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**INTERAÇÕES ENTRE ARANHAS, FORMIGAS E
PLANTAS COM NECTÁRIOS EXTRAFLORAIS:
REDES ECOLÓGICAS E EFEITOS RECÍPROCOS**

LARISSA NAHAS DOMINGUES DE OLIVEIRA

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Tese apresentada à Universidade Federal de
Uberlândia, como parte das exigências para
obtenção do título de Doutor em Ecologia e
Conservação de Recursos Naturais

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Uberlândia, MG

Outubro de 2015

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Doutoranda: Larissa Nahas Domingues de Oliveira

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**Uberlândia, MG,
Outubro de 2015**

A Deus

Meu amor maior

Aos meus pais

Paulo Henrique e Anice

Ao meu esposo

Marlus Leonardo

Ao meu irmão

Paulo Júnior

...Dedico

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RESUMO

Nahas, L. 2015. Interações entre aranhas, formigas e plantas com nectários extraflorais: redes ecológicas e efeitos recíprocos. Tese de doutorado. Universidade Federal de Uberlândia. Uberlândia, MG, Brasil. 82p. Orientação: Prof. Dr. Kleber Del Claro.

Interações ecológicas, como aquelas estabelecidas entre plantas com nectários extraflorais e artrópodes predadores, são muito importantes para a manutenção da diversidade de espécies e estrutura das teias tróficas nos ecossistemas. Nesse contexto, este estudo se propôs a conhecer e caracterizar as interações envolvendo plantas com nectários extraflorais, aranhas e formigas. Buscamos também verificar os impactos desses predadores sobre os herbívoros e a herbivoria das plantas. Objetivamos ainda, por meio de testes em laboratório, comprovar o consumo de néctar por aranhas errantes e construtoras de teia. Todo o estudo foi conduzido em área de cerrado sentido restrito no Clube de Caça e Pesca Itororó em Uberlândia, Minas Gerais, no período de agosto de 2012 a fevereiro de 2014. Em um levantamento inicial, nós encontramos 35 espécies de formigas e 74 espécies de aranhas associadas às 19 espécies de plantas com nectários extraflorais avaliadas. As redes de interações entre aranhas e plantas e entre formigas e as mesmas plantas foram significativamente aninhadas e com baixa especialização, como em outros sistemas mutualísticos facultativos. Esse padrão se manteve no período diurno e noturno, apesar das mudanças na composição e posições das espécies nas redes. Por meio de experimentos de exclusão de predadores, nós constatamos que houve um impacto emergente de aranhas e formigas sobre herbívoros em duas espécies com alto índice de importância na comunidade estudada, *Heteropterys pteropetala* (Malpighiaceae) e *Ouratea spectabilis* (Ochnaceae). No entanto, a herbivoria nessas espécies foi reduzida somente na presença de aranhas. Por meio de análises químicas em laboratório, constatamos que cerca de 88% das aranhas coletadas em plantas com nectários extraflorais consomem néctar. Os resultados positivos abrangeram adultos e imaturos, aranhas errantes e construtoras de teia. Esse estudo realça a importância de estudar interações ecológicas em uma perspectiva mais ampla, em nível de comunidade, a fim de conhecermos padrões e processos gerais inerentes às diversas interações.

Palavras-chave: Cerrado, interações ecológicas, predadores, herbívoros, herbivoria.

ABSTRACT

Nahas, L. 2015. Interactions among spiders, ants and extrafloral nectary bearing plants: ecological networks and reciprocal effects. Ph D. Thesis. Universidade Federal de Uberlândia. Uberlândia, MG, Brasil. 82p. Adviser: Prof. Dr. Kleber Del Claro.

Ecological interactions, such as those established between extrafloral nectary-bearing plants and predator arthropods, are very important for the maintenance of species diversity and structure of food webs in ecosystems. In this context, this study aimed to know and characterize the interactions involving extrafloral nectary-bearing plants, spiders and ants. We also verify the impacts of these predators on plant's herbivores and herbivory. We aimed still, by laboratory tests, to prove nectar consume by wandering and web weaver spiders. All studies took place in cerrado sensu stricto area in Clube de Caça e Pesca Itororó, located in Uberlândia County, Minas Gerais State, Brazil, between August 2012 and February 2014. In an initial survey, we found 35 ant species and 74 spider species associated to 19 extrafloral nectary-bearing plant species. The interaction networks between spiders and plants and ants and the same plants were significantly nested and with low specialization, as in other facultative mutualistic systems. This pattern of interactions occurred in the diurnal and nocturnal period, despite changes in the composition and position of species in the networks. We found an emergent impact of spiders and ants on herbivores in two plant species with high importance index in the studied community, *Heteropterys pteropetala* (Malpighiaceae) and *Ouratea spectabilis* (Ochnaceae), by experiments of exclusion of predators. However, the herbivory in these species were only reduced in the presence of spiders. We found that about 88% of collected spiders in extrafloral nectary-bearing plants consume nectar, by chemical analyses in laboratory. The positive results covered adults and immatures, wandering and web weaver spiders. This study highlights the importance of studying ecological interactions in broader perspective, in the community level, in order to know general patters and processes inherent to the interactions.

Palavras-chave: Cerrado, ecological interactions, predators, herbivores, herbivory.

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INTRODUÇÃO GERAL

A natureza está repleta de interações entre espécies e o sucesso da vida na Terra representa o sucesso das interações bióticas entre organismos (Del-Claro 2012). Assim, a história da biodiversidade é fundamentalmente a história das interações ecológicas (Thompson 1999). É portanto, reconhecida a importância dessas interações na manutenção da diversidade de espécies e estrutura das teias tróficas nos mais diversos ecossistemas. Interações estabelecidas entre plantas e animais são especialmente importantes dada a sua alta abundância (Vázquez *et al.* 2009) e em uma perspectiva mais recente e realística, a biodiversidade deve ser vista e avaliada de forma que abranja a riqueza de interações plantas-animais (Price 2002). A natureza e os resultados dessas interações são altamente variáveis espacial e temporalmente, podendo variar de formas antagônicas a mutuamente benéficas (Del-Claro 2012). Nesse contexto, tem sido amplamente discutido que tais interações devem ser estudadas em uma perspectiva mais ampla, em nível de comunidade, a fim de conhecermos padrões e processos gerais inerentes às diversas interações (Vázquez *et al.* 2009).

Estudos mostram que em comunidades locais, a biodiversidade está organizada em complexas redes ecológicas de interações entre espécies (Hagen *et al.* 2012). Nessa perspectiva, a análise de redes ecológicas é um meio formal de visualizar, explorar e abordar os padrões comuns que estão por trás das inúmeras interações que ocorrem em comunidades. Estudos mostram que as redes ecológicas podem estruturar-se de várias formas, sendo geralmente aninhadas, compartimentadas ou compostas (Lewinsohn & Prado 2006). Redes mutualísticas (como os sistemas plantas-polinizadores ou plantas-dispersores) tendem a estruturar-se de maneira aninhada, onde espécies generalistas interagem entre si formando um núcleo coeso (core) na rede e especialistas interagem com generalistas (Guimarães Jr *et al.* 2006). Por outro lado, redes de interações antagônicas geralmente são compartimentadas, com a presença de grupos coesos (compartimentos) de espécies que interagem, com relativamente poucas interações entre esses grupos (Guimarães Jr *et al.* 2006). As análises recentes de teias alimentares utilizando redes complexas têm realçado princípios básicos gerais que influenciam a sua estabilidade e as possibilidades de recuperação após distúrbios severos, como a supressão de predadores-chave ou perda de espécies generalistas, que compõem o core das interações (Jordano 2010).

De acordo com Price *et al.* (1980), todas as comunidades terrestres baseadas em plantas são compostas por pelo menos três níveis tróficos que interagem entre si: plantas, herbívoros e os inimigos naturais desses herbívoros. Assim, o papel do terceiro nível trófico deve ser considerado em estudos de interações multitróficas para um melhor entendimento da estrutura e dinâmica de comunidades. Os efeitos diretos de predadores sobre densidade ou biomassa de

suas presas via predação ou risco de predação podem ser transmitidos indiretamente aos níveis tróficos mais baixos em uma cascata trófica (Romero & Koricheva 2011). Tais forças topo-base, exercidas por invertebrados predadores sobre herbívoros, e seus efeitos cascata nas plantas são extremamente importantes na estruturação de comunidades (Schmitz *et al.* 2000; Halaj & Wise 2001; Sanders *et al.* 2008). Predadores exercem impacto na densidade, distribuição espacial e diversidade de herbívoros alterando os padrões e níveis de herbivoria nas plantas (Thomas 1989; Romero & Vasconcellos-Neto 2004). De fato, vários estudos comprovam tal benefício indireto da presença de predadores para plantas (e.g. Oliveira 1997; Ruiz *et al.* 2009). Romero & Koricheva (2011) fizeram uma meta análise para verificar a magnitude do efeito de carnívoros sobre plantas e constataram que carnívoros significativamente contribuem para aumentar o fitness das plantas via supressão de herbívoros. Esses autores encontraram ainda que os efeitos de predadores foram mais fortes para plantas que ofereciam recompensas, como o néctar extrafloral.

O néctar extrafloral é secretado por nectários extraflorais (NEFs), glândulas que não estão associadas à polinização. Essa substância geralmente contém açúcares, aminoácidos, proteínas, lipídeos e outros compostos orgânicos (González-Teuber & Heil 2009). Os nectários extraflorais estão presentes em mais de 108 famílias de plantas (Weber *et al.* 2013) e de fato, muitos estudos mostram que o néctar extrafloral, por atrair predadores, pode exercer um papel importante na defesa das plantas contra ação de herbívoros (Heil *et al.* 2001). Formigas são os organismos mais frequentemente observados visitando NEFs e estudos demonstram que quando é dada uma escolha, forrageiam preferencialmente em plantas com NEFs (Oliveira *et al.* 1999; Heil *et al.* 2001). Em muitos habitats, especialmente nas regiões tropicais, formigas compreendem a maior parte da fauna de artrópodes encontrada na vegetação (Oliveira & Freitas 2004). Muitos estudos têm demonstrado que formigas visitantes destes nectários promovem redução na herbivoria e aumento do sucesso reprodutivo das plantas (e.g. Koptur 1979, 1984; Ferreira 1993; Del-Claro *et al.* 1996; Oliveira 1997; Oliveira *et al.* 1999). Outros estudos, no entanto, não detectaram tal efeito (e.g. O'Dowd & Catchpole 1983; Rashbrook *et al.* 1992; Mody & Linsenmair 2004). Tais exemplos podem constituir exceções ou simplesmente refletir a grande variação temporal e espacial prevista para mutualismos de proteção facultativos, além de refletir também a grande variedade de espécies de plantas e artrópodes associados (Heil & Mckey 2003). Formigas também podem se beneficiar na interação com plantas com NEFs. Byk & Del-Claro (2010) demonstraram experimentalmente que o consumo de néctar extrafloral de *Chamaecrista desvauxii* (Caesalpinaceae) tem efeito positivo sobre a aptidão das colônias de

Cephalotes pusillus (Myrmicinae), no que diz respeito ao número de indivíduos por colônia, pesos corporais e número de ovos.

Nectários extraflorais, no entanto, não são visitados apenas por formigas. Estudos mostram que vários outros artrópodes também forrageiam nessas estruturas, dentre eles vários predadores, como vespas (Cuautle & Rico-Gray 2003), neurópteros (Limburg & Rosenheim 2001) e aranhas (Taylor & Pfannenstiel 2008; Nahas *et al.* 2012). Aranhas, assim como formigas, são artrópodes predadores extremamente abundantes na vegetação (Foelix 1996). Dessa forma, podem também indiretamente beneficiar plantas por meio da supressão de herbívoros. Considerando especificamente interações entre aranhas e plantas com NEFs, estudos demonstram que o forrageamento de aranhas em tais plantas, muitas vezes, é relacionado com diminuição de herbivoria e aumento da aptidão das plantas. Por exemplo, Ruhren & Handel (1999) encontraram correlação positiva entre a presença de salticídeos que se alimentavam de néctar extrafloral e o número de frutos e sementes produzidos em *Chamaecrista nictitans* (Fabaceae). Whitney (2004) constatou aumento na produção de sementes em *Acacia ligulata* (Fabaceae) contendo ninhos da aranha subsocial *Phryganoporus candidus* (Desidae). Apesar das análises impossibilitarem a conclusão de que o néctar extrafloral é um importante recurso alimentar para essa espécie de aranha, o número de colônias por planta e volume do ninho foram maiores em *Acacia ligulata* do que em outras hospedeiras sem NEFs. Esses dados juntamente com observações de aranhas se alimentando em NEFs (e.g. Ruhren & Handel 1999; Taylor & Pfannenstiel 2008) sugerem que a presença de néctar extrafloral pode mediar interações mutualísticas entre aranhas e plantas, como ocorre para formigas.

Apesar de muitos estudos mostrarem efeitos positivos da presença de um ou outro tipo de predador sobre plantas, frequentemente múltiplos predadores, e não só um determinado grupo ou guilda, estão presentes nas plantas. No caso específico de aranhas e formigas, esses dois grupos frequentemente coocorrem nas plantas com nectários extraflorais (e.g. Nahas *et al.* 2012; Stefani *et al.* 2015). Como tais grupos são potenciais competidores e predadores mútuos (Sanders & Platner 2007), os resultados das interações com plantas dependerão, além de outros fatores, da natureza das interações entre os predadores. De fato, vários estudos têm demonstrado que múltiplos predadores frequentemente têm efeitos sobre presas que não podem ser previstos simplesmente pela soma de seus impactos individuais. Tais efeitos são chamados emergentes (Sih *et al.* 1998). Por exemplo, Rosenheim *et al.* (2004) constataram que a presença de dois predadores (uma espécie de coleóptero da família Coccinellidae e uma espécie de aranha da família Theridiidae) não acarretou diminuição de herbívoros devido à predação de besouros por

aranhas. Nahas *et al.* (2012) constataram que há um impacto emergente de aranhas e formigas sobre herbivoria de uma espécie arbórea com nectários extraflorais, *Qualea multiflora* (Vochysiaceae) em área de Cerrado, ou seja, em plantas com a presença de ambos os predadores, a herbivoria foi reduzida em maior extensão do que seria esperado pela soma de seus impactos individuais. Esses resultados contrastantes entre estudos mostram que interações multitróficas são altamente complexas e variam dependendo de uma série de fatores espaciais, temporais e inerentes às espécies envolvidas.

