Universidade Federal de Uberlândia

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Seasonality and extrafloral nectaries shape plant–spider networks

Philip Brian J. Hall

2026

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Dissertação apresentada à Universidade Federal de Uberlândia, como parte das exigências para obtenção o título de Mestre em Ecologia e Conservação de Recursos Naturais".

Orientadora

Profa. Dra. Vanessa Stefani Sul Moreira

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APROVADO em 29 de Julho de 2026

Profa. Dra. Denise Lange	UTFPR
Prof. Dr. Sérgio Hayato Seike	IFTM

Profa. Dra. Vanessa Stefani Sul Moreira

UFU

(Orientadora)

UBERLÂNDIA

Julho – 2026

Dedicado à Edwin Kent Hall, obrigado por me ensinar a cultivar minha curiosidade pelo mundo e por sempre me incentivar a buscar novos aprendizados.

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Índice

RESUMO

Nectários extraflorais (NEFs) são características vegetais fundamentais que mediam interações multitróficas, contudo seu papel na estruturação de comunidades de plantas e aranhas em nível de rede permanece pouco compreendido. Aqui, fornecemos a primeira análise empírica de redes de interações entre plantas e aranhas no Cerrado brasileiro, avaliando como os NEFs e a sazonalidade climática moldam a arquitetura das interações e a composição da comunidade. De outubro de 2020 a setembro de 2021, quantificamos as ocorrências de aranhas em plantas com flores com e sem NEFs ao longo das estações seca e chuvosa e construímos redes quantitativas bipartidas de interações. Analisamos conectância, aninhamento, modularidade e especialização, comparando os valores observados com as expectativas de modelos nulos. As redes de plantas e aranhas apresentaram estruturas altamente não aleatórias, com plantas portadoras de NEFs promovendo menor especialização e modularidade significativa ao longo das estações. Contrariamente às expectativas, a conectância foi maior durante a estação seca, enquanto os modelos nulos revelaram que restrições biológicas limitaram a realização das interações mesmo sob alta disponibilidade de recursos. Mudanças sazonais alteraram fortemente a topologia da rede, mas não afetaram a composição das espécies de aranhas, que permaneceu estável entre os tipos de plantas e as estações. Nossos resultados indicam que os NEFs atuam como elementos estruturais centrais que reorganizam os padrões de interação sem promover substituição de espécies, destacando a importância das interações facultativas e da flexibilidade comportamental na manutenção da estabilidade da comunidade em ecossistemas de savana sazonais.

Palavras-chave: Redes Ecológicas; Aranhas; Nectários Extraflorais; Ecologia de Comunidades.

ABSTRACT

Extrafloral nectaries (EFNs) are key plant traits mediating multitrophic interactions, yet their role in structuring plant–spider communities at the network level remains poorly understood. Here, we provide the first empirical network analysis of plant–spider interactions in the Brazilian Cerrado, evaluating how EFNs and climatic seasonality shape interaction architecture and community composition. From October 2020 to September 2021, we quantified spider occurrences on flowering plants with and without EFNs across dry and rainy seasons and constructed quantitative bipartite interaction networks. We analyzed connectance, nestedness, modularity, and specialization, comparing observed values against null model expectations. Plant–spider networks exhibited highly non-random structures, with EFN-bearing plants promoting lower specialization and significant modularity across seasons. Contrary to expectations, connectance was higher during the dry season, while null models revealed that biological constraints limited interaction realization even under high resource availability. Seasonal shifts strongly altered network topology but did not affect spider species composition, which remained stable across plant types and seasons. Our results indicate that EFNs act as structural hubs that reorganize interaction patterns without driving species turnover, highlighting the importance of facultative interactions and behavioral flexibility in maintaining community stability in seasonal savanna ecosystems.

Keywords: Ecological Networks; Spiders; Extra-floral Nectaries; Community Ecology.

INTRODUCTION

In natural communities, the connections among species extend beyond simple predator–prey dynamics. Plants, spiders, and other arthropods interact through mutualistic, antagonistic, and commensal relationships, and resources such as extrafloral nectaries (EFNs) mediate many of these interactions (De Oliveira Dias and Stefani 2024; Gonçalves-Souza et al. 2008; Bronstein 1994). Analyzing these interactions through ecological networks provides a comprehensive view of how species coexist and influence one another within these complex systems (Bascompte 2009; Pascual and Dunne 2006). Understanding how species interact and how these interactions shape the structure and functioning of ecological communities is a central goal in ecology (Montoya et al. 2006; Bascompte 2009). From the early recognition of food chains and specific pairwise interactions, such as those between plants and spiders, to the development of complex network theory, ecologists have increasingly sought integrative ways to represent and study the relationships that underpin biodiversity (Bascompte 2007).

Spiders are widespread across terrestrial ecosystems and occur in environments ranging from deserts to caves and high-elevation habitats (Santos et al. 2017). Spiders are abundant arthropods in vegetation that interact with plants in several ways, most prominently as predators of plant-associated arthropods, but also through facultative associations involving plant-provided resources (Romero and Vasconcellos-Neto 2005; Nyffeler and Birkhofer 2017). Plants therefore play a fundamental role for spiders: they provide support for webs (Vasconcellos-Neto et al. 2017), shelter from predators (Romero and Vasconcellos-Neto 2005; Uetz 1991), sites for reproduction (Vasconcellos-Neto et al. 2017), and nesting sites for egg protection (Romero and Vasconcellos-Neto 2005). In addition, plants provide food resources both indirectly, through herbivorous prey (Romero and Vasconcellos-Neto 2003; Nyffeler and Birkhofer 2017), and directly, for example through nectar produced by EFNs (Nahas et al. 2017).

