fine-mesh bags (litter bags) and submerged in the stream for seven days, the same period used for larval acclimatization in the laboratory. This incubation period is necessary for leaching and colonization of the leaves by microorganisms, forming a biofilm associated with the leaves. In the laboratory, the leaves were cut to obtain 1.4 cm diameter discs, which were used to feed the larvae during the experimental period. The cut was made to avoid the major leaf veins (Graça et al. 2001).

3. *Leaf consumption*

For the experiment, plastic aquariums (height = 09 cm; diameter = 15 cm; volume = 1L) were used, containing calcined sand (height ~1 cm) and water from the stream where the *Phylloicus* were captured. Aquariums were prepared with and without the presence of shredder larvae (*Phylloicus*). In the first case, the treatments were used to determine the total consumption (shredder consumption + microbial consumption) of each leaf species. In the second case, the control aimed to determine microbial consumption and adjust leaf consumption by shredders (shredder consumption = total consumption – microbial consumption).

To determine consumption, leaf discs were dehydrated and weighed on a precision scale before and after the experiment. For the determination of dry mass, the leaf discs were dried in a climate-controlled room with relative air humidity below 20%. This humidity level is common in the study region during the dry season, which is when the experiment took place. To establish the time needed for the discs to reach a constant weight, three discs of each species were weighed on a precision scale (0.000g) every hour for seven hours. From the fourth hour (fourth weighing), the weight stabilized and remained constant. Thus, four hours were considered the minimum time needed for the dehydration of leaf discs to achieve stabilized weight, considering the precision scale used. Nonetheless, to minimize any potential weighing and drying biases, the leaf discs were weighed before and after exposure after seven hours of drying under the aforementioned conditions.

Thus, total consumption was determined in the aquariums containing shredders and calculated by dividing the ingested leaf mass (initial mass – final mass) by the survival period of the individuals, in days. Microbial consumption was determined by dividing the leaf mass ingested by microorganisms (initial mass – final mass) in the aquariums without shredders by the duration of the experiment. Shredder consumption was determined by the difference between total consumption and microbial consumption (Martins et al. 2017).

4. *Experimental design of the study*

For the experiment, 15 individuals of *Phylloicus* were used, collected from the wild and acclimated. After acclimatization, all specimens were individually placed in plastic aquariums with a water