No Cerrado, plantas com nectários extraflorais são comuns (Oliveira & Leitão-Filho 1987; Oliveira & Oliveira-Filho 1991). Por exemplo, de 15,4 a 25,5 % das espécies lenhosas amostradas em 10 áreas de Cerrado no Mato Grosso e São Paulo possuem NEFs (Rico-Gray & Oliveira 2007). Tais plantas constituem bons modelos para o estudo de como interações multitróficas podem moldar a organização de comunidades naturais (Del-Claro 2004). Estudos em áreas de Cerrado têm mostrado que formigas e aranhas são abundantes nas plantas com NEFs e podem estabelecer relações mutualísticas com tais plantas (e.g. Nahas *et al.* 2012; Stefani *et al.* 2015). Observações de aranhas próximas aos NEFs e algumas vezes se alimentando de néctar são comuns nesse ambiente (Nahas *et al.* 2012), o que o torna um bom modelo para o estudo de interações envolvendo plantas com nectários extraflorais, herbívoros e predadores. Segundo Rudgers & Gardener (2004), em comunidades ricas em plantas com NEFs, o néctar pode ser um recurso vital nas teias alimentares.

Nesse contexto, o objetivo geral da presente tese foi conhecer e caracterizar as interações multitróficas envolvendo plantas com NEFs e artrópodes predadores (aranhas e formigas) em uma área de cerrado (Figura 1). Buscamos também verificar possíveis impactos emergentes de aranhas e formigas sobre herbívoros e herbivoria em duas espécies vegetais altamente abundantes e importantes para a comunidade estudada. Ainda objetivamos, por meio de testes em laboratório, corroborar o consumo de néctar por aranhas em ambiente de cerrado.

Para alcançar tal objetivo e melhor apresentar os resultados, a tese está apresentada em três capítulos, redigidos em formato de artigos científicos, conforme seus objetivos a seguir:

CAPÍTULO 1. Interações entre formigas, aranhas e plantas com nectários extraflorais em uma savana tropical: estrutura das redes e composição noite-dia

Objetivo específico: descrever e comparar para uma área de cerrado no triângulo mineiro, as redes de interações entre 19 espécies de plantas com nectários extraflorais abundantes e representativas na área e as formigas e aranhas que as visitam, verificando variações entre os períodos diurno e noturno.

CAPÍTULO 2. Efeitos de múltiplos predadores (aranhas e formigas) sobre herbívoros e herbivoria em duas espécies de plantas com nectários extraflorais

Objetivo específico: avaliar possíveis efeitos emergentes de aranhas e formigas sobre herbívoros e herbivoria em *Heteropoterys pteropetala* (Malpighiaceae) e *Ouratea spectabilis* (Ochnaceae), duas espécies vegetais com NEFs e com alto índice de importância na comunidade estudada.

CAPÍTULO 3. Consumo de néctar por aranhas errantes e construtoras de teia em nectários extraflorais na savana neotropical

Objetivo específico: determinar através de análises químicas e observações de campo se aranhas que visitam plantas com nectários extraflorais no cerrado efetivamente consomem néctar.



Figura 1. (A) Área de cerrado *sensu stricto* (contorno de linha amarela) dentro do Clube de Caça e Pesca Itororó em Uberlândia, MG. Fonte: Google Earth 2015. (B) Fotografia da área de estudo.

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CAPÍTULO 1

INTERAÇÕES ENTRE FORMIGAS, ARANHAS E PLANTAS COM NECTÁRIOS EXTRAFLORAIS EM UMA SAVANA TROPICAL: ESTRUTURA DAS REDES E COMPOSIÇÃO NOITE-DIA

Interactions among ants, spiders and extrafloral nectary-bearing plants in a tropical savanna: network structures and night-day composition

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Resumo

Interações ecológicas entre animais e plantas são muito importantes para a estrutura e dinâmica de ecossistemas. Algumas plantas oferecem néctar extrafloral para predadores, tais como formigas e aranhas. Esses predadores podem proteger tais plantas contra herbivoria e contribuir para o sucesso reprodutivo das plantas. Essas associações entre plantas e artrópodes predadores interferem diretamente na dinâmica de redes de interações. Nesse estudo, descrevemos as redes de interações entre artrópodes predadores e plantas com nectários extraflorais e comparamos as variações nos períodos diurno e noturno. Encontramos 35 espécies de formigas e 74 espécies de aranhas interagindo com as 19 espécies de plantas avaliadas. As redes formiga-planta e aranha-planta foram significativamente aninhadas, assimétricas e tiveram baixa especialização. Nós observamos que há uma mudança nas espécies de formigas do core generalista entre os períodos do dia, o que não ocorre para as aranhas. Nossos dados também mostraram que formigas foram encontradas mais frequentemente nas plantas no período diurno, enquanto aranhas foram mais frequentes nas plantas no período noturno. Esse estudo realça a necessidade de conhecer as interações entre espécies de vários grupos em diferentes períodos do dia. Espécies estão interconectadas e estudos sobre interações feitos de modo isolado no espaço ou tempo podem não ser realísticos em proporcionar uma visão geral de processos importantes responsáveis por estruturar os ecossistemas.

Palavras-chave: redes formiga-planta; redes aranha-planta; nectários extraflorais; aninhamento; período diurno; período noturno.

Abstract

Ecological interactions between animals and plants are very important to the structure and dynamics of ecosystems. Some plants offer extrafloral nectar to predators such as ants and spiders. In turn, these predators can protect these plants from herbivory and help increasing plants' reproductive success. These interactions between plants and arthropod predators directly affect the dynamics in interaction networks. As for the current study, we describe the predator arthropods - EFN-bearing plants interaction networks and compare its variations during the diurnal and nocturnal periods. We found 35 ant species and 74 spider species interacting with the 19 plant species evaluated. The ant-plant and spider-plant networks were significantly nested, asymmetric and with low specialization. We observed ant species turnover in the generalist core between the two different periods of the day, but the same was not recorded for spiders. Our data also showed that ants were more often found on plants during the diurnal period, whereas spiders were more often seen on them during the nocturnal period. The current study highlights the need for knowing the interactions among species from several groups in different periods of the day. Species are interconnected and studies on isolated interactions in space and time may be unrealistic in offering an overview on important processes responsible for structuring the ecosystems.

Key Words: ant-plant networks; spider-plant network; nestedness; diurnal period; nocturnal period; generalist core.

Introduction

Ecological interactions are among the fundamental processes responsible for the structure and dynamics of ecosystems (Brown *et al.* 2001). Therefore, it is necessary knowing not just who interacts with whom in the communities, but also the factors that affect these interactions (Blüthgen 2012; Lange & Del-Claro 2014). Associations set between plants and animals are very abundant and complex within natural environments, and they may present drastic outcome variations in a spatial and temporal perspective (Del-Claro 2012). According to Price *et al.* (1980), plants form the basis of trophic units in most ecosystems, and all living plant-based terrestrial communities are composed of at least three interacting trophic levels: plants, herbivores and herbivores' natural enemies. Therefore, these interactions are especially

important for the understanding of ecological systems as well as for their management and conservation (Vázquez *et al.* 2009).

The extrafloral nectar is a resource produced by glands, namely extrafloral nectaries (EFNs). This nectar is often rich in carbohydrates, amino acids, lipids, phenols, alkaloids and volatile organic compounds (González-Teuber & Heil 2009). EFNs have been recorded for at least 3941 species distributed in 108 families of vascular plant species (Weber *et al.* 2013) from both tropical and temperate regions, although they are more commonly found in the flora of tropical areas (Oliveira & Pie 1998). EFN-bearing plants may account for up to 31% of the woody individuals and for 25% of the woody species in the Brazilian tropical savanna (Cerrado) (Oliveira & Oliveira-Filho 1991; Oliveira & Pie 1998). Several studies have shown that EFN-bearing plants are intensively patrolled by many nectar-gathering ants in this ecosystem, both during the day and the night (Oliveira & Freitas 2004; Dáttilo *et al.* 2014a). The extrafloral nectar, however, can attract other arthropods, such as spiders (Nahas *et al.* 2012) and wasps (Cuautle & Rico-Gray 2003). These arthropods, which are mostly predators, may protect plants from herbivores and help increasing their reproductive success (Ruhren & Handel 1999; Cuautle & Rico-Gray 2003; Nascimento & Del-Claro 2010; Nahas *et al.* 2012; Lange & Del-Claro 2014), although it is not a universal true (e.g. Alves-Silva *et al.* 2014; Assunção *et al.* 2014). Ants and spiders are considered to be key predators in most terrestrial ecosystems (Sanders & Platner 2007). The frequency and abundance of these predators on plants may change due to a number of factors, including climatic and biotic factors, such as abundance of competitors, predators or parasites; availability of extrafloral nectar; and other plant features (for ants see: Lange *et al.* 2013; Dáttilo *et al.* 2014a; Lange & Del-Claro 2014; for spiders see: Romero & Vasconcellos-Neto 2007a; Souza 2007; Stefani *et al.* 2015).

Studies show that ant - EFN-bearing plants interactions are often organized in structurally nested networks (Bascompte & Jordano 2007; Dáttilo *et al.* 2014a). Dáttilo *et al.* (2014a) showed that this pattern is often the same, regardless of the period of the day and of the involved ant species. The nested pattern is characterized by a core of generalist plants and animals that interact to each other. Therefore, few species may be involved in a big number of interactions and there are many species involved in few interactions (Almeida-Neto *et al.* 2008). Thus, the nested networks present generalist character, and they tend to be very robust to random disturbances (loss of a node), but very sensitive to selective node losses at the core (Bascompte & Jordano 2007; Jordano 2010). This pattern is common in mutualistic networks involving facultative associations, which are often less specialized than those of more obligate associations, particularly in ant-plant networks (Blüthgen *et al.* 2007). Interactions between

spiders and plants, especially EFN-bearing plants, are still somehow unknown. Several studies show facultative mutualisms between spider and these plant species (e.g. Ruhren & Handel 2001; Whitney 2004; Nahas *et al.* 2012). However, there is no information available on the structure of spider – EFN-bearing plants according to the ecological networks perspective.

The current study aimed describe and compare the predator arthropod – EFN-bearing plant interaction networks. We seek to give a detailed description of the ant - EFN-bearing plant and spider – EFN-bearing plant interactions by considering that both groups hold diurnal and nocturnal habit species. Thus, we compared the web structure between spiders, ants and plants (and within the same taxa) considering the periods of the day (morning / night). Our hypothesis were:

1. Predator-plant interaction networks are generalist and present low specialization. Interactions between predator arthropods and extrafloral nectary-bearing plants tend to be opportunistic and dependent on the availability of resources on the plant. There is low fidelity in this association. Thus, we expect interactions to present a nested and generalist character with low specialization.
2. There are changes in the composition and specific positions of ants and spiders within networks when the diurnal and nocturnal periods are compared, but the structure of the networks does not change.

Methods

Study Area

We conducted the fieldwork between August 2012 and July 2013 in the ecological reserve (230 ha) of Clube de Caça e Pesca Itororó (CCPIU) (18°58' S, 48°17' W) in Uberlândia County, Minas Gerais State, Brazil. The vegetation in this area is dominated by cerrado stricto sensu. It is characterized by the presence of shrubs and trees in a proportion that varies between 10 and 60 percent in a grass dominated area. Similarly to other Cerrado areas, there is a dry season (between May and September) and a rainy season (between October and April) (Oliveira & Marquis 2002).

Data collection

We tagged all EFN-bearing plant individuals measuring one to two meters high, which were found in 15 parallel 50 x 5 m transects, placed 50 m from one another. We found 19 EFN-

bearing plant species in the transects: *Caryocar brasiliense* (Caryocaraceae); *Licania humilis* (Chrysobalanaceae); *Enterolobium gummiferum*, *Plathymenia reticulata*, *Stryphnodendron adstringens*, *S. polyphyllum* (Fabaceae), *Lafoensia pacari* (Lythraceae); *Banisteriopsis campestris*, *B. laevifolia*, *B. malifolia*, *Heteropterys pteropetala*, *Peixotoa tomentosa* (Malpighiaceae); *Eriotheca gracilipes* (Malvaceae); *Ouratea hexasperma*, *O. spectabilis* (Ochnaceae); *Tocoyena formosa* (Rubiaceae); *Qualea grandiflora*, *Q. multiflora* and *Q. parviflora* (Vochysiaceae) (Figure 1) (check on Appendix 1 for more details about abundance and nectary site).

We inspected all marked plants in a monthly basis during the diurnal (07:00 - 11:00 h) and nocturnal periods (18:00 - 22:00 h), since the first until the last studied month. The same researcher (LN) recorded the abundance and richness of ants and spiders on the plants, in each inspection. Whenever necessary, specimens were collected for identification in laboratory.

Data analyses

We developed incidence matrices (ants per plants and spiders per plants) to describe the network structures of each taxa (spiders and ants) and plants. The matrices considered the frequency of interactions at each evaluated period of the day and at the total matrix. Therefore, we generated three quantitative matrices (day, night and total matrix =day + night) for each taxa.