Extrafloral nectaries are plant structures that produce carbohydrate-rich nectar (Koptur 1994) and are central to indirect defense strategies against herbivory (González-Teuber and Heil 2009; Stefani et al. 2015; De Oliveira Dias and Stefani 2024). Although classically associated with mutualisms involving ants, which defend plants in exchange for food (Del-Claro et al. 1996; Nascimento and Del-Claro 2010), spiders also utilize EFNs. Typically known as predators in these systems (Nyffeler and Birkhofer 2017), some spiders complement their diet by feeding directly on extrafloral nectar (Nahas et al. 2017). By obtaining resources from plants, spiders can also function as defensive partners, contributing to reduced herbivory and, in some systems, increased plant productivity and seed production (Ruhren and Handel 1999; Stefani et al. 2015; Silva et al. 2020; De Oliveira Dias and Stefani 2024). Because EFNs are used by several groups of arthropods, plants bearing these structures may become an important focus of interactions within ecological communities (Marazzi et al. 2013).

EFN-bearing plants may therefore concentrate interactions by providing a relatively predictable food resource to plant-associated arthropods (Guimarães Jr. et al. 2007; da Silva Filho et al. 2024). This system, involving multiple plant and spider species, forms a complex ecological network that requires approaches capable of assessing patterns beyond individual pairwise associations. Network approaches provide a framework for representing species as nodes and their associations as links, allowing patterns to be examined from individual interactions to entire communities (Delmas et al. 2019). Because persistent resources can influence the distribution of interactions within ecological networks (Olesen et al. 2008; Dáttilo et al. 2014), EFNs may alter the degree to which interactions are integrated, specialized, nested, or compartmentalized across the plant–spider community.

Quantitative interaction matrices provide a means of representing these associations and evaluating their emergent organization at the community level (Bascompte and Jordano 2007; Delmas et al. 2019). These networks can be quantified using topological metrics that

describe how species (nodes) are connected through their interactions (links) (Fath et al. 2007), revealing structural properties such as connectance, nestedness, specialization, and modularity. Network theory therefore provides a useful framework for understanding the organization of plant–spider associations in biodiverse and environmentally heterogeneous systems such as the Cerrado.

The Cerrado, the world's most diverse savanna, is a particularly suitable system for investigating these processes because of its pronounced climatic seasonality (Alvares et al. 2013; Calixto et al. 2021; da Silva Filho et al. 2024), with a hot and rainy season from October to March and a cooler, dry season from April to September. Seasonal changes in water availability, plant phenology, prey abundance, and plant-provided resources may alter the distribution and availability of resources used by spiders, potentially modifying both their interaction breadth and their relative use of EFN-bearing plants. Such temporal variation may consequently be reflected in network topology, allowing us to evaluate whether resource-rich and resource-poor periods differ in the organization of plant–spider associations (Kaiser-Bunbury et al. 2010).

While specific interactions between spiders and plants, including those involving EFNs, are well documented (e.g., Ruhren and Handel 1999; Nahas et al. 2012; da Silva Filho et al. 2024; De Oliveira Dias and Stefani 2024), a gap remains in our understanding of how these associations are organized at the community level. The aim of this study is to describe and compare plant–spider interaction networks in a Neotropical savanna and assess how EFN presence and climatic seasonality are associated with network architecture and spider assemblage composition. To address this, we tested three specific hypotheses.

First, we hypothesized (H1) that interaction networks associated with EFN-bearing plants would be more integrated and less compartmentalized than those associated with plants lacking EFNs. Because EFNs provide a relatively stable, carbohydrate-rich resource that can

attract a broad range of arthropod visitors, we predicted that EFN subnetworks would exhibit higher connectance and nestedness, together with lower specialization and modularity than non-EFN subnetworks. Second, we hypothesized (H2) that climatic seasonality would shift network topology. We predicted that resource scarcity during the dry season would increase the concentration and breadth of interactions around the resources that remain available, resulting in higher connectance, nestedness, and specialization, whereas greater resource availability during the rainy season would promote higher modularity. We further predicted that seasonal changes in network structure would be more pronounced among EFN-bearing plants because EFNs may represent a comparatively persistent resource during the dry season. Finally, we hypothesized (H3) that EFN presence and seasonality would be associated with differences in spider assemblage composition. We predicted taxonomic differentiation between spiders associated with EFN and non-EFN plants, as well as differences in assemblage composition between the dry and rainy seasons.

MATERIALS AND METHODS

Study site and data collection

This study was conducted in a ≈640 ha Cerrado fragment at the Reserva Ecológica do Clube de Caça e Pesca Itororó (CCPIU; 48°17′W, 18°58′S) in Uberlândia, Minas Gerais, Brazil. The vegetation consists of open grasslands and Cerrado *sensu stricto* (Ratter et al. 1997), and the climate is a seasonal tropical savanna (Köppen Aw), with a rainy season from October to March and a dry season from April to September.

We established ten 1,000 m² (50 × 20 m) plots, separated by at least 50 m. From October 2020 to September 2021, a single researcher surveyed all plots every two weeks between 07:00 and 11:00. To minimize potential bias associated with survey order, the sequence of plot surveys alternated between consecutive sampling occasions, proceeding from plot 1 to plot 10 in one survey and from plot 10 to plot 1 in the next. Within each plot, we

identified all plants with open inflorescences (and/or flowers) up to a maximum height of 2.5 meters. Then visually inspected their reproductive structures (from base to apex) to record the number of spiders. Temperature and humidity were also recorded during each survey.

While the search effort per individual plant was standardized, the total time per plot varied with floral availability. Voucher specimens of spiders (70% ethanol) and plants (Herbarium of Universidade Federal de Uberlândia - UFU) were collected for identification.

Network metrics

To characterize the architecture of plant–spider interaction networks, we quantified four complementary network-level metrics: connectance (C), nestedness (NODF), specialization (H_2'), and modularity (Q). Connectance represents the proportion of realized interactions relative to all possible links in the network and therefore provides a measure of overall interaction density (Calabrese and Fagan 2004).