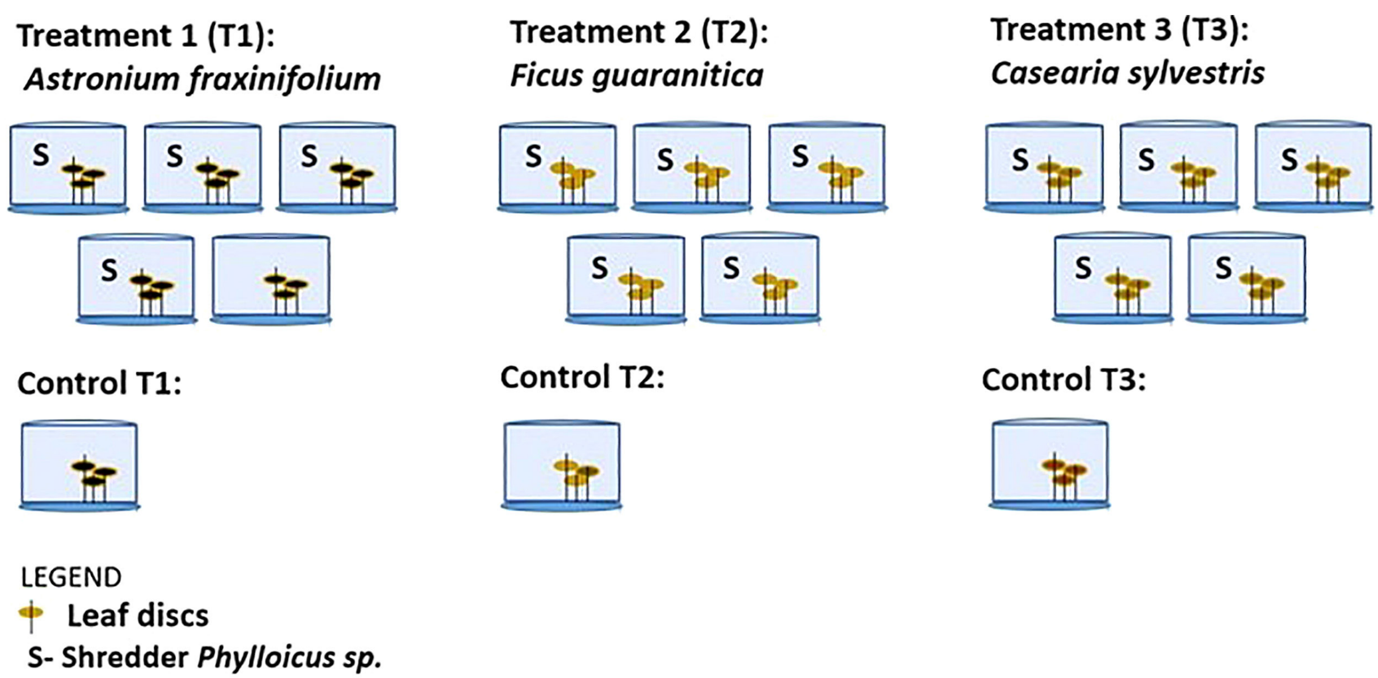

Figure 2. Experimental design set up to evaluate the leaf consumption by *Phylloicus* sp. on three Cerrado plant species (leaves). In treatment 1, leaves of the species *Casearia sylvestris* were provided; in treatment 2, leaves of the species *Astronium fraxinifolium* were provided; in treatment 3, leaves of the species *Ficus guaranitica* were provided. Control = one aquarium for each leaf species and without shredder. S = *Phylloicus* sp. shredder.

Figure 3. Images of some stages of the process: A and B refer to acclimatization in the laboratory. C refers to the incubation period of the leaves in the stream. D refers to the cutting and drying of leaf discs. E and F refer to the assembly of the experiments. G refers to an assembled aquarium and H is an image of a leaf fragmented by *Phylloicus*.

capacity of 1L. At the beginning of the experiment, three leaf discs were provided as food for the *Phylloicus*, replaced every five days. To standardize the size of the individuals used in the experiment, a visual selection was made, avoiding the inclusion of very large individuals, those close to emergence, or very young and fragile ones.

The aquariums were randomly divided into three groups: Group A (T1), consisting of five aquariums, each receiving three leaf discs of the plant species *Casearia sylvestris* Sw. and one *Phylloicus* in each; Group B (T2), comprising five aquariums, each receiving three leaf discs of the plant species *Astronium fraxinifolium* and one *Phylloicus*; Group C (T3), composed of five aquariums, each receiving three leaf discs of the plant species *Ficus guaranitica* and one *Phylloicus*. As a control, an aquarium containing only the plant species, without the shredder, was added to each treatments (Figure 2 and 3).

5. Data analysis

This approach allows us to assess larval preference based on the quantity of leaves consumed for each exposed plant species. To randomize the treatments in the aquariums, the sample function in the R program was employed. At the end of the experiment, we obtained 15 sample units distributed across three treatments (leaf A, B, and C). To assess which plant species was most consumed under these conditions, we conducted a one-way Analysis of Variance (ANOVA), considering the quantity of consumed leaves as the response variable and the three exposed leaf types as the categorical predictor variable. Subsequently, the Tukey test was employed to pairwise compare each combination of plant species: A and B, A and C, B and C.

Results

1. Phylloicus survival

There was no mortality of *Phylloicus* individuals during the experiment period (11 days). However, one individual transformed into a pupa on the sixth day, shortly after the first weighing, and two other individuals transformed into pupae on the eleventh day, after the second weighing. This information was considered to adjust the final value of consumption for the time during which the individuals were in conditions to perform fragmentation, avoiding any damage or bias to the results.