We used several metrics to check network properties: degree of species, mean degree (for plants and animals) (sensu Dunne 2006), connectance (sensu Jordano 1987), network specialization index (sensu Blüthgen *et al.* 2006), nestedness index (sensu Almeida-Neto *et al.* 2008) and web asymmetry (sensu Vázquez *et al.* 2007). We classify the species in the networks as belonging to the generalist or to the peripheral core sensu Dáttilo *et al.* (2013a). This categorization enabled us to evaluate the temporal turnover in specific positions of animals within each network (e.g., shifting from peripheral to generalist core between the diurnal and nocturnal networks).

We also verified the importance of the plant species to its interaction with ants and spiders; that is why we calculated the importance index (sensu Murray 2000). This index values range from 0 to 1.0, tending to 1, when a plant species has many interactions in the community or has a big number of exclusive interactions.

We computed Jaccard's similarity index (JSI) for all networks (spiders and ants) to check species composition turnover in the diurnal and nocturnal networks. This index was

calculated as follows: $JSI_{(DN)}=C/(A+B+C)$, wherein A is the number of species found only in the diurnal period, B is the number of species found only in the nocturnal period and C is the number of ant species shared between the two sampling periods (Dáttilo *et al.* 2014a).

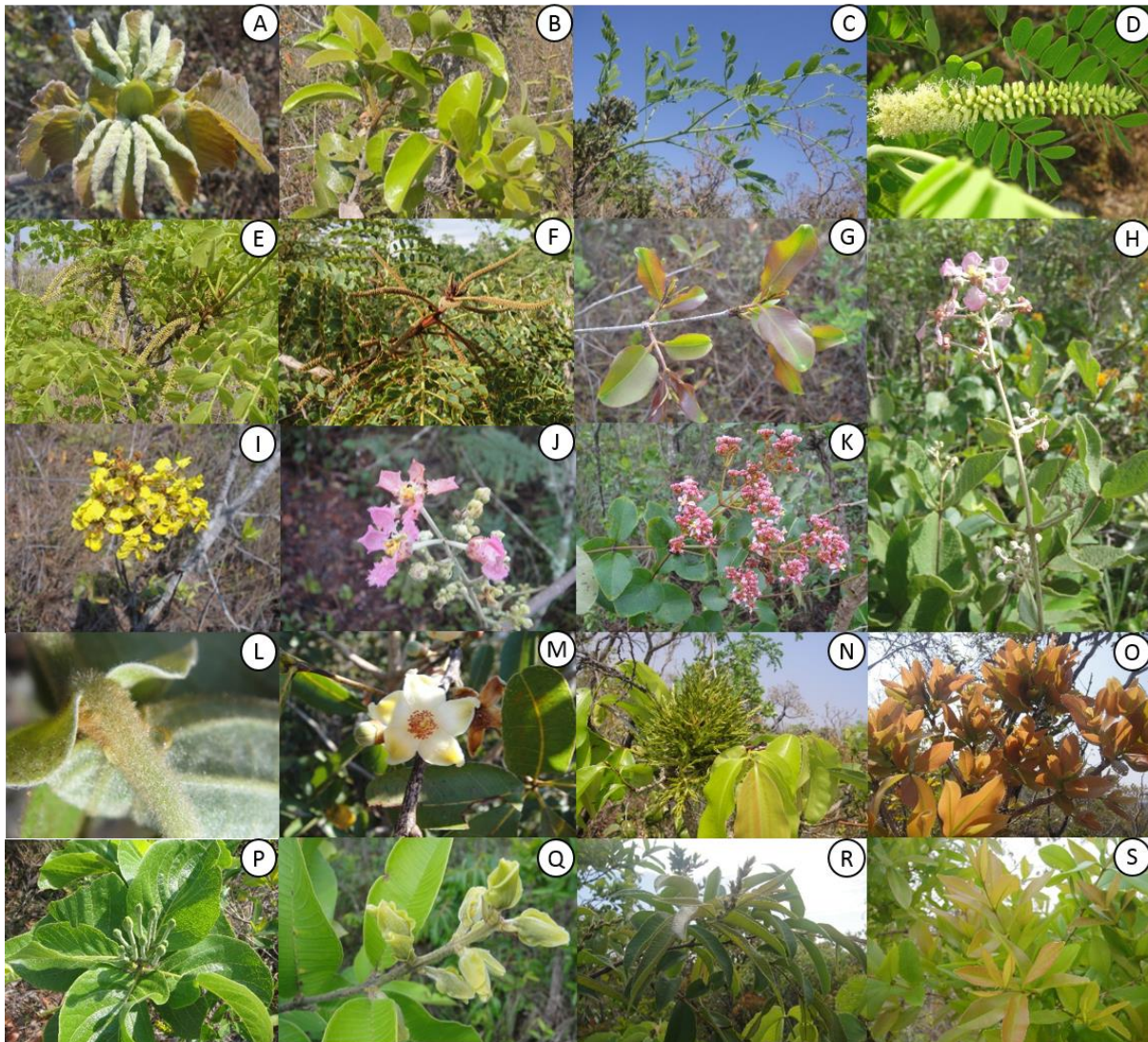


Figure 1. Extrafloral nectary-bearing plants found in the transects. (A) *Caryocar brasiliense* (Caryocaraceae); (B) *Licania humilis* (Chrysobalanaceae); (C) *Enterolobium gummiferum* (Fabaceae); (D) *Plathymenia reticulata* (Fabaceae); (E) *Stryphnodendron adstringens* (Fabaceae); (F) *S. polyphyllum* (Fabaceae); (G) *Lafoensia pacari* (Lythraceae); (H) *Banisteriopsis campestris* (Malpighiaceae); (I) *B. laevifolia* (Malpighiaceae); (J) *B. malifolia* (Malpighiaceae); (K) *Heteropterys pteropetala* (Malpighiaceae); (L) *Peixotoa tomentosa* (Malpighiaceae); (M) *Eriotheca gracilipes* (Malvaceae); (N) *Ouratea hexasperma* (Ochnaceae); (O) *O. spectabilis* (Ochnaceae); (P) *Tocoyena formosa* (Rubiaceae); (Q) *Qualea grandiflora* (Vochysiaceae); (R) *Q. multiflora* (Vochysiaceae) and (S) *Q. parviflora* (Vochysiaceae).

Results

Ant-plant networks

We found 35 ant species interacting with all evaluated plant species (Table 1; Appendix 2). In the diurnal period, we recorded 26 ant species (14 of them exclusive from this period) from 13 genera and six subfamilies foraging on all plant species. As for the nocturnal period, we recorded 21 ant species (nine of them exclusive from this period) that represent 11 genera and five subfamilies on all plant species. There was low similarity (Jaccard similarity index=0.343) between ant – EFN-bearing plant networks in the diurnal and nocturnal periods. Moreover, the ant species present in the generalist core totally changed in the two sampling periods. *Camponotus crassus*, *Cephalotes pusillus*, *Pseudomyrmex gracilis* and *P. flavidulus* were part of the generalist core in the diurnal network, whereas *Camponotus bonariensis*, *C. renggeri* and *C. atriceps* were part of the generalist core in the nocturnal network (Figure 2).

We only observed one ant species (*C. crassus*) interacting with all evaluated plant species during the diurnal period (Figure 2). In this period, *H. pteropetala* was the plant species in association with higher number of ant species (53.85% of all ant species; n=14). *E. gummiferum* was the plant species in association with the lowest ant richness (11.54% of all ant species; n=3). We did not observe ant species interacting with all plant species during the nocturnal period. The ant species with bigger number of interactions was *C. bonariensis* (Figure 2). At night, the highest ant richness was observed on following plants: *C. brasiliense*, *Q. multiflora* and *O. spectabilis* (57.14% of all ant species; n=12). The lowest ant richness was recorded for *S. adstringens*, *B. campestris* and *B. laevifolia* (4.76% of all ant species; n=1) (Figure 2).

The ant – EFN-bearing plant networks in both evaluated periods of the day showed significantly nested topology (diurnal period: 52.56; nocturnal period: 59.22 and p-values <0.001 – Table 1). We found more than twice the number of interactions in the diurnal period than in the nocturnal period. The networks presented higher connectance in the nocturnal period than in the diurnal one (32.08% and 28.74%, respectively). The specialization degree ranged from 0.183, in the diurnal period, to 0.234, in the nocturnal one. Networks were asymmetric in both periods, with more animal than plant species (Table 1).

The plant species' importance index to ants changed in the diurnal and nocturnal samplings. The plant species with higher importance index to ants in the diurnal sampling was *L. humilis* and that in the night sampling was *H. pteropetala*. The species *H. pteropetala*, *P.*

tomentosa and *O. spectabilis*, were among the seven most important plant species to diurnal and nocturnal ants (Table 2).

Spider-plant networks

We found 74 spider species interacting with all evaluated plant species (Table 1; Appendix 3). In the diurnal period, we recorded 49 ant species (12 exclusive from this period) from nine different families; they foraged on the 19 plant species. In the nocturnal period, we recorded 62 spider species (25 exclusive from this period) from 19 different families; and they foraged on 18 plant species (we did not find spiders interacting with *P. tomentosa* at night). Despite the low similarity (Jaccard similarity index = 0.5) between the diurnal and nocturnal networks, the spider assemblage in the generalist core was similar (Figure 2). All spider species in the generalist core found in the diurnal sampling were also present in the generalist core in the nocturnal sampling: *Chira lanei* (Salticidae), *Tmarus* sp2 (Thomisidae), *Araneus venatrix* (Araneidae), *Oxyopes macroscelides* (Oxyopidae), *Euophryinae* sp1 (Salticidae), *Tmarus* sp3 (Thomisidae) and *Aysha* sp1 (Anyphaenidae). The species *Cryptachaea hirta* (Theridiidae) and *Hamataliwa* sp1 (Oxyopidae) were restricted to the generalist core in the nocturnal sampling. However, the species' positions changed within the networks when the diurnal and nocturnal periods were compared to each other (Figure 2).

We did not observe any spider species interacting with all studied plant species. The species presenting the bigger number of interactions during the diurnal period was *C. lanei* (Figure 2). In this period, *O. spectabilis* presented the greatest richness of associated spider species (43.94% of all spider species; n=23), and the lowest ant richness was recorded for *B. campestris* (2.04% of all spider species; n=1). In the nocturnal period, *Tmarus* sp2 was the spider species presenting more interactions (Figure 2). In this period, the greatest richness of associated spider species was recorded for *H. pteropetala* (45.16% of all spider species; n=28) and the lowest spider richness was recorded for *B. campestris* and *E. gummiiferum* (4.84% of all spider species; n=3) (Figure 2).

Spider-plant networks showed significantly nested topology in both periods of the day (diurnal period: 34.39; nocturnal period: 44.22 and p-values < 0.001 – Table 1). We found twice associations in nocturnal spider-plant networks than in the diurnal period. The specialization degree ranged from 0.25 in the nocturnal period, to 0.43, in the diurnal one. Connectance was lower in the diurnal period, thus presenting 17.83% possible interactions in comparison to

23.39%, in the nocturnal period. The networks were asymmetric in both periods of the day (Table 1).

Plant species' importance index to spiders changed when we compared the diurnal and nocturnal periods. The most important plant species to spiders during the diurnal period was *P. tomentosa*, whereas for the nocturnal period it was *H. pteropetala*. The species *O. spectabilis*, *L. humilis*, *H. pteropetala* and *Q. multiflora* were among the seven most important plant species to diurnal and nocturnal spiders (Table 2).

Comparisons between ant-plant and spider-plant networks

When we compared the networks set between EFN-bearing plants and the two predator groups, we found bigger number of spider species associated to plants than ant species (Table 1). We also observed ant species turnover in the generalist core when the two periods of the day were compared to each other; however, the same did not occur among spiders.

We more often found ants in the diurnal period, whereas spiders were usually seen on the plants during the nocturnal period. We also noticed that although both networks were significantly nested, nestedness and connectance were higher in the ant-plant networks. Spider and ant species degree was higher during the nocturnal period. Plant species degree in the spider-plant network was higher in the nocturnal period. As for the ant-plant network, plant species degree was higher in the diurnal period (Table 1).

When we analyzed the importance of plant species to networks considering both periods, we observed that some plant species are very important for the maintenance of the two analyzed animal taxa, for example, *O. spectabilis* and *H. pteropetala* (Table 2). If we just take into account the number of interactions, our results show that each animal taxa was more intensely associated to different plant species; except for the plant species *Q. multiflora*, which was important for both the spiders and the ants during the nocturnal period and in the networks considering the whole day (Figure 2).

By considering ant and spider richness, we observed a great overlap between these two predator groups on some plant species, for example *O. spectabilis*, *H. pteropetala* and *Q. multiflora* (Figure 2).

Table 1. The metrics of the interaction networks for ants, spiders and extrafloral nectary bearing plants in cerrado reserve of Clube de Caça e Pesca Itororó in Uberlandia County, Minas Gerais State, Brazil, during the period from August 2012 to July 2013.