Nestedness was quantified using NODF, which measures the extent to which the interaction partners of species with fewer links form subsets of those associated with more highly connected species. NODF ranges from 0, indicating no nested structure, to 100, indicating complete nestedness (Almeida-Neto et al. 2008; Payrató-Borràs et al. 2020). We quantified network-level specialization using H_2', an entropy-based index that accounts for differences in interaction frequencies and partner availability. H_2' ranges from 0, indicating generalized interactions, to 1, indicating high specialization (Blüthgen et al. 2006).

Finally, modularity (Q) was used to quantify network compartmentalization, with higher values indicating stronger subdivision into groups of plant and spider taxa that interact more frequently within than among modules (Barber 2007; Fortuna et al. 2010). Together, these metrics describe complementary dimensions of network organization, ranging from overall interaction density and hierarchical structure to specialization and compartmentalization.

Data analysis

All analyses were performed in R (R Core Team 2024). We constructed quantitative bipartite matrices in which rows represented plant species, columns represented spider taxa, and cell values corresponded to the number of spider individuals recorded on each plant species. For the overall network, observations from the dry and rainy seasons were pooled within plant species. We calculated connectance (C), nestedness (NODF), network-level specialization (H_2'), and modularity (Q) using the bipartite package (Dormann et al. 2008). To examine variation associated with extrafloral nectaries (H1) and seasonality (H2), we subsequently constructed subnetworks for plants with and without EFNs, for dry and rainy seasons, and for each combination of EFN presence and season.

For each network configuration, we compared the observed metrics with null-model expectations. We generated 1,000 randomized quantitative matrices using the r2dtable algorithm, which preserves the marginal totals of the interaction matrix (Patefield 1981). For each metric, we calculated the mean and 95% interval of the null distribution and a standardized effect size (SES), defined as the difference between the observed value and the null mean divided by the standard deviation of the null distribution. Statistical significance was assessed using two-tailed empirical probabilities, with a +1 correction, and values of $p < 0.05$ were considered significant. These null-model analyses evaluate whether each individual network departs from its own random expectation and were therefore not interpreted as direct statistical tests between subnetworks.

Because the number of recorded interactions differed substantially among EFN categories and between seasons, we additionally evaluated the robustness of between-network differences to sampling intensity using a complementary bootstrap procedure. For each comparison, both networks were resampled with replacement to a common number of interactions corresponding to the smaller observed network, with 500 bootstrap replicates

generated for each network. Connectance, NODF, and H_2' were recalculated for each bootstrap replicate, and 95% confidence intervals were obtained for each metric and for the difference between networks. A contrast was considered robust to differences in interaction frequency when the 95% confidence interval of the between-network difference did not include zero. Modularity was not included in this procedure because of the substantially greater computational demand of repeated module detection. The bootstrap was treated as a sensitivity analysis for sampling effort rather than as biological replication.

To evaluate whether EFN presence and seasonality were associated with spider assemblage composition (H3), we used multivariate analyses implemented in the vegan package (Oksanen et al. 2022). Plant species × season combinations with no recorded spiders were excluded prior to analysis, and Bray–Curtis dissimilarities were calculated from the quantitative abundance matrix. Compositional patterns were visualized using two-dimensional non-metric multidimensional scaling (NMDS) with no automatic transformation of abundances, and ordination quality was assessed from the stress value.

We tested the effects of EFN presence, season, and their interaction on spider assemblage composition using permutational multivariate analysis of variance (PERMANOVA; 9,999 permutations). The full model included EFN presence, season, and the EFN × season interaction, with terms evaluated sequentially. Because the design was unbalanced, we also repeated the model with the order of the two main effects reversed to assess whether inference was sensitive to term order. Homogeneity of multivariate dispersion was evaluated using PERMDISP (betadisper) with bias adjustment and 9,999 permutations for EFN categories, seasons, and the four EFN × season combinations.

Finally, because the set of flowering plant species differed between seasons, we conducted a complementary sensitivity analysis restricted to plant species represented in both dry and rainy seasons. For this analysis, seasonal differences in spider assemblage composition

were tested by PERMANOVA while restricting permutations within plant species. This analysis was used to assess whether the overall seasonal result could be attributed primarily to turnover in the plant species represented in each season.

RESULTS

The complete plant–spider network comprised 21 plant species and 41 spider taxa, totaling 1,072 spider records distributed across 160 realized links. Overall connectance was low (C = 0.186; Figure 1), indicating that only a small fraction of all potential plant–spider associations was realized. The complete network differed significantly from its null expectation for all four structural metrics. Connectance (SES = −5.75, p = 0.002) and nestedness (NODF; SES = −4.59, p = 0.002) were lower than expected, whereas network-level specialization (H_2'; SES = 15.39, p = 0.002) and modularity (Q; SES = 16.41, p = 0.002) were higher than expected (Table S1).