2. Leaf consumption

The leaf consumption of *Casearia sylvestris* by *Phylloicus* was 0.0208 ± 0.003 (mean ± standard deviation) mg/day, the consumption of *Ficus guaranitica* was 0.012 ± 0.006 mg/day, and the consumption of *Astronium fraxinifolium* was 0.008 ± 0.0009 mg/day. There was a significant difference in leaf consumption comparing the three plant species (ANOVA: $F_{2,15} = 9.75$; $p = 0.004$) (Figure 4; Table 1), with the highest consumption observed in *Casearia sylvestris*, significantly different from *Ficus guaranitica* ($p = 0.012$) and *Astronium fraxinifolium* ($p = 0.007$). However, the consumption of *Ficus guaranitica* and *Astronium fraxinifolium* did not differ ($p = 0.961$; Table 1). The assumptions of variance homogeneity (Levene Test; $F = 2.656$; $p = 0.118$) and data normality (Shapiro Test; $W = 0.965$; $p = 0.836$) were met for the ANOVA.

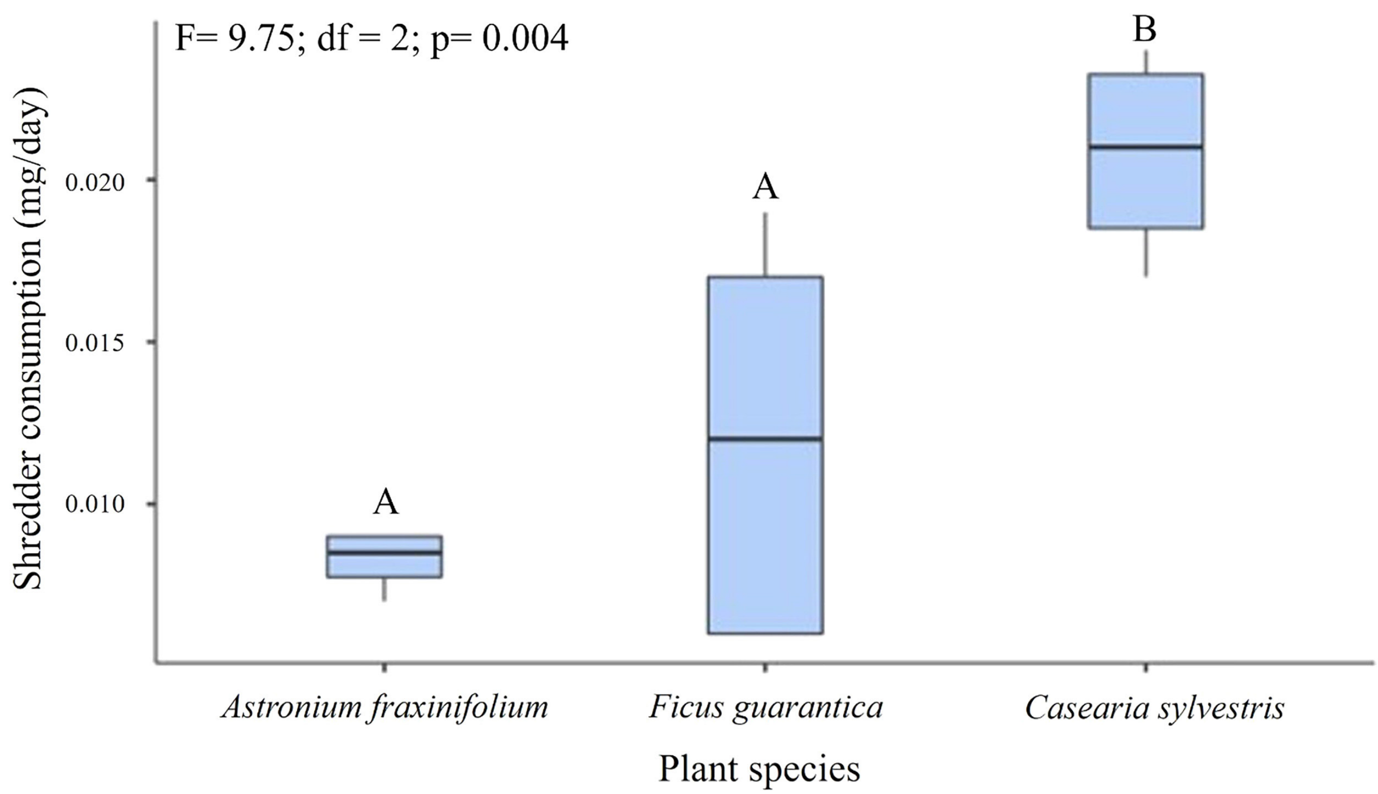

Figure 4. Leaf consumption of three species of Cerrado plants, *Astronium fraxinifolium*, *Ficus guaranitica*, and *Casearia sylvestris* by the shredder *Phylloicus* sp.