Network metrics	Day	Night	Whole-Day
Ant-plant			
Number of ant species	26	21	35
Number of associations	752	305	1057
Degree of plant species (Average degree±SE)	7.47±0.7	6.74±0.93	11.89±1,18
Degree of ant species (Average degree±SE)	5.46±1	6.09±0,95	6.46±0,91
Network connectance	28,74%	32,08%	33,98%
H2' index	0.183	0.234	0.152
Web asymmetry	-0.15	-0.05	-0.3
Nestedness value (NODF)	52.56; p<0.001	59.22; p<0.001	59.7; p<0.001
Spider-plant			
Number of spider species	49	62	74
Number of associations	282	576	859
Degree of plant species (Average degree±SE)	8.74±1.25	14.5±1.86	17.89±2.26
Degree of spider species (Average degree±SE)	3.39±0.44	4.21±0.52	4.59±0.52
Network connectance	17.83%	23.39%	24.18%
H2' index	0.43	0.251	0.248
Web asymmetry	-0.44	-0,55	-0.59
Nestedness value (NODF)	34.39 ; p<0.001	44.22 p<0.001	56.68 p<0.001

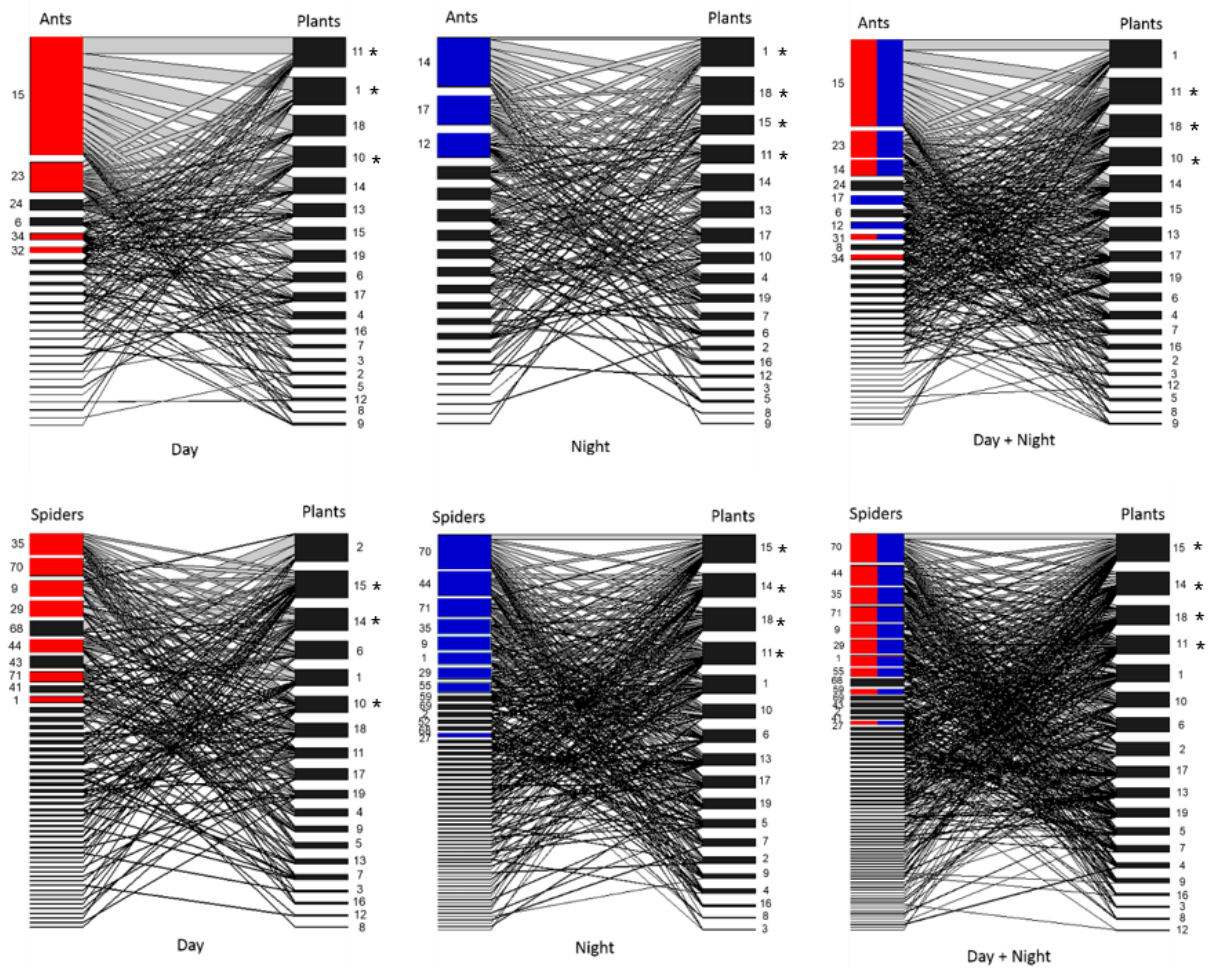


Figure 2. Ant-plant and spider-plant networks sampled in cerrado reserve of Clube de Caça e Pesca Itororó in Uberlandia, Minas Gerais, Brazil, during the period from August 2012 to July 2013. For each animal group we built networks using diurnal, nocturnal, and both together sampling. Within each network, node represents one animal species (right) or plant species (left), and lines represent animal–plant interactions. The nodes are arranged according to the species’ number of interactions. Rectangle height is proportional to the number of interactions of each species. Red and blue rectangles represent ant and spider species that were present in the generalist core. * represent plants in the generalist core.

Table 2. Importance index (Murray) of plant species in the two interaction networks (day, night and both together), in cerrado reserve of Clube de Caça e Pesca Itororó in Uberlandia County, Minas Gerais State, Brazil, during the period from August 2012 to July 2013.

Plant species	Ant-plant interaction			Spider-plant interaction		
	Day	Night	Day + night	Day	Night	Day + night
<i>C. brasiliense</i>	0.254	0.201	0.177	0.271	0.181	0.161
<i>L. humilis</i>	0.338	0.100	0.206	0.414	0.238	0.207
<i>E. gummiferum</i>	0.079	0.120	0.0827	0.100	0.097	0.080
<i>P. reticulata</i>	0.085	0.101	0.091	0.113	0.204	0.183
<i>S. adstringens</i>	0.106	0.071	0.096	0.113	0.272	0.219
<i>S. polyphyllum</i>	0.154	0.232	0.224	0.192	0.202	0.191
<i>L. pacari</i>	0.078	0.106	0.084	0.189	0.201	0.135
<i>B. campestris</i>	0.169	0.071	0.124	0.200	0.111	0.101
<i>B. laevifolia</i>	0.088	0.167	0.083	0.143	0.139	0.127
<i>B. malifolia</i>	0.186	0.155	0.133	0.329	0.232	0.258
<i>H. pteropetala</i>	0.229	0.246	0.214	0.319	0.341	0.254
<i>P. tomentosa</i>	0.287	0.202	0.247	0.625	-	0.625
<i>E. gracilipes</i>	0.091	0.227	0.162	0.263	0.212	0.188
<i>O. hexasperma</i>	0.127	0.134	0.109	0.293	0.314	0.271
<i>O. spectabilis</i>	0.208	0.221	0.208	0.423	0.237	0.294
<i>T. formosa</i>	0.184	0.114	0.129	0.292	0.069	0.155
<i>Q. grandiflora</i>	0.171	0.112	0.140	0.259	0.276	0.240
<i>Q. multiflora</i>	0.208	0.123	0.160	0.329	0.233	0.191
<i>Q. parviflora</i>	0.230	0.114	0.158	0.347	0.195	0.196

Discussion

Our results showed that the ant-plant and spider-plant networks followed the nested pattern displayed by facultative mutualistic interactions, thus corroborating our first hypothesis. In addition, the general nestedness and asymmetric patterns occurred in both periods of the day and in both the ant-plant and spider-plant networks. On the other hand, the nestedness index changed depending to the period of the day, mainly in spider-plant networks. Despite such variation, the nestedness index was always significant. Some studies show that nestedness tends to grow as richness increases (Wright & Reeves 1992; Guimarães Jr *et al.* 2006). It was evident in the spider-plant networks, because there was greater species richness and nestedness in these interactions during the nocturnal period. Nestedness was also higher in the ant-plant networks during the nocturnal period; however, in this case, species richness was lower. According to Guimarães Jr. *et al.* (2006) the nested asymmetrical specialization variation among EFNs networks is not totally explained by differences in species richness or by differences in the ratio between animals and plants. These differences may result from the sampling bias, because generalist and more connected organisms are more likely to be sampled. However, these authors observed that still some communities differed in the specialization level even after they were controlled for preferential sampling of generalists. Therefore, it is suggested that other

ecological variables may lead to a particular nestedness degree in any particular assemblage. Dáttilo *et al.* (2014b) showed that plant and ant species presenting the highest number of interactions within networks had more interactions among themselves than it was expected by the abundance-based assumption and by the random mixing of individuals. They suggested that these ant species' dominance occurs because they are able to beat other ant species when they feed on extrafloral nectaries. Dáttilo *et al.* (2014c) also showed that the competition and monopolization of the resources used by ants are important factors for keep the nested pattern in ant–plant mutualistic networks. In addition, the abiotic factors that affect the availability and quality of food resources, for example soil features, may have important effects on nestedness in mutualistic networks (Dáttilo *et al.* 2013b).

The specialization index of ant-plant and spider-plant networks evaluated in the current study was low. Both interactions present a generalist character, because extrafloral nectaries usually are easily accessible structures both several species of ants like other arthropods. There is often low fidelity in these associations (Blüthgen 2012). Such index values corroborate that of other studies that have evaluated pollinator-plant (Blüthgen 2012; Ramírez-Flores *et al.* 2015) and ant-plant interactions (Lange *et al.* 2013; Lange & Del-Claro 2014). There are no studies approaching spider – EFN-bearing plant networks. However, spiders are generalist predators that mostly forage in solitude (Lang 2003; Romero & Vasconcellos-Neto 2007b) and often do not establish specific associations with plants (but see Romero & Vasconcellos-Neto 2005; Vasconcellos-Neto *et al.* 2006). This fact can lead to the generalist character of these interactions. We can observe important differences between ant-plant and spider-plant networks despite similar patterns in relation to nestedness, asymmetry and low specialization. For example, while spiders are more abundant on plants, ants are more frequent. Our results show that each plant species receive more spider species than ant species but in a lower frequency. On the other hand, ants visit more plant species. Such data suggest that spiders can be very important to plant defense in determinate periods when they are more abundant. Ants can be very important because they are more frequent on plants. Thus, ants and spiders can have complementary effects on plant defense, as showed by some studies (Nahas *et al.* 2012; Stefani *et al.* 2015).

Our results showed that although diurnal and nocturnal networks have displayed similar nestedness patterns and low specialization, we found some differences between the two periods of the day and it corroborated our second hypothesis. We recorded important differences regarding the species composition and its positions in the networks, fact that shows a temporal dynamics in the interactions. We observed an important difference among networks when we

compared the diurnal and nocturnal periods, there was drastic ant species turnover in the generalist core. On the other hand, the spider composition in the generalist core was very similar in both the diurnal and the nocturnal periods. Such feature can be particularly advantageous since these spiders may be effective herbivore repellents because they remain longer on the plants. This long staying on plants is probably due to the fact that the vegetal substrate is important for foraging as well as for the refuge, resting, mating and oviposition of these predators (Souza 2007). Even by considering that these spider species' activities may be higher in a given period of the day, their presence on the plants may scare herbivores, since they may offer predation risks (Lima 1998). On the other hand, ants return to their nests after foraging (Belchior *et al.* 2012). The advantage of replacing the ant fauna is a change in foraging strategies that may help to the suppressing different herbivore types depending on the ant species found on the plants (Del-Claro & Oliveira 2000). Some ant species may be more efficient in protecting the plant against small herbivores and from eggs, whereas other ant species may preferably attack bigger enemies (Byk & Del-Claro 2010; Blüthgen 2012).

Other species' position within the networks was different in the diurnal and nocturnal periods, in both studied interactions. According to Pigozzo & Viana (2010), the properties of the networks seem to be more important than the species' identity, because the system keeps on running regardless of its composition, since the processes are kept even if there were changes in community composition. Dáttilo *et al.* (2014a) compared the structure of networks between ants and three EFN-bearing plant species during the diurnal and the nocturnal period. The aforementioned authors found that the network specialization was similar in both periods and that, despite the high similarity in ant species composition in both the diurnal and nocturnal networks, ant assembly in the generalist core totally changed in the two sampling periods. As for the present study, the change in the network position of some species in the periods of the day may be responsible for the increased connectance in the nocturnal period in both the spider-plant and ant-plant interactions. We observed that the network was composed of more connected species in the nocturnal period in the ant-plant interaction. Although ant richness was lower at night, the degree of species remained similar, and it helps increasing connectance in this period. The species were also more connected in the nocturnal period in the spider-plant interaction. We observed that the generalist core in the diurnal period was composed of seven spider species, whereas the generalist core in the nocturnal period was composed of nine spider species. We recorded higher connectance values in the ant-plant networks in both periods in comparison to the spider-plant networks. Although we have recorded almost twice the number

of spider species foraging on plants than that of ants, the number of spider-plant associations was smaller considering both day periods.

In the nocturnal period, we found more spider species as well as twice the number of associations than in the diurnal period. It can explain the higher degree of plant species in the nocturnal period. It is likely that spiders preferably forage during the nocturnal period due to physiological reasons and/or to the possible overlap effect with ants. Spiders' nocturnal foraging in Cerrado may be especially advantageous, since several spider species are susceptible to desiccation (Gillespie 1987; Henschel *et al.* 1992; Schmalhofer 1999). These species may find more favorable temperature conditions in the nocturnal period as well as the possibility to forage for longer without facing prolonged sun exposure in a relatively hot environment such as Cerrado. In addition to the abiotic factors, the biotic ones may also influence the species' foraging period choice. There are evidences that spiders tend to avoid the presence of ants on plants, since these two predator groups are potential competitors and mutual predators (e.g. Nahas *et al.* 2012; Stefani *et al.* 2015). Other authors suggest that the effects on ants and spiders are asymmetrical. So, ants may suppress the number of spiders in a larger extent than spiders may limit ants' density, probably because of ants' social behavior (Moya-Laraño & Wise 2007). We observed that besides the greater foraging in the nocturnal period, if one considers the number of interactions in the two interaction networks, spiders seem to more intensely forage on plants that are less visited by ants. However, when we analyzed the interaction networks by taking under consideration the number of species on plants, we noticed that a large number of spider and ant species use the same plant species. The ants' effect on spiders might depend on the involved species, and such effect might be stronger on some spider species. It was corroborated by the differences in the use of plants when we just considered the number of interactions and not the number of species. In addition, the potential prey abundance may be a factor influencing predators' foraging on plants.