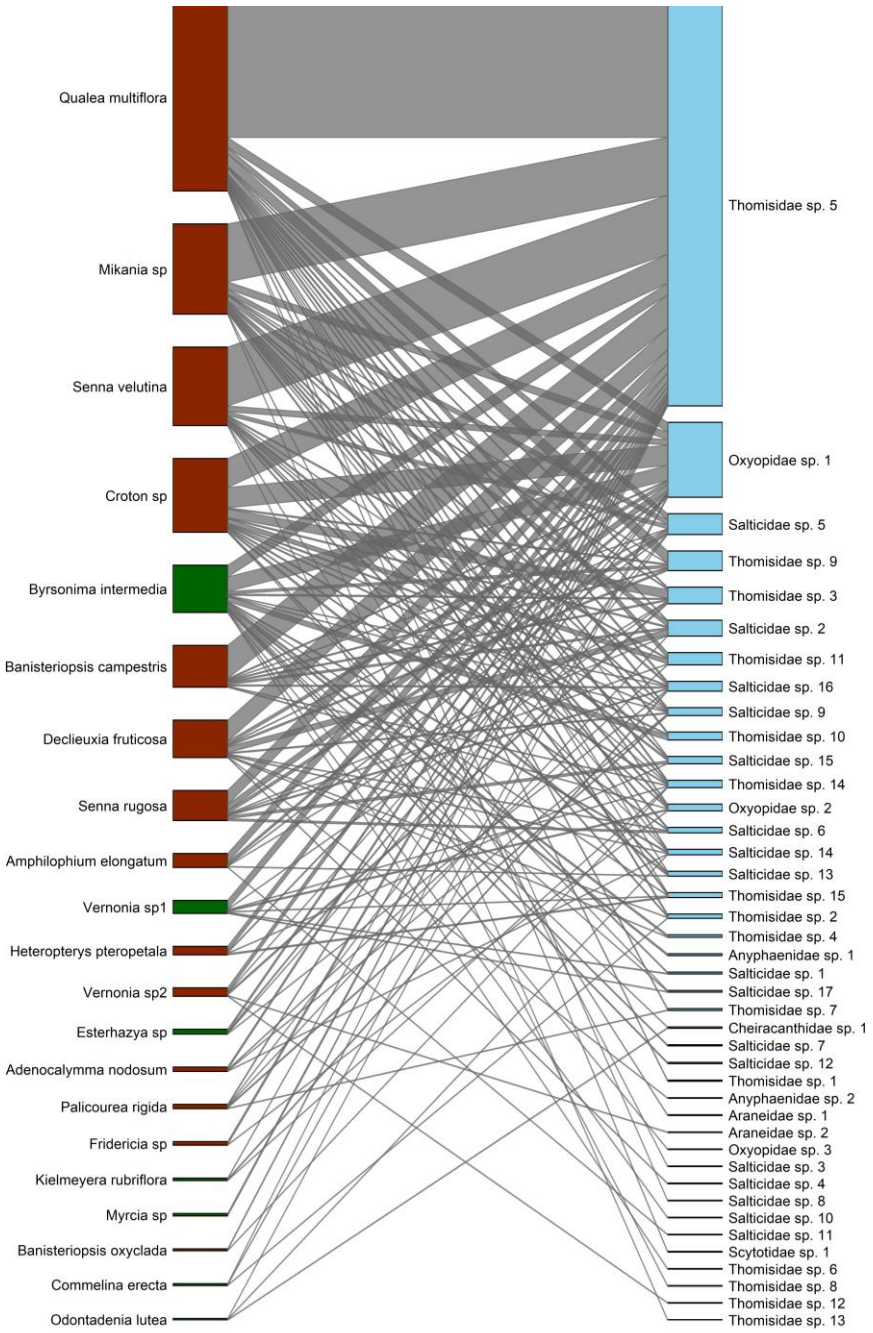

Figure 1. Overall quantitative bipartite network of interactions between flowering plants and spiders recorded in the Cerrado community. Plant species (left) are colored according to the presence of extrafloral nectaries (EFNs), dark orange bars represent EFN-bearing plants and green bars represent plants without EFNs. Spider species (right) are represented by blue bars. Species are ordered by decreasing interaction frequency from left to right to visualize the network's structure and central hubs. The width of each bar indicates species abundance, while link width represents interaction frequency.

Our first hypothesis (H1), which predicted greater integration and lower compartmentalization in networks associated with EFN-bearing plants, received partial support. EFN-bearing plants showed slightly higher connectance (C = 0.254 vs. 0.242) and nestedness (NODF = 51.13 vs. 43.97), together with lower specialization (H_2' = 0.159 vs. 0.244) and modularity (Q = 0.157 vs. 0.296) than non-EFN plants (Figures 2 and 3; Table S2). However, after standardizing interaction frequency between the two subnetworks, bootstrap confidence intervals for differences in connectance, nestedness, and specialization all overlapped zero, indicating that these contrasts were sensitive to differences in sampling intensity (Table S4). Null-model analyses nevertheless showed that EFN networks departed significantly from random expectations for all four metrics, whereas in non-EFN networks connectance, specialization, and modularity differed from null expectations, but nestedness did not (Table S2).