Table 1. Analysis of Variance (ANOVA) results for leaf consumption of the three plant species by *Phylloicus* sp., including Tukey's test results.

	ANOVA			Tukey		
	F	**GL**	**P-valor**	***Casearia sylvestris***	***Astronium fraxinifolium***	***Ficus guaranitica***
Leaf consumption	9.75	2	0.004	0.0208 ± 0.003 **(a)**	0.008 ± 0.0009 **(b)**	0.012 ± 0.006 **(b)**

F = F-statistic value; GL = Degrees of freedom; P-valor = statistical significance; a, b, c = pairwise comparison results.

Discussion

The lack of knowledge about basic aspects of species biology is a barrier that hinders scientific development (Hortal et al. 2015). For instance, simple questions such as diets are still poorly understood for several species (Freitas et al. 2021). These knowledge gaps impede or delay the progress of studies that rely on the maintenance of live animals in the laboratory. Here, we found that *Casearia sylvestris* is a plant species that stands out as a good option for feeding *Phylloicus* in laboratory experiments.

The feeding preference of aquatic shredder insects is closely associated with the quality of leaves (Gessner & Chauvet 1994, Hladyz et al. 2009). Although little is known about the physical and nutritional characteristics of the studied species, it is acknowledged that the crude protein content (13%) in the leaves of *Casearia sylvestris* is suitable for cattle, and the species is an important forage plant in the Pantanal (Carvalho 2007). This may indicate the nutritional quality of the leaves of this plant species and justify the preference by the studied shredder. However, as these are distinct organisms, studies assessing the nutritional and physical quality of this plant are essential for making more robust inferences in the future.

On the other hand, the species *Astronium fraxinifolium*, which was not preferred by *Phylloicus*, is rich in secondary compounds and belongs to the Rhoeae tribe (Carmello-Guerreiro & Paoli 2000), one of the three tribes of Anacardiaceae known to have toxic species (Correia et al. 2006). Secondary compounds are generally produced by plants as a defense mechanism. There are reports of the toxic effect of extracts from organs of *Astronium* species (e.g., *Astronium urundeuva*) on larvae (Napoleão et al. 2012; Souza et al. 2012, Souza et al. 2015), pupae, and eggs of other insects (Souza et al. 2012). Studies evaluating the effect of extracts from parts of *Astronium fraxinifolium* have demonstrated antifungal activity (Bonifácio et al. 2015) and larvicidal properties (Santos & Mesquita 2018). Thus, the low levels of leaf consumption by *Phylloicus* larvae for this species may be associated with the presence of these toxic compounds.

We did not find literature mentioning studies on leaf composition or toxicity of *Ficus guaranitica* specifically. However, it is known that the genus has latex that serves as a defense to inhibit other organisms and is traditionally used as a vermifuge in South America (Hansson et al. 1986). Analyses conducted with *Ficus insipida* have already demonstrated its potential as an anthelmintic (Gonzales, Santos, & Tavares-Dias 2019). Furthermore, a comprehensive review of the pharmacological literature on the Genus *Ficus* highlighted its cytotoxic and antimicrobial action (Salehi et al. 2021). Therefore, we believe that the lower consumption of *Ficus guaranitica* by *Phylloicus* may be related to these chemical defense characteristics present in its genus.

Although the information obtained in this study is modest, it has already opened horizons, laying the groundwork for the development of new experimental work involving fragmenters of the genus *Phylloicus* and the ecosystem services they perform. A good example would be the use of these animals as models for laboratory studies on the effects of climate change and land use on aquatic ecosystems in the Middle Araguaia River region.

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Leandro Schlemmer Brasil: Conceptualization; Data curation; Formal Analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Writing – original draft; Writing – review & editing.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

Ethics

This study did not involve human subjects or clinical trials which require authorization by an Institutional Committee.

Data Availability

The datasets generated during and/or analyzed during the current study are available at: https://doi.org/10.48331/scielodata.YWZWC9

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