We found more ant species and twice the number of associations during the diurnal period than in the nocturnal period. Both the abiotic (such as temperature throughout the day) and biotic factors, such as extrafloral nectar (Lange *et al.* 2013) and honeydew (Blüthgen *et al.* 2000) availability and the abundance of competitors (Blüthgen & Fiedler 2004), may affect the ants' foraging on plants. According to Heinrich (1993) ants' body temperature is dictated by the physical environment outside the nest. Thus, ants have activity peaks and avoid the hotter periods. Cerdá *et al.* (1998) showed that temperature primarily determines the temporal activity patterns found in ant communities from open Mediterranean habitats and that subordinate ant species foraging in these habitats are more influenced by temperature than by competition,

although the effect of dominants on subordinates has been shown in few cases. Besides the physiological issues, other factors may be involved in ant occurrence on plants. For example, EFNs often show high nectar flow variation during the day and among seasons (Fiala 1990; but see O'Dowd 1979; Schemske 1982). We did not compare the nectar amount and the concentration in the two periods of the day in the current study. Some studies show that nectaries' productivity is often higher during the nocturnal period (e.g. Heil *et al.* 2000; Dáttilo *et al.* 2015). In a study from the same area of the present one, Lange *et al.* (2015) showed that the nectar volume is higher at night. However, the authors of such study found more ant species that exclusively forage during the diurnal period than ant species exclusively forage at night. They also found higher frequency of ants in the morning than in the afternoon and in the evening.

It is likely that other factors influence ant's activity in the studied system, for instance, overlap with spiders and other ant species on the plants. Sanders & Platner (2007) showed that intraguild interactions between spiders and ants are important structuring forces in a dry grassland. Moreover, the competition among ant species may influence the foraging period. More competitive species may dominate the resources. It can make other species forage in other periods of the day. Studies using baits to evaluate the competition for food resources among ants often show that inferior competitors are displaced by more dominant species (Parr & Gibb 2010). We did not investigate competition effects among species in the present study. However, we observed that only three ant species made part of the generalist core in the nocturnal period. These species appeared to be aggressive and they dominated plants due to their big number of individuals. We rarely observed other ant species on plants when these particular species were found on the plants. We found more ant species occupying the same plants in the diurnal period. It is possible that coexistence is higher in the diurnal period. More aggressive species may dominate the resource (e.g. extrafloral nectar) during the nocturnal period, time when nectaries' productivity is often higher. Additional studies on these factors are necessary in order to get more conclusions about the most concerning information to ant occurrence on the studied plant species.

Ecological interaction analysis in community level based on a broader perspective is important because it allows us to know the key species in communities. Such species are very important for the permanence and stability of the ecological systems. Therefore, our study helps better understanding the spider and ant communities associated to extrafloral nectary-bearing plants in Cerrado, to show the key plant and predator species in this system. Data showed that certain plant species are extremely important for the maintenance of the two studied animal

taxa and of their interactions, as it is the case of *Ouratea spectabilis* (Ochnaceae) and *Heteropterys pteropetala* (Malpighiaceae). Results in the current study reveal that it is worth considering the nocturnal samplings in ant-plant and spider-plant interaction studies, because it may lead to the more realistic understanding of interaction networks in a given environment. Future studies should focus on the mechanisms behind the observed patterns in the herein studied system, for example, focused on the actual role of the competition and physiological factors responsible for the distribution of predator species on the plants throughout the day. Moreover, it is important to investigate how predator communities affect herbivores, herbivory, pollinators and productivity on the studied plant species.

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Appendix 1: Plant species, codes, the location of nectary on the plant (according Oliveira & Leitão-Filho 1987) and information about abundance and distribution in 15 transects in cerrado stricto sensu in Clube de Caça e Pesca Itororó, Uberlândia County, Minas Gerais State, Brazil.

Plant species	Codes	Nectary site	Number of individuals	Number of transects
Caryocaraceae				
<i>Caryocar brasiliense</i> (Cambess)	1	Cálix	30	10
Chrysobalanaceae				
<i>Licania humilis</i> (Cham & Schl.)	2	Leaf blade	2	2
Fabaceae				
<i>Enterolobium gummiferum</i> (Mart.) J.F. Macbr	3	Rachis	5	3
<i>Plathymenia reticulata</i> Benth.	4	Stem	16	9
<i>Stryphnodendron adstringens</i> (Mart.) Coville	5	Rachis	9	5
<i>Stryphnodendron polyphyllum</i> (Mart.)	6	Rachis	23	12
Lythraceae				
<i>Lafoensia pacari</i> (A. St.-Hil.)	7	Leaf blade	18	9
Malpighiaceae				
<i>Banisteriopsis campestris</i> (A. Juss.) Little	8	Leaf blade	8	5
<i>Banisteriopsis laevifolia</i> (A. Juss.) B. Gates	9	Leaf blade	15	5
<i>Banisteriopsis malifolia</i> (Ness & Mart.) B. Gates	10	Leaf blade	31	11
<i>Heteropterys pteropetala</i> (A. Juss)	11	Leaf blade	28	12
<i>Peixotoa tomentosa</i> A. Juss	12	Leaf blade	12	8
Bombacaceae				
<i>Eriotheca gracilipes</i> (K. Schum.) Robyns	13	Petiole	18	6
Ochnaceae				
<i>Ouratea hexasperma</i> (A. St.-Hil.) Baill	14	Cataphylls	34	15
<i>Ouratea spectabilis</i> (Mart.) Engl.	15	Cataphylls	30	13
Rubiaceae				
<i>Tocoyena formosa</i> (Cham. & Schltdl.) K. Schum.	16	Cálix	16	8
Vochysiaceae				
<i>Qualea grandiflora</i> Mart.	17	Stem, pedicel	21	8
<i>Qualea multiflora</i> Mart.	18	Stem, pedicel	33	12
<i>Qualea parviflora</i> Mart.	19	Stem, pedicel	26	11

Appendix 2. Species codes of ants recorded in extrafloral nectary-bearing plants in diurnal and nocturnal period in the Brazilian Neotropical Savanna in Clube de Caça e Pesca Itororó, Uberlândia County, Minas Gerais State, Brazil.

Ant species	Codes	Diurnal period	Nocturnal period
Dolichoderinae			
<i>Azteca</i> sp1	1	X	X
<i>Azteca</i> sp2	2		X
Ectatomminae			
<i>Ectatomma brunneum</i> Smith, 1858	3	X	
<i>Ectatomma edentatum</i> Roger, 1863	4		X
<i>Ectatomma planidens</i> Borgmeier, 1939	5		X
<i>Ectatomma tuberculatum</i> (Olivier, 1792)	6	X	X
<i>Gnamptogenys semiferox</i> Brown, 1958	7	X	
Formicinae			
<i>Brachymyrmex</i> sp1	8	X	X
<i>Brachymyrmex</i> sp2	9	X	
<i>Brachymyrmex</i> sp3	10	X	
<i>Brachymyrmex</i> sp4	11		X
<i>Camponotus atriceps</i> (Smith 1858)	12		X
<i>Camponotus blandus</i> (Smith, 1858)	13	X	
<i>Camponotus bonariensis</i> Mayr, 1868	14	X	X
<i>Camponotus crassus</i> Mayr, 1862	15	X	X
<i>Camponotus melanoticus</i> Emery, 1894	16	X	X
<i>Camponotus renggeri</i> Emery, 1894	17		X
<i>Camponotus trapeziceps</i> Forel, 1908	18	X	
<i>Nylanderia</i> sp1	19		X
Myrmicinae			
<i>Atta sexdens</i> (Linnaeus, 1758)	20		X
<i>Atta</i> sp1	21	X	
<i>Cephalotes maculatus</i> (Smith, 1876)	22	X	
<i>Cephalotes pusillus</i> (Klug, 1824)	23	X	X
<i>Crematogaster crinosa</i> Mayr, 1862	24	X	X
<i>Crematogaster quadriformis</i> Roger, 1863	25		X
<i>Crematogaster</i> sp1	26	X	
<i>Nesomyrmex asper</i> (Mayr, 1887)	27	X	
<i>Pheidole radoszkowskii</i> Mayr, 1884	28	X	X
<i>Pheidole</i> sp1	29	X	X
<i>Solenopsis</i> sp1	30	X	X
Ponerinae			
<i>Neoponera villosa</i> (Fabricius, 1804)	31	X	X
Pseudomyrmecinae			
<i>Pseudomyrmex flavidulus</i> (Smith, 1858)	32	X	
<i>Pseudomyrmex gr. elongatus</i> sp. 1	33	X	
<i>Pseudomyrmex gracilis</i> (Fabricius, 1804)	34	X	
<i>Pseudomyrmex tenuis</i> (Fabricius, 1804)	35	X	

Appendix 3. Species codes of spiders recorded in extrafloral nectary-bearing plants in diurnal and nocturnal period in the Brazilian Neotropical Savanna in Clube de Caça e Pesca Itororó, Uberlândia County, Minas Gerais State, Brazil.

Spider species	Codes	Diurnal period	Nocturnal period
Anyphaenidae			
<i>Aysha</i> sp1	1	X	X
<i>Aysha</i> sp2	2	X	X
<i>Sanogasta</i> sp1	3		X
<i>Wulfilopsis</i> sp1	4		X
Anyphaenidae sp1	5	X	X
Anyphaenidae sp2	6	X	X
Anyphaenidae sp3	7		X
Araneidae			
<i>Alpaida</i> sp1	8	X	X
<i>Araneus venatrix</i> (C. L. Koch, 1838)	9	X	X
<i>Araneus</i> sp1	10	X	X
<i>Argiope argentata</i> (Fabricius, 1775)	11	X	X
<i>Cyclosa</i> sp1	12	X	X
<i>Eustala</i> sp1	13		X
<i>Gasteracantha cancriformis</i> (Linnaeus, 1758)	14	X	X
<i>Manogea porracea</i> (C. L. Koch, 1838)	15	X	
<i>Micrathena plana</i> (C. L. Koch, 1836)	16	X	
<i>Micrathena</i> sp1	17		X
<i>Parawixia</i> sp1	18		X
<i>Verrucosa</i> sp1	19	X	X
<i>Wagneriana</i> sp1	20	X	X
Clubionidae			
Clubionidae sp1	21		X
Clubionidae sp2	22		X
Corinnidae			
Corinnidae sp1	23		X
Corinnidae sp2	24		X
Eutichuridae			
<i>Cheiracanthium inclusum</i> (Hentz, 1847)	25		X
Mimetidae			
<i>Gelanor</i> sp1	26	X	X
Oxyopidae			
<i>Hamataliwa</i> sp1	27	X	X
<i>Hamataliwa</i> sp2	28	X	X
<i>Oxyopes macrosclides</i> Mello-Leitao, 1929	29	X	X
<i>Oxyopes salticus</i> Hentz, 1845	30	X	X
Oxyopidae sp1	31	X	
Oxyopidae sp2	32	X	
Pisauridae			
<i>Architis</i> sp1	33		X

Prodidomidae			
Prodidomidae sp1	34		X
Salticidae			
<i>Chira lanei</i> Soares & Camargo, 1948	35	X	X
<i>Helvetia</i> sp1	36	X	X
<i>Lyssomanes</i> sp1	37	X	X
<i>Synemosyna</i> sp1	38	X	
<i>Synemosyna</i> sp2	39	X	
<i>Thiodina</i> sp1	40	X	X
Dendryphantinae sp1	41	X	X
Dendryphantinae sp2	42	X	
Dendryphantinae sp3	43	X	X
Euophryinae sp1	44	X	X
Salticidae sp1	45	X	X
Salticidae sp2	46	X	
Salticidae sp3	47		X
Scytodidae			
<i>Scytodes</i> sp1	48		X
Selenopidae			
<i>Selenops</i> sp1	49		X
Senoculidae			
<i>Senoculus</i> sp1	50		X
Sparassidae			
Sparassidae sp1	51	X	X
Sparassidae sp2	52		X
Tetragnathidae			
<i>Leucauge</i> sp1	53	X	X
Theridiidae			
<i>Anelosimus</i> sp1	54		X
<i>Cryptachaea hirta</i> Taczanowski, 1873	55	X	X
<i>Episinus</i> sp1	56		X
<i>Euryopsis</i> sp1	57		X
<i>Latrodectus geometricus</i> C. L. Koch, 1841	58	X	X
<i>Theridion</i> sp1	59	X	X
<i>Theridion</i> sp2	60	X	X
Theridiidae sp1	61		X
Theridiidae sp2	62	X	
Theridiidae sp3	63	X	
Theridiosomatidae			
Theridiosomatidae sp1	64		X
Thomisidae			
<i>Aphantochilus rogersi</i> O. P. Cambridge, 1870	65	X	
<i>Misumenops</i> sp1	66	X	X
<i>Misumenops</i> sp2	67	X	
<i>Synema</i> sp1	68	X	X
<i>Tmarus</i> sp1	69	X	X

<i>Tmarus</i> sp2	70	X	X
<i>Tmarus</i> sp3	71	X	X
Thomisidae sp1	72	X	X
Trechaleidae			
Syntrechalea sp1	73		X
Trechaleidae sp1	74		X

CAPÍTULO 2

EFEITOS DE MÚLTIPLOS PREDADORES (ARANHAS E FORMIGAS) SOBRE HERBÍVOROS E HERBIVORIA EM DUAS ESPÉCIES DE PLANTAS COM NECTÁRIOS EXTRAFLORAIS

Multiple predators (spiders and ants) effects on herbivores and herbivory in two extrafloral nectary-bearing plants

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Resumo

Aranhas e formigas são potenciais competidores e predadores mútuos, então, quando coocorrem no mesmo substrato na vegetação os resultados de suas interações com plantas dependem da natureza das interações entre esses dois tipos de predadores. No presente estudo, nós experimentalmente avaliamos os efeitos de aranhas e formigas sobre herbivoria e herbívoros encontrados em duas plantas com nectários extraflorais, *Heteropterys pteropetala* (Malpighiaceae) and *Ouratea spectabilis* (Ochnaceae). Nós dividimos as plantas (n=60) em quatro grupos experimentais, dependendo da presença ou ausência de formigas e aranhas, em ambas as espécies. Nós comparamos os efeitos de cada tratamento sobre a riqueza e abundância de herbívoros mastigadores e sugadores assim como sobre a herbivoria (perda de área foliar). A presença de formigas reduziu a abundância e riqueza de aranhas, mas aranhas não afetaram a abundância e riqueza de formigas. A remoção de aranhas acarretou um aumento significativo na herbivoria. No entanto, nós encontramos uma interação significativa do efeito de formigas e aranhas sobre abundância e riqueza de herbívoros, o que indica um efeito emergente de múltiplos predadores. O presente estudo realça a importância de conhecer os impactos das diferentes guildas de predadores sobre herbívoros e plantas, já que não é possível prever os resultados de cascatas tróficas conhecendo somente os efeitos de uma ou outra guilda de predador.