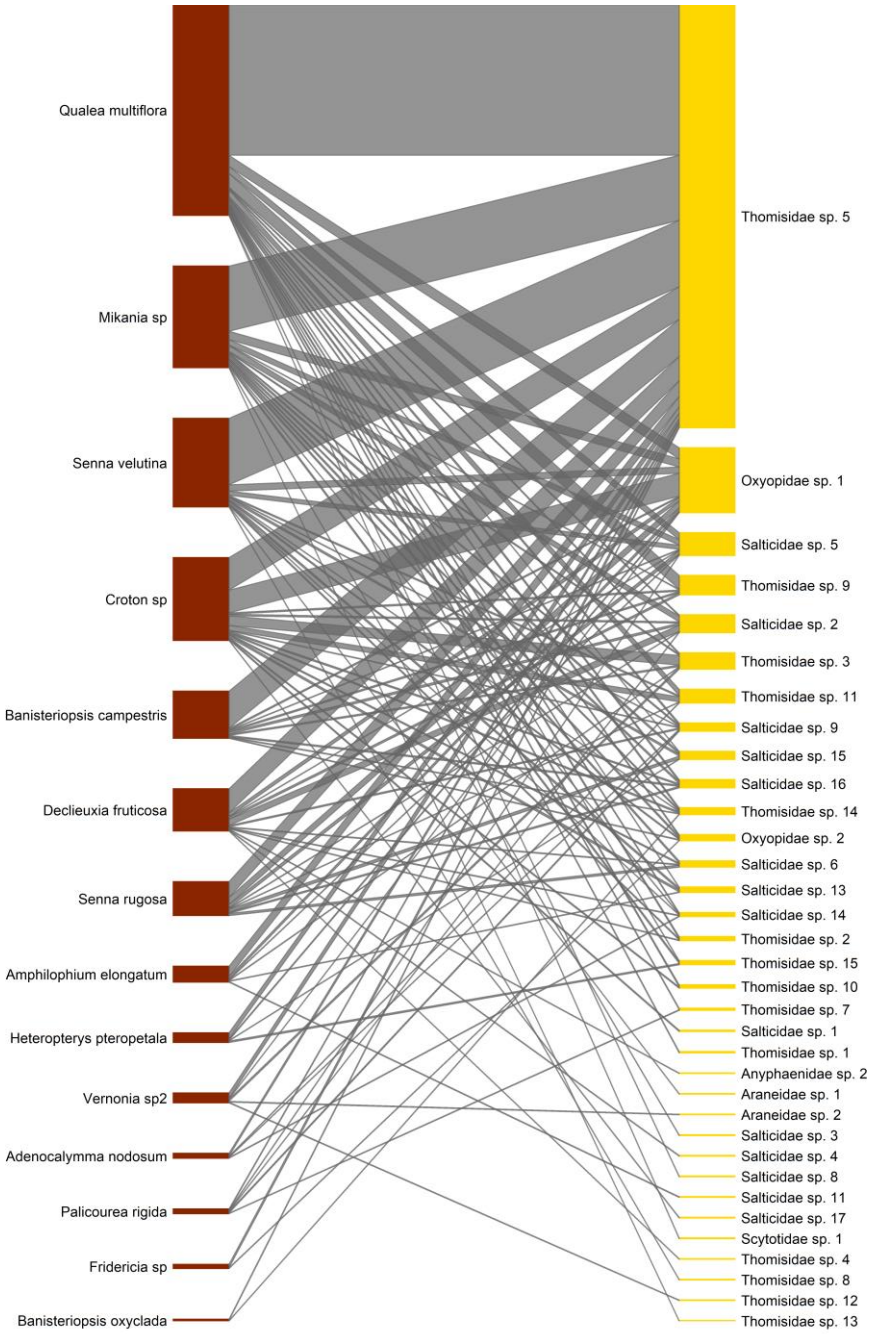

Figure 2. Quantitative bipartite network of interactions between spiders and flowering plants bearing extrafloral nectaries (EFNs). Plant species (left) are represented by dark orange bars, and spider species (right) by yellow bars. Species are ordered by decreasing interaction frequency from left to right to highlight central hubs and network structure. The width of each bar indicates species abundance, while link width represents interaction frequency.