Palavras-chave: impacto emergente, néctar extrafloral; múltiplos predadores; guilda de predador.

Abstract

Spiders and ants are potential competitors and mutual predators, therefore when they co-occur on the same vegetation substrate the outcomes regarding their interaction with the plant depends on the nature of the interactions between these two predators. In the current study, we experimentally evaluated the spiders and ants effects on herbivory and herbivores found in two extrafloral nectary-bearing plants, *Heteropterys pteropetala* (Malpighiaceae) and *Ouratea spectabilis* (Ochnaceae). We divided the plants (n=60) into four experimental groups, depending on the presence or absence of ants and spiders, in both species. We compared the effects of each treatment on the richness and abundance of chewing and sucking herbivores as well as on herbivory (leaf area loss). The presence of ants reduced the abundance and richness of spiders, but spiders did not affect the abundance and richness of ants. A significant increase in herbivory resulted from spiders' removal. However, we found a significant interaction effect of ants and spiders on herbivores abundance and richness, and it indicates an emergent multiple predator effect. The current study highlights the importance of knowing predator guilds impacts on herbivores and plants, since we could not predict trophic cascades outcomes just by being aware of the effects of one or another predator guild.

Key words: emergent impact; extrafloral nectar; multiple predators; predator guild.

Introduction

Ecological interactions studies, mainly those focused on plant-animal interactions, are pointed out as the basis to understand how communities are established and organized. These interactions are essential to species diversity maintenance and to food web structures (Del-Claro 2012; Bascompte & Jordano 2013). All terrestrial communities based on living plants are composed of at least three interacting trophic levels, namely: plants, herbivores and natural herbivores enemies (Price *et al.* 1980). The third trophic level must be considered as being part of a set of defenses plants have against herbivores (Price *et al.* 1980).

Associations between extrafloral nectary-bearing plants and predator arthropods, given its high abundance in many environments, such as Cerrado (e.g. Oliveira & Leitão-Filho 1987; Koptur *et al.* 1998; Lange & Del-Claro 2014), have been widely studied. These arthropods can consume extrafloral nectar and help their host plants by preying or repelling herbivores (Nascimento & Del-Claro 2010; Whitney 2004). Extrafloral nectar secretion is done by specialized glands known as extrafloral nectaries (EFNs) that are not involved in pollination

(Bentley 1977). The extrafloral nectar is rich in carbohydrates, amino acids, proteins, lipids, and other organic compounds (Gonzalez-Teuber & Heil 2009). Ants are among the main EFNs visitors and nectar consumption can improve their adaptive value (Byk & Del-Claro 2011). Ants' presence on plants may decrease herbivory and increase these plants reproductive success (e.g. Del-Claro *et al.* 1996; Oliveira *et al.* 1999; Nascimento & Del-Claro 2010). However, extrafloral nectaries attract not only ants but also other arthropods, both the herbivores and the predators, including spiders (Rudgers & Gardener 2004), which are very abundant predators on vegetation and can reduce herbivores damages on plants (Nahas *et al.* 2012).

Spiders and ants are potential competitors and mutual predators (Sanders & Platner 2007). When they co-occur on the same vegetation substrate, the outcomes of their interactions with the plants depends on the nature of the interactions between these two predators. Several studies have shown that multiple predators often lead to emergent impacts on prey (Rosenheim *et al.* 2004; Nahas *et al.* 2012). Emergent impact occur when multiple predator effects can not be predicted simply by summing the effects of single predator types. Understanding the emergent multiple predator effects (MPEs) is a critical issue for community ecology, given that natural communities typically have multiple predators that feed on prey (Sih *et al.* 1998). In the Cerrado, spiders and ants are abundant on extrafloral-nectary bearing plants; they often co-occur on the same plants (Nahas *et al.* 2012). These authors also showed that ants and spiders had complementary impact on herbivory found in a plant with EFNs, *Qualea multiflora* (Vochysiaceae), in the same Cerrado area therein studied.

By considering the co-occurrence of ants and spiders in the same EFNs-bearing plants in Cerrado savanna, we hypothesized that spiders and ants emergent effect on herbivory found in *Qualea multiflora* by Nahas *et al.* (2012) could also occur in other plants with EFNs in the same study site. We used the shrub *Heteropterys pteropetala* (Malpighiaceae) and the tree *Ouratea spectabilis* (Ochnaceae) - which are also visited by both predator groups (Nahas pers. obs.) - as models. We investigated the following questions: (1) Do ants and spiders have significant effect on herbivory, on herbivores richness and abundance and on productivity in *H. pteropetala* and *O. spectabilis*? (2) Do these possible effects differ when ants and spiders act simultaneously and separately? (3) Does spider exclusion affect ants and vice versa, in these plants?

Methods

Study site

The study was conducted from September 2013 to February 2014 in the Cerrado reserve (127 ha) of Clube de Caça e Pesca Itororó (CCPIU) (18°56'21.04"S, 48°16' 14.33"W), Uberlândia County, Minas Gerais State, Brazil. The vegetation in this area is known as cerrado (stricto sensu), and it is featured by the prevalence of 3–8-m-tall trees and shrubs with more than 30% crown cover; however with a fair amount of herbaceous vegetation between them (Oliveira & Marquis 2002). Like other Cerrado areas, it is marked by two distinguished seasons, a dry one between May and September (autumn-winter), and the rainy season, between October and April (spring-summer).

Plant species

Heteropterys pteropetala A. Juss, is a shrub commonly found in the study site. The species has two extrafloral nectaries at the base of its leaves, at the inflorescences bracts and at the base of its bud pedicels (Assunção *et al.* 2014) (Figure 1A, B). There are from two to four nectaries located on the same leaf region in young leaves growing with inflorescences. These nectaries in young leaves are more active than those in mature leaves (Réu & Del-Claro 2005). *Heteropterys pteropetala* is a deciduous shrub, which loses its leaves at the end of September; its leaves resprout in October, and flowering takes place between January and March (Assunção *et al.* 2014).

Ouratea spectabilis is a semideciduous tree with extrafloral nectaries in the stipules and in the basal region of the pedicel. EFNs are mainly active in young leaves, which are produced all over the year but mostly during the dry/cold season (Figure 1C, D). The leaf expansion phase lasts 15–20 days. The extrafloral nectar in EFNs, during this period, contains 16.75% sucrose in average (Byk & Del-Claro 2010). *Ouratea spectabilis* is the tree species presenting the biggest population and greatest importance index within the study site (Faleiro 2007). It flourishes between August and September and the fruits ripen in October and November (Lorenzi 2002).

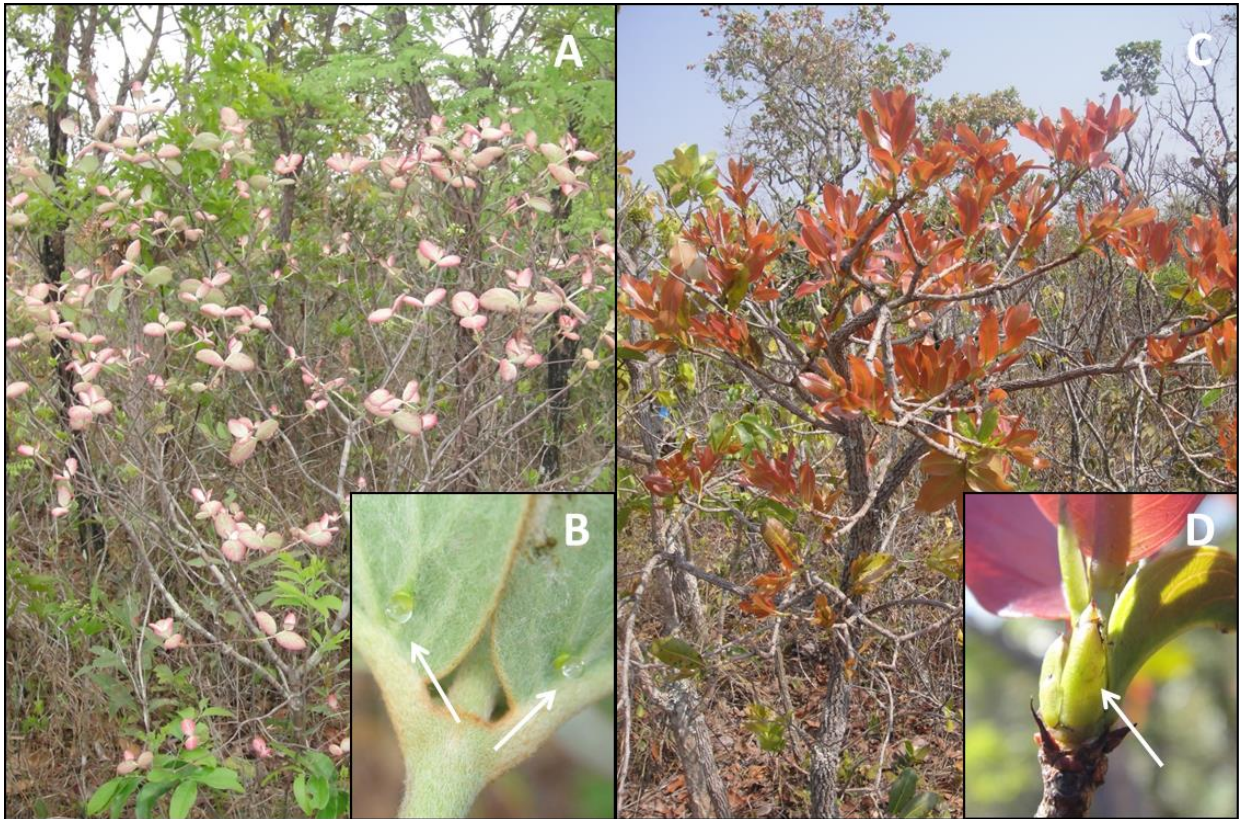


Figure 1. (A) Photograph of an individual of *Heteropterys pteropetala* used in experiment. (B) Extrafloral nectaries in *H. pteropetala*. (C) Photograph of an individual of *Ouratea spectabilis* used in experiment. (D) Extrafloral nectary in *O. spectabilis*.

Procedures

Experiments were designed according to Nahas *et al.* (2012). We tagged 60 *H. pteropetala* and 60 *O. spectabilis* individuals presenting similar phenological state (*H. pteropetala* – without leaves; *O. spectabilis* – sprouting young leaves), tree height (*H. pteropetala* 1.65 ± 0.21 m; *O. spectabilis*: 1.76 ± 0.19 m) and stem number (*H. pteropetala* 5 stems ± 3 ; *O. spectabilis*: 9 stems ± 4) in August 2013. The selected individuals were at least 5 m apart each other. Plants of each species were then randomly divided into four treatments with 15 individuals each. Plants from different groups did not differ in their phenological aspect, height (*H. pteropetala*; $F=0.463$ $p=0.709$; *O. spectabilis* $F= 1.299$ $p=0.284$) and number of stems (*H. pteropetala*; $F=0.230$ $p=0.875$; *O. spectabilis* $F=0.211$ $p=0.889$) when the experiment began.

Plants were not subjected to manipulation in the first group (*Control*), and ants and spiders had free access to all plant parts. Both the ants and the spiders were removed from the plants in the second group (*Exclusion*). Only spiders were removed from the plants in the third

group (*Ants*) and only ants were removed in the fourth treatment (*Spiders*). Ants and spiders were removed using forceps and small brushes. We removed all the spiders found during visual inspections (groups ‘Exclusion’ and ‘Ants’) every 3 days. This time interval was established during the pre-experimental phase, when we removed the spiders from seven trees, we monitored their resettlement and daily inspected these plants for four days. Recolonization during this experiment was slow and the mean number of spiders on plants was significantly lower than the original one, even after 4 days (*H. pteropetala*: $F= 10.667$ $p= 0.007$; *O.spectabilis*: $F= 6.769$ $p=0.002$ Figure 2). The visual inspection appeared to be an effective method to find the arthropods due to the size of trees used in the experiment and to the number of leaves and stems in each one of them. We manually removed the ants from plants in the ‘Exclusion’ and ‘Spider’ groups. After their removal, we covered the trunks with 5-cm large adhesive paper strap and applied a sticky resin layer over it (Tanglefoot ®; The Tanglefoot Company, Rapids, Michigan, U.S.A.) to avoid the ants to climb up. We removed all the structures from other plants that ants could use as bridges to get access to the experimental plants. We also covered ‘Control’ and ‘Ants’ treatment trunks with a paper strap and the resin was applied only in one side of the trunk, in order to control the effects of sticky resin.

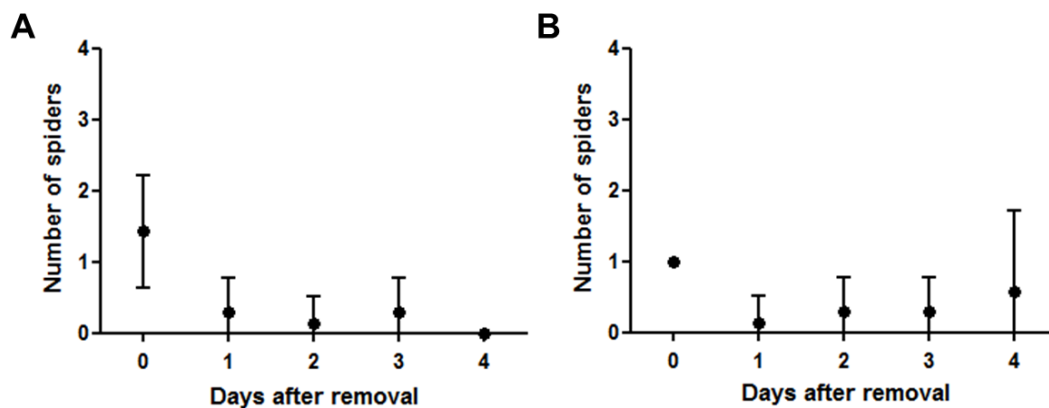


Figure 2. Recolonization of individuals of (A) *Heteropterys pteropetala* and (B) *Ouratea spectabilis* (N = 7) by spiders during four days after the experimental removal. Mean \pm SD of individuals located. Day 0 represents the day that spiders were removed.

Herbivory and plant reproduction evaluation

We inspected the individuals in all groups from both plant species three times per month (every 9 or 10 days), from the first to the last month of experiments. Experiments involving *H. pteropetala* were conducted from October 2013 to February 2014 and those involving *O. spectabilis*, from September 2013 to January 2014; it totaled five months of experiments for each species. The same researcher (LN) recorded ants, spiders and herbivores abundance and richness on plants during each inspection. Voucher specimens were collected from non-experimental plants in order to be identified. We quantified the herbivory (leaf area loss) in a monthly basis, always in the first week of the month. We recorded data from 12 leaves per plant - four from the most apical stem, four from a middle stem and four from the most basal stem - to set the monthly mean leaf area loss. This procedure was done without leaf removal. Herbivory on leaves were assessed by placing the leaves on a transparent grid (divided into millimeters). An index of herbivory from each leaf was calculated as the proportion of points in the grid falling within damaged and undamaged areas of the leaf blade (see Dirzo & Domínguez 1995). We recorded the plant phenology in fortnightly basis (young and mature leaf presence and the number of floral buds, flower and fruits produced by each plant) (e.g. Torezan-Silingardi & Oliveira 2004).

Statistical analyses

The total amount of leaf damage, herbivores richness (number of morphospecies) and abundance (in total and separated in chewing and sucking) and the number of buds and flowers were compared by two-way ANOVA (Two-way Analysis of Variance) - the presence/absence of each predator group (ants and spiders) was treated as a separate factor. The significant interaction among these factors would indicate the occurrence of an emergent effect. Parametric test assumptions were tested using the Levene's test for variance equality and the Lilliefors' test was used to check normality. Whenever it was necessary, data were log or square root transformed in order to perform the analysis. We used the permutational ANOVA in the PERMANOVA software when parametric tests assumptions were violated and it was not possible to perform data transformation (e.g. abundance of sucking herbivores).

We compared the number of morphospecies and ants abundance in the 'Control' and 'Ants' groups to evaluate the influence of spiders on ants, using t-test. The same procedure was

adopted to analyze ants influence on spider' richness and abundance, comparing 'Control' and 'Spiders' groups.

Spiders were divided into five guilds according to Heikkinen & MacMahon (2004) and to Halaj *et al.* (2000): orb weavers (e.g. Araneidae, Tetragnathidae), cobweb spiders (e.g. Theridiidae), jumpers (e.g. Salticidae and Oxyopidae), ambushers (e.g. Thomisidae) and pursuers (e.g. Anyphaenidae). The abundance of each guild was compared between the "Control" and "Spiders" groups using the t-test or the Mann Whitney test, when it was not possible the data transformation. The number of records of spiders and ants on plants found in each month were compared by Chi square test in Bioestat 5.0 software. All other analyses were performed using the Systat 10.2 software. In all tests, significance was established at $p \leq 0.05$.

Results

The exclusion experiments showed that spiders presence resulted in significantly lower herbivory in both the *H. pteropetala* and the *O. spectabilis* (Tables 1 and 2). However, spiders and ants had emergent impact on herbivore richness and abundance in both plant species (Tables 1 and 2). When the herbivores were separated into the chewing and sucking group, the analysis showed that spiders and ants emergent effect on *H. pteropetala* was marginally significant for sucking herbivores, whereas on *O. spectabilis*, the effect appeared to be significant for the chewing herbivores only (Tables 1 and 2). As for *H. pteropetala*, the most abundant chewing herbivores were the Chrysomelidae beetles, and the most abundant sucking herbivores were the bugs from the family Coreidae. With regards to *O. spectabilis*, the most abundant chewing herbivores were the Chrysomelidae beetles, Lepidoptera larvae (e.g. *Udranomia spitzii*: Hesperidae) and the Acrididae; the most abundant sucking herbivores were the leafhoppers from the family Cicadellidae (Appendix 1 for all herbivores found on plants).

Table 1. Results of two-way ANOVAs and Permutational Anova (*) on data used to compare the effects of spiders, ants and both predators on herbivory, richness and abundance of herbivores (total and separated in chewing and sucking) on *Heteropterys pteropetala*.

Source	SS	df	F	P
Herbivory (log transf. data)				
Spiders	1.183	1	8.958	0.004
Ants	0.000	1	0.001	0.973
Spiders x Ants	0.002	1	0.018	0.893
Error	7.398	56		
Herbivore richness (square root transf. data)				
Spiders	1.198	1	2.417	0.126
Ants	0.925	1	1.865	0.178
Spiders x Ants	1.936	1	3.904	0.053
Error	27.765	56		
Abundance of herbivores (square root transf. data)				
Spiders	1.618	1	1.977	0.165
Ants	0.833	1	1.018	0.317
Spiders x Ants	3.383	1	4.133	0.047
Error	45.833	56		
Abundance of chewing herbivores (square root transf. data)				
Spiders	0.184	1	0.357	0.553
Ants	0.013	1	0.025	0.876
Spiders x Ants	1.136	1	2.203	0.143
Error	28.877	56		
Abundance of sucking herbivores *				
Spiders	1862.2992	1	0.732	0.440
Ants	6864.8731	1	2.699	0.081
Spiders x Ants	7879.7384	1	3.098	0.059
Residual	141436.0101	56		

Table 2. Results of two-way ANOVAs and Permutational Anova (*) on data used to compare the effects of spiders, ants and both predators on herbivory, richness and abundance of herbivores (total and separated in chewing and sucking) on *Ouratea spectabilis*.

Source	SS	df	F	P
Herbivory				
Spiders	372.740	1	10.581	0.002
Ants	9.405	1	0.267	0.607
Spiders x Ants	15.857	1	0.450	0.505
Error	1937.579	55		
Herbivore richness (log transf. data)				
Spiders	0.003	1	0.046	0.830
Ants	0.048	1	0.882	0.352
Spiders x Ants	0.237	1	4.378	0.041
Error	2.975	55		
Abundance of herbivores (square root transf. data)				
Spiders	0.030	1	0.096	0.758
Ants	0.293	1	0.947	0.335
Spiders x Ants	1.224	1	3.949	0.052
Error	17.050	55		
Abundance of chewing herbivores (square root transf. data)				
Spiders	0.014	1	0.063	0.802
Ants	0.946	1	4.181	0.046
Spiders x Ants	1.093	1	4.834	0.032
Error	12.441	55		
Abundance of sucking herbivores *				
Spiders	450.7109	1	0.160	0.798
Ants	229.0069	1	0.081	0.888
Spiders x Ants	1641.3526	1	0.583	0.483
Residual	146187.6692	55		

Ants negatively affected spiders' richness (*H. pteropetala* $t = -3.655$; $p = 0.001$; *O. spectabilis* $t = -2.330$, $p = 0.028$) and abundance (*H. pteropetala* $t = -4.153$; $p < 0.001$; *O. spectabilis* $t = -2.240$, $p = 0.034$) in both plant species (Figures 3 and 4). When each spider guild was separately analyzed, we observed that ants presence negative effect on spider abundance was just significant for the "Ambushers" in *H. pteropetala* ($U = 43500$, $p = 0.003$; Figure 3) and for the "Jumpers" in *O. spectabilis* ($t = -2.168$, $p = 0.039$; Figure 4). We found 26 spider species foraging on *H. pteropetala* and 40 species foraging on *O. spectabilis* (Appendix 1 for all spider species found on plants). *Chira lanei* (Salticidae), *Oxyopes macroscelides* (Oxyopidae) and *Tmarus sp2* (Thomisidae) were among the most abundant spider species in both plant species.

The spider presence did not affect ants' richness (*H. pteropetala* $t = -1.266$ $p = 0.216$; *O. spectabilis* $t = 0.221$ $p = 0.827$) and abundance (*H. pteropetala* $t = -1.094$ $p = 0.283$; *O. spectabilis* $t = 0.135$ $p = 0.894$) in any of the plant species. We found 16 ant species foraging on *H. pteropetala* and 15 foraging on *O. spectabilis* (Appendix 1 for all ant species found on plants). The most frequent species on *H. pteropetala* were: *Camponotus crassus*, *Ectatomma tuberculatum*, *Cephalotes pusillus* and *Camponotus blandus*. The main species on *O. spectabilis* were: *Camponotus crassus*, *Cephalotes pusillus* and *Camponotus blandus*.

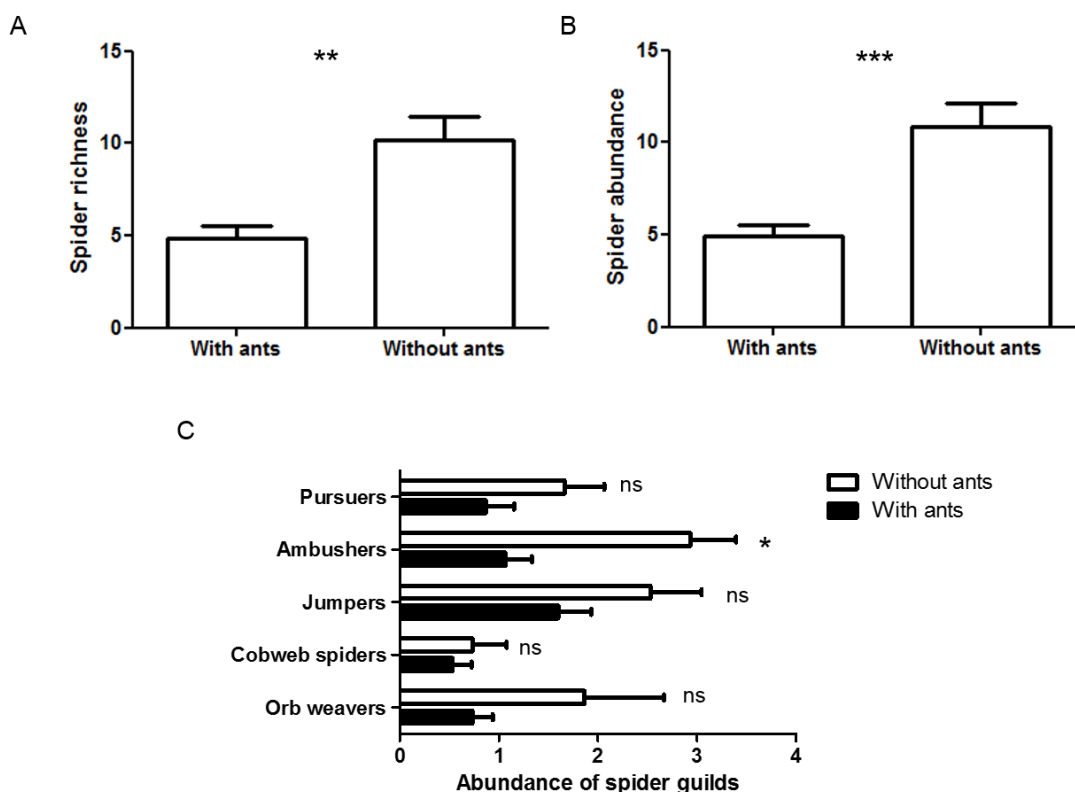


Figure 3. Spider richness (A), abundance (B), and the abundance of different spider guilds (C) in *H. pteropetala* plants with (control group) and without ants (spider group). Richness refers to the number of species per tree and abundance refers to the number of individuals per tree. Means + 1SE are presented. * $P < 0.005$, ** $P < 0.003$, *** $P < 0.001$. (ns indicates no difference between samples).