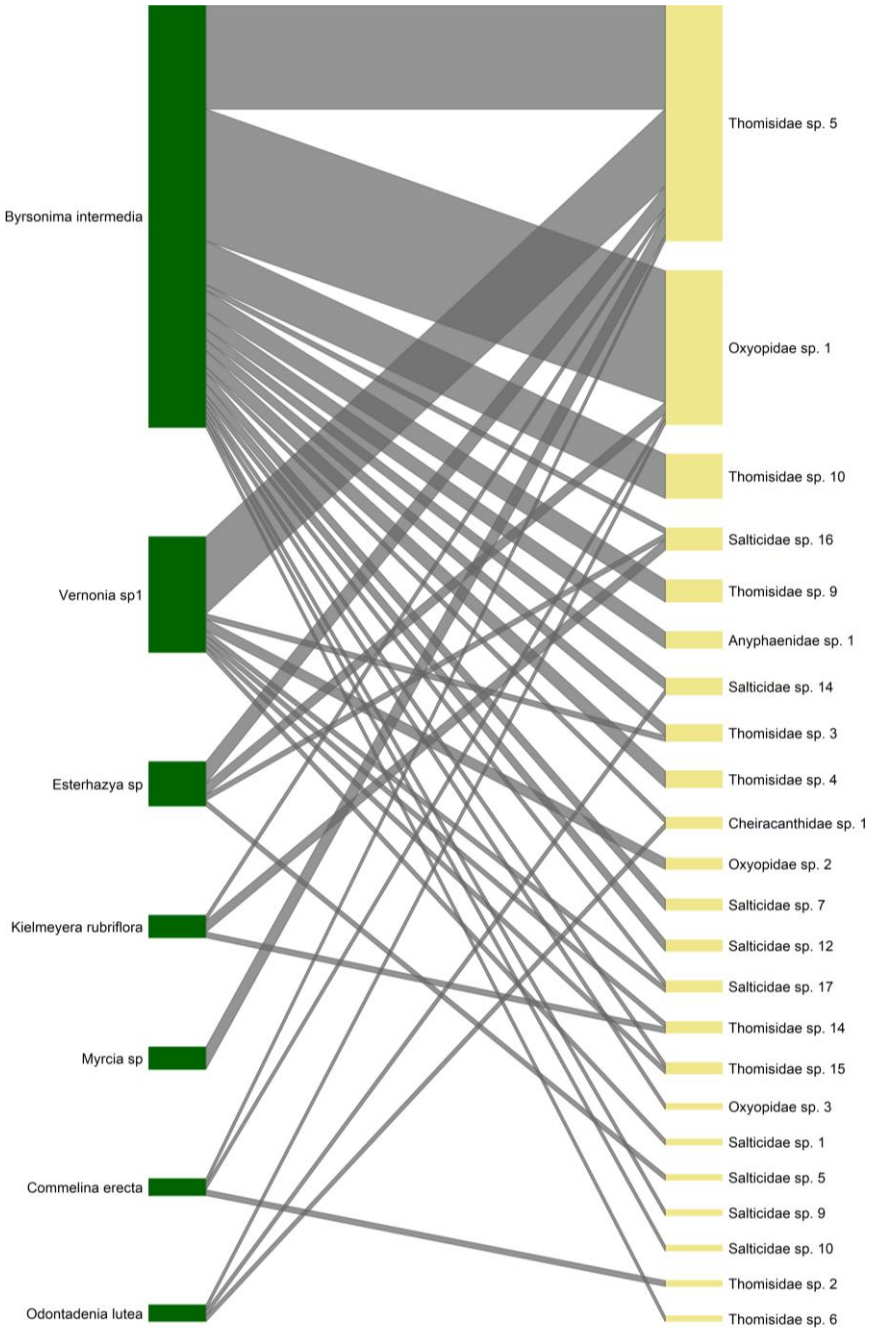

Figure 3. Quantitative bipartite network of spider associations with flowering plants lacking extrafloral nectaries. Plant species (left) are represented by dark green bars, and spider species (right) by khaki bars. Species are ordered by decreasing interaction frequency from left to right to visualize network architecture. The width of each bar indicates species abundance, while link width reflects the frequency of spider occurrences on each plant species.

Seasonal variation in network architecture provided partial support for H2. The dry-season network exhibited higher connectance (C = 0.283 vs. 0.206) and nestedness (NODF = 58.28 vs. 48.72), together with lower specialization ($H_2' = 0.147$ vs. 0.198) and modularity (Q = 0.160 vs. 0.204) than the rainy-season network (Table S2). After standardizing both seasonal networks to the same number of interactions, connectance remained higher during the dry season (mean difference, rainy − dry = −0.078, 95% CI = −0.147 to −0.016), whereas confidence intervals for nestedness and specialization included zero (Table S4). Thus, the predicted increase in dry-season connectance was robust to differences in interaction frequency, whereas the predicted increase in nestedness was not, and specialization changed in the opposite direction to that predicted. Null-model analyses further showed that rainy-season networks differed from random expectations for all four metrics, whereas during the dry season only specialization and modularity departed significantly from null expectations (Table S2).

The additional prediction within H2 that EFN effects would become stronger during the dry season was not supported. When EFN presence and season were considered jointly, connectance was higher during the dry season in both EFN and non-EFN subnetworks. Among EFN-bearing plants, connectance increased from 0.272 in the rainy season to 0.375 in the dry season, while specialization decreased from $H_2' = 0.173$ to 0.131 and modularity from Q = 0.172 to 0.136. Among non-EFN plants, connectance likewise increased from 0.316 to 0.375, but specialization increased from $H_2' = 0.303$ to 0.347 and modularity from Q = 0.123 to 0.292. Departures from null expectations were strongest for EFN-bearing plants during the rainy season, when all four network metrics differed from their corresponding null distributions. In contrast, no consistent increase in non-random network structure associated with EFNs was detected during the dry season (Table S3).

In contrast to H3, neither EFN presence nor seasonality was associated with detectable

shifts in spider assemblage composition. The NMDS ordination showed substantial overlap among spider assemblages associated with EFN-bearing and non-EFN plants and between dry and rainy seasons, with a good two-dimensional representation of Bray–Curtis dissimilarities (stress = 0.107; Figures 4 and 5). Consistent with this pattern, PERMANOVA detected no significant effect of EFN presence ($F_{1,22} = 1.36$, $R^2 = 0.055$, $p = 0.201$), season ($F_{1,22} = 0.80$, $R^2 = 0.033$, $p = 0.565$), or their interaction ($F_{1,22} = 0.50$, $R^2 = 0.020$, $p = 0.862$) on spider assemblage composition. Multivariate dispersion also did not differ among EFN categories, seasons, or their combinations (all $p > 0.22$). A complementary analysis restricted to the five plant species recorded in both seasons yielded the same conclusion, with no detectable seasonal differentiation in spider assemblage composition ($F_{1,8} = 0.63$, $R^2 = 0.073$, $p = 0.688$).