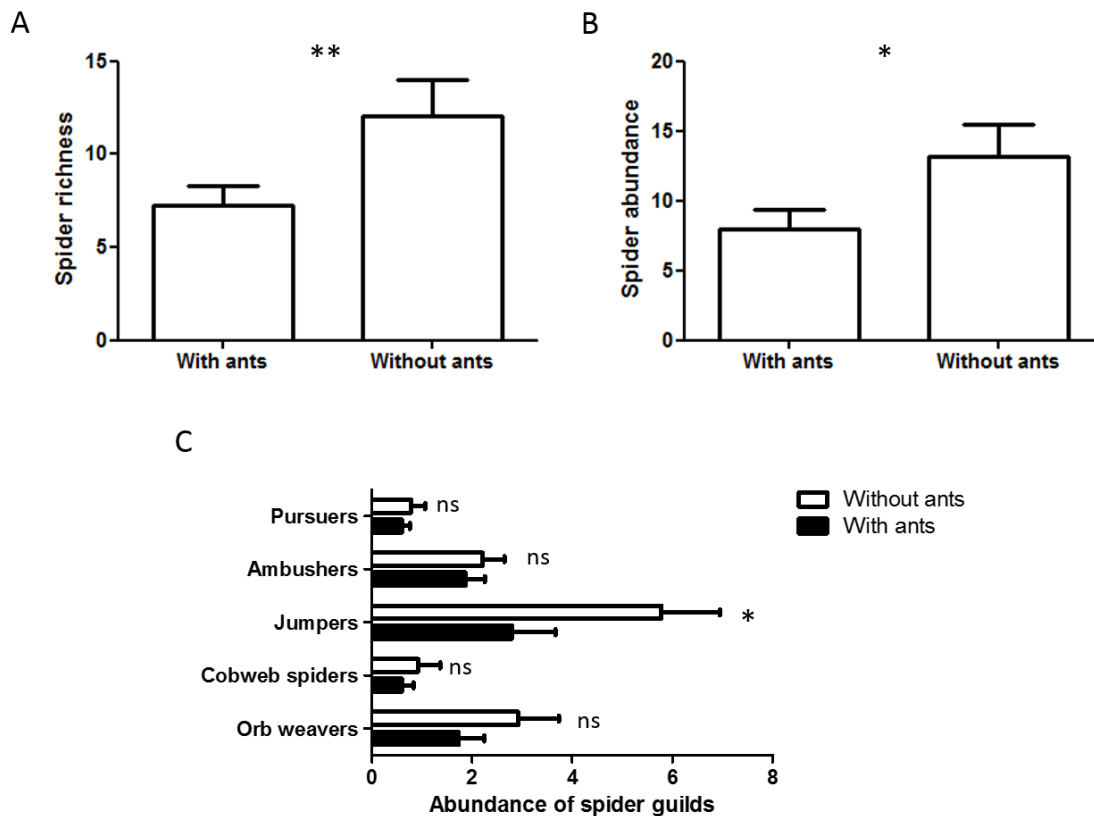


Figure 4. Spider richness (A), abundance (B), and the abundance of different spider guilds (C) in *O. spectabilis* plants with (control group) and without ants (spider group). Richness refers to the number of species per tree and abundance refers to the number of individuals per tree. Means + 1SE are presented. * $P < 0.05$, ** $P < 0.03$. (ns indicates no difference between samples).

Ants were more abundant on *H. pteropetala* in October and November, whereas herbivores and spiders were more abundant in December and January (Figure 5). Ants frequency on these plants was higher at the beginning of the experiments, whereas spiders were more frequent in the last months of them (Figure 6). Herbivores abundance on *O. spectabilis* did not change much over the months. Ants were more abundant in the first months, whereas spiders were more abundant on these plants in the last month of the experiment (January) (Figure 5). Ants frequency on plants was higher at the beginning of the experiments, whereas the spiders were more frequent in the last months of them (Figure 6).

We could not analyze spiders and ants influence on buds, flowers and fruits production, as well as on the ratio of fruits generated per buds in *O. spectabilis*, since only five plants produced buds, flowers and fruits. There was no predators' effect on the number of buds and flowers produced in *H. pteropetala*. It was not possible to analyze productivity (fruits generated per produced buds) in this species, since only three plants bore fruits. Rainfall was lower in the months of October, November and December (2013) than in the previous year. Moreover,

hemipterans from the family Coreiidae and beetles from the family Chrysomelidae (*Chlamisus minax*) were quite abundant on *H. pteropetala*. These herbivores intensively attacked the buds when they began to emerge. These factors may have contributed to the low productivity recorded for the plants during the studied period.

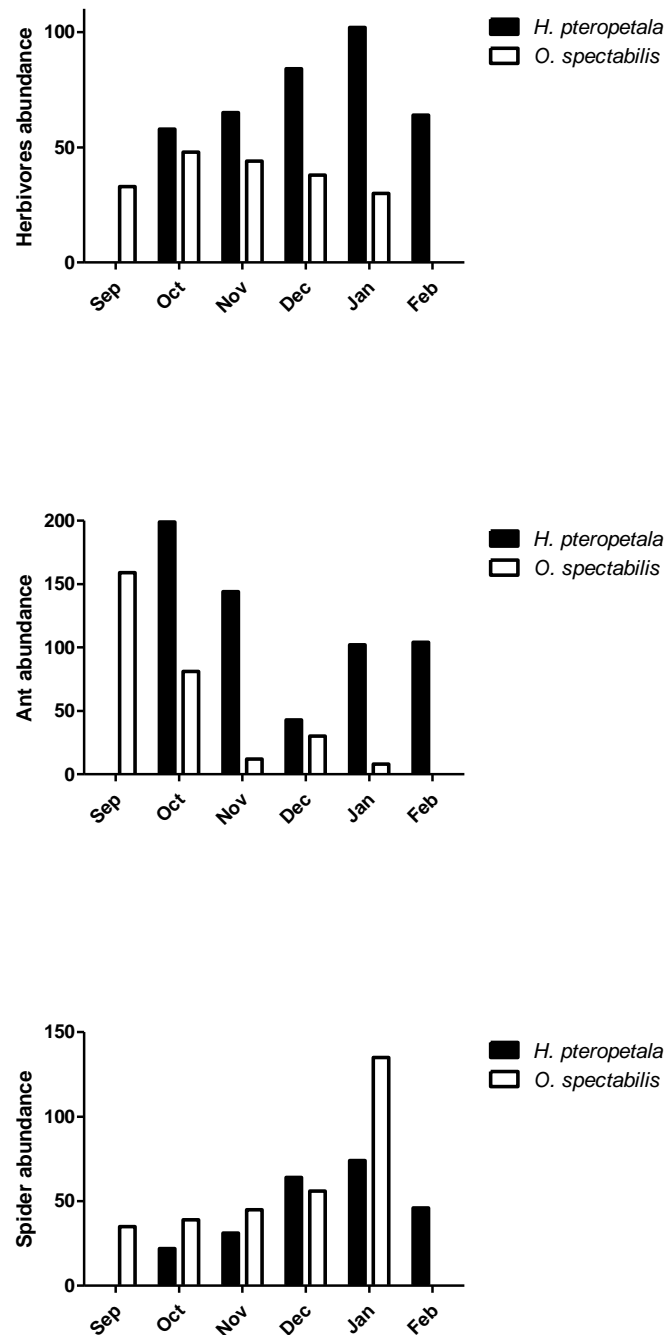


Figure 5. Abundance (number of individuals) of herbivores, ants and spiders in *H. pteropetala* (Malpigiaceae) and *O. spectabilis* (Ochnaceae) between September 2013 and February 2014 in the cerrado study site.

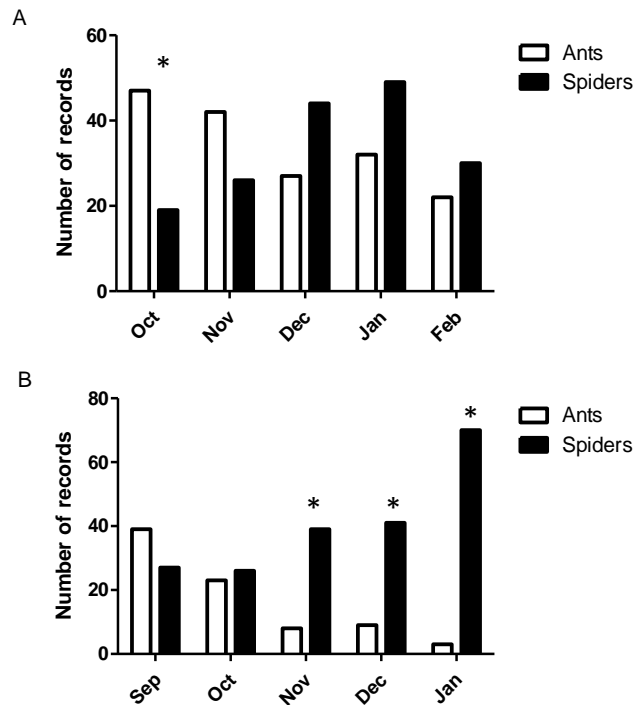


Figure 6. Number of records of ants and spiders between September 2013 and February 2014 in the cerrado savanna study site in (A) *Heteropterys pteropetala* (Malpighiaceae) and (B) *Ouratea spectabilis* (Ochnaceae). * represents significant statistical difference between number of records of ants and spiders (Qui cuadrado $p < 0.05$).

Discussion

Herbivore and herbivory suppression may vary when multiple predators are involved in interactions with plants and the resulting effects can not be predicted simply by knowledge of the individual impacts from each of these predators (Griffen 2006; Nahas *et al.* 2012). The effects of these multiple predators (MPE) is often emergent, in other words, it can be higher or lower than the expected by adding the effects of each predator (e.g. Cuautle & Rico-Gray 2003; Denno *et al.* 2004, Nahas *et al.* 2012; Stefani *et al.* 2015). Unlike our initial hypothesis, which had established that both the spiders and the ants would have an emergent impact on herbivory, only spiders affected plants herbivory in both studied systems (*H. pteropetala* – herbivores – spiders and ants and *O. spectabilis* – herbivores – spiders and ants). Nahas *et al.* (2012), showed an emergent impact of spiders and ants on herbivory in another system studied in the same study site. Stefani *et al.* (2015) found a significantly higher number of fruits per buds, seeds per fruits and seed viability in the combined presence of spiders and ants on *Eriotheca gracilipes* (Malvaceae). Several factors may have led to the lack of benefit brought by ants to plants in the present study. First, although ants were abundant and frequent on plants at the beginning of the

experiments, they were not abundant during all the studied period. In the last months, we observed that ant frequency was lower than spider frequency, mainly on *O. spectabilis*. It is possible that spiders have become more important to the biological defense because they were more frequent on the plants. According to Bronstein (1998) the magnitude of benefits to an organism (in this case, plants) interacting with another one (in this case, ants and spiders) depends not just on whether any partner is present, but also on how many partners are present, in other words, the abundance of interacting individuals is important to the final outcomes of these interactions.

There was an emergent effect of spiders and ants on herbivore richness and abundance. Herbivores were more intensely suppressed than the expected in the presence of both predator groups. Studies show that greater predator diversity may decrease herbivores density. In this case, predators would have a complementary effect on herbivores either because different predators consume different herbivores or because different predators cause different herbivores to hide or to leave the plants (Byrnes *et al.* 2006). Cardinale *et al.* (2003) showed that in the presence of three predator guilds, aphid species density was more reduced than it was predicted due to the impact of each predator, and it reveals an emergent impact, just as we have evidenced in the current study. However, another aphid species was not affected by the predators, and it reveals that the impact may differ depending on the herbivore identity. As for the present study, we expected that the emergent effect on herbivores would result in an emergent effect on herbivory, mainly on *O. spectabilis*, since the analyses in this species showed that the chewing herbivores were affected by both predator groups and these herbivores were obviously those that have contributed to herbivory as it was evaluated. However, our expectation was not fulfilled. Therefore, we can suggest that herbivores affected by ants were not the same herbivores responsible for the herbivory. For example, the lepidopteran larvae *Udranomia spitzii* (Hesperiidae) is very common on *O. spectabilis*, and in their initial stages they are more vulnerable to ants than they are in their final stages (Bächtold *et al.* 2012). This chewing herbivore was mainly found on plants without ants in the current study. On the other hand, it was not very abundant on plants, and probably did not significantly contribute to the recorded herbivory. Moreover, the nocturnal herbivores were not sampled. These herbivores may also have contributed to the recorded herbivory, and they may have been affected by spiders. We cannot state that we sampled all herbivores that contributed to herbivory, since they were sampled three times per month on both the *H. pteropetala* and the *O. spectabilis*. Predators can affect different herbivores in different magnitudes. Denno *et al.* (2003) showed that

Lycosidae spiders were effective to suppress a sucking herbivore species abundance, but they were not effective in other three phytophagous species feeding on plants.

Spiders and ants emergent effect on sucking herbivores in *H. pteropetala* was marginally significant. These herbivores did not contribute to the herbivory as it was evaluated. The analyses showed that chewing herbivores were not affected on this plant species. One of the most abundant chewing herbivore on this plant was *Chlamisus minax* (Chrysomelidae). Réu & Del-Claro (2005) showed that this herbivore is not affected by the presence of ants on this plant. Adults found by ants simply fly away or fall on the ground, below the host plant, whereas the larvae retract into the case, and the strongly sclerotised head capsule forms a secure seal. It is possible that herbivores that consumed the leaves were not completely sampled or that although their richness and abundance was not affected, their activity was impaired in the presence of spiders only. Predators can have positive effects on plants by directly reducing herbivores density due to predation and by indirectly reducing their feeding activity, fact that causes them to increase vigilance and to change their behavior in response to the predation risk. In both cases, predators reduce herbivores ability to damage plants (Lima 1998; Schmitz & Suttle 2001). Several biotic and abiotic factors can affect herbivore occurrence on plants, as the differences observed in present study in relation to herbivores presents and herbivores affected by ants in each plant species. For example, Bachtold *et al.* (2014a) showed that plant structure plays a major role to the herbivore *U. spitzi* skipper choice. The two studied vegetal species have very different architectures, and it may have led to the herein observed differences. Predators' effects may also differ depending on the involved species. For example, Bachtold *et al.* (2014b) showed that myrmecophilous lycaenids eggs are significantly more abundant on the *Heteropterys byrsonimifolia* branches with ants on them, especially those with *Camponotus crassus* and *Camponotus blandus* on them. These two ant species are known to attend to lycaenids.

Ants were more abundant in the beginning of the experiments, on both the *H. pteropetala* and the *O. spectabilis*. The extrafloral nectaries were active in both species during this period. Ants abundance abruptly decreased in *O. spectabilis* mainly in the third month of the experiment. This species has nectaries in the stipules and from the third month of the experiments, the leaves were mature and had no extrafloral nectar. Moreover, there was no nectar in