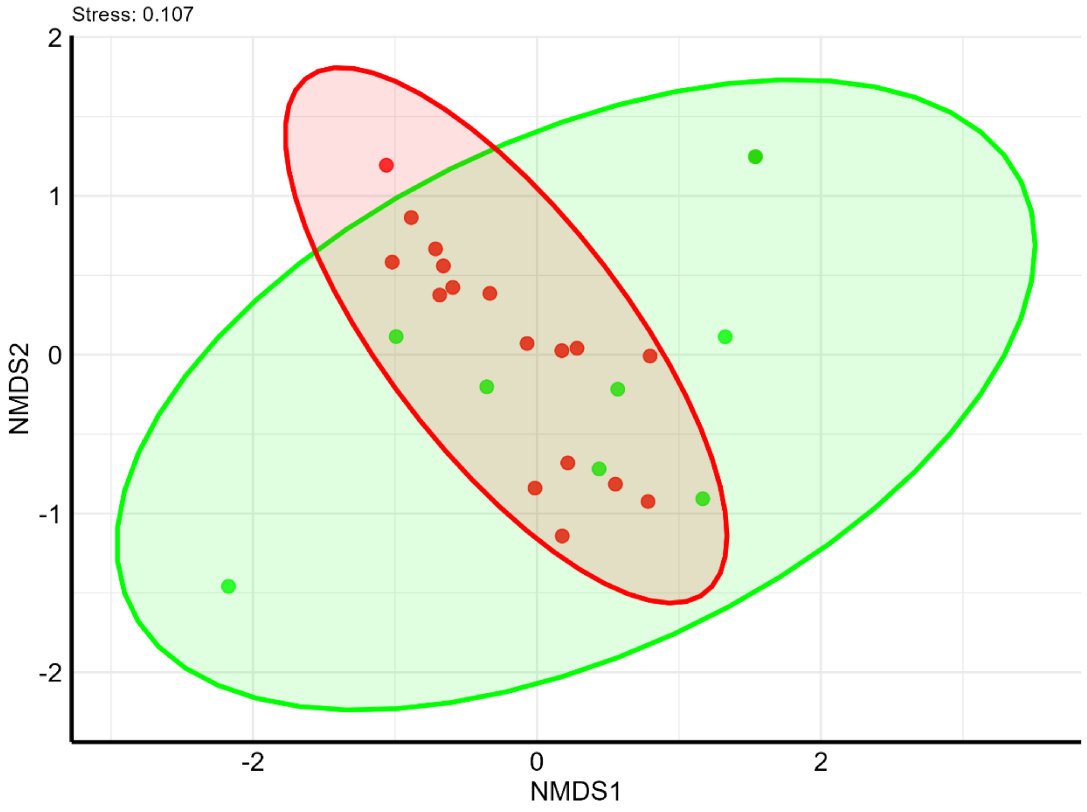

Figure 4. NMDS ordination of spider assemblages associated with flowering plants with EFNs (red) and without EFNs (green), based on Bray–Curtis dissimilarity. The overlap among groups indicates similarity in species composition.

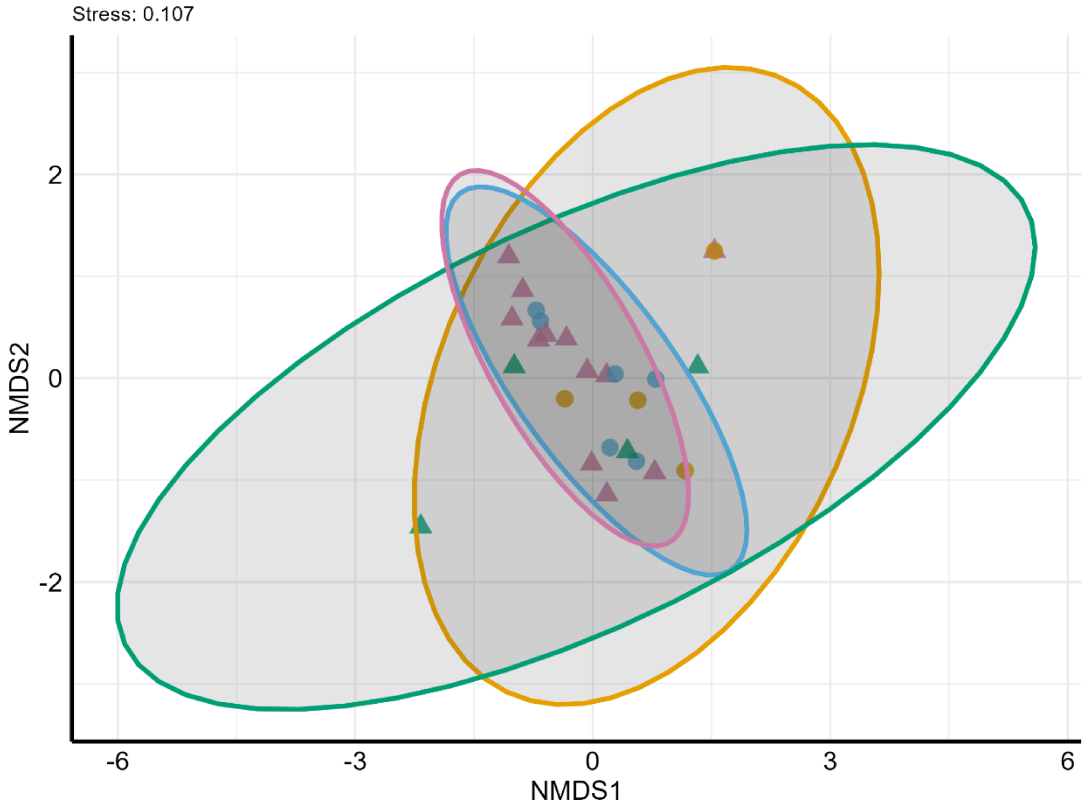

Figure 5. NMDS ordination showing the distribution of spider assemblages according to seasonality and EFN presence. Pink points represent plants with EFNs during the rainy season, blue points plants with EFNs during the dry season, green points plants without EFNs during the rainy season, and yellow points plants without EFNs during the dry season.

DISCUSSION

By offering sugar-rich nectar, EFN plants attract a diversity of predatory spiders and integrate them into the plant–arthropod web (Ruhren and Handel 1999; Silva et al. 2020; De Oliveira Dias and Stefani 2024). This study provides the first empirical network analysis of plant–spider interactions in the Neotropical savanna, showing that seasonal variation was associated with clearer changes in interaction architecture than EFN presence, while neither factor produced detectable shifts in spider assemblage composition.

The complete plant–spider network was characterized by low connectance, indicating that only a small fraction of all possible plant–spider interactions was realized. Moreover, connectance and nestedness were significantly lower than expected under the null model, whereas specialization and modularity were higher than expected. This pattern indicates that interactions were more restricted and compartmentalized than would be expected from the marginal distribution of interactions alone, with spiders concentrating their associations on a subset of available flowering plants rather than using plant species proportionally or indiscriminately.

Regarding our first hypothesis, EFN-bearing plants showed descriptive differences consistent with greater network integration and reduced compartmentalization, but the evidence was weaker than initially expected. EFN subnetworks exhibited slightly higher connectance and nestedness, combined with lower specialization and modularity than non-EFN networks. However, after interaction frequency was standardized between subnetworks, bootstrap confidence intervals for connectance, nestedness, and specialization all overlapped zero. Thus, these contrasts were sensitive to sampling intensity and cannot be interpreted as robust direct effects of EFN presence. Null-model analyses nevertheless showed that all four metrics differed from random expectations in the EFN network, whereas nestedness did not differ from null expectations in the non-EFN network. Because these null models compare each network with its own random expectation, they do not provide a direct test between EFN and non-EFN networks. Predictable, carbohydrate-rich subsidies may attract a broad visitor assemblage (Koptur 1994; Marazzi et al. 2013), and our results remain consistent with the possibility that EFNs contribute to network integration. However, they do not provide sufficient evidence to characterize EFN-bearing plants as structural connector hubs. H1 was therefore only partially supported.

The direction of the observed differences agrees with the expectation that stable resources may promote integration and reduce compartmentalization (Olesen et al. 2008; Dáttilo et al. 2014), although these contrasts were not robust to standardized sampling intensity. This is broadly consistent with other resource-mediated systems in which predictable food sources may reduce interaction selectivity and promote network integration, while differing from ant–plant mutualisms that can involve more specialized and often obligate associations (Del-Claro et al. 1996; Nascimento and Del-Claro 2010).

Our second hypothesis, predicting seasonal variation in network structure, was also partially supported. Connectance was higher during the resource-scarce dry season in the overall network, and the same descriptive trend occurred within both EFN-bearing and non-EFN plants. Importantly, higher dry-season connectance remained evident after both seasonal networks were standardized to the same number of interactions. This pattern is consistent with resource limitation promoting broader interaction breadth rather than resource-rich periods necessarily producing denser networks. Nestedness was descriptively higher during the dry season, but this difference was not robust after standardization, while specialization was lower rather than higher during the dry season, contrary to our original prediction. Modularity likewise showed a modest decline. Null-model analyses further indicated that all four metrics departed from random expectations during the rainy season, whereas only specialization and modularity differed from their null expectations during the dry season. Seasonal variation therefore affected individual dimensions of network architecture differently rather than producing a uniform transition from one structural state to another.

The additional prediction that the effect of EFNs on network metrics would become stronger during the dry season was not supported. Although connectance

increased from the rainy to the dry season in both EFN and non-EFN networks, this pattern alone does not demonstrate a stronger EFN effect under dry-season conditions. Modularity and specialization showed contrasting seasonal responses between plant types: both decreased in EFN networks but increased in non-EFN networks. However, subgroup null-model analyses provided little evidence that dry-season EFN and non-EFN configurations consistently departed from random expectations. The joint effects of EFN presence and season therefore appear more context dependent than originally predicted. More generally, increased connectivity under resource scarcity may reflect broader use of the resources that remain available, whereas greater resource availability may distribute interactions among a wider set of potential partners (Olesen et al. 2008). In our study, the robust dry-season increase in connectance is consistent with spiders broadening their realized associations when resources become more restricted. Potential mechanisms include seasonal shifts in prey availability, plant phenology, or microhabitat conditions, all of which may influence spider foraging decisions. Network topology can respond dynamically to such resource fluctuations, with generalist species often increasing interaction breadth during periods of scarcity (Rivera-García et al. 2017). Physiological constraints during resource-poor periods may also alter foraging strategies and increase the use of alternative resources (Dáttilo et al. 2014; Rico-Gray et al. 2012). Thus, the strongest evidence for H2 was the increase in connectance during the dry season rather than coordinated changes across all network metrics.

Finally, H3 was not supported. Despite seasonal variation in network architecture and descriptive differences between plant types, spider assemblage composition did not differ detectably among EFN categories or between seasons. Thus, the observed variation in interaction architecture occurred without a corresponding detectable differentiation in overall spider assemblage composition.

The absence of detectable compositional differences suggests that changes in network architecture do not necessarily require major taxonomic turnover in the spider assemblage. One possible mechanism is behavioral flexibility expressed through shifts in interaction frequency or partner use rather than species replacement (Wäckers et al. 2005). However, our data do not directly measure behavior, and variation in network architecture may also reflect changes in spider relative abundance, plant availability, or resource distribution among seasons. Direct observations are therefore needed to determine whether spiders alter web-site selection, activity patterns, or their relative use of nectar and prey as environmental conditions fluctuate (CaraDonna et al. 2021; Toju et al. 2024). This interpretation is consistent with ecological theory on behavioral plasticity and niche flexibility in generalist predators (Wäckers et al. 2005; Heil 2015). In facultative interactions, behavioral flexibility may allow assemblages to persist despite temporal variation in resource availability (Bronstein 1994). This contrasts with more obligate ant–plant mutualisms, in which resource availability can be associated with stronger partner specificity and turnover in associated fauna (Del-Claro et al. 1996; Nascimento and Del-Claro 2010). In spider–plant systems, the facultative nature of the interaction may reduce dependence on any single plant resource and contribute to the limited compositional differentiation observed here. Nevertheless, the absence of significant multivariate differences does not imply complete absence of species-level turnover; rather, any such turnover was insufficient to produce detectable differences in overall assemblage composition. The ability of generalist predators to exploit multiple resource types and microhabitats provides a plausible mechanism for this pattern (Uetz 1991; Vasconcellos-Neto et al. 2017).

Our findings demonstrate that seasonal variation, particularly the dry-season increase in connectance, was associated with changes in plant–spider interaction

architecture, whereas differences associated with EFN presence were more sensitive to sampling intensity. These patterns occurred without detectable differences in overall spider assemblage composition. This decoupling suggests that network-level responses to environmental variation can occur without major compositional change, potentially through shifts in interaction frequencies, resource use, or other ecological processes (Bronstein 1994; Wäckers et al. 2005). The Cerrado plant–spider system illustrates how facultative interactions, mediated by resource variation and potentially by behavioral plasticity, can generate dynamic networks across seasonal environments. Future research should integrate direct behavioral observations with network analysis to identify the mechanisms underlying spider foraging decisions and their consequences for plant fitness and community assembly. This approach will improve our understanding of how ecological networks respond to environmental change and how biodiversity is maintained in seasonal ecosystems.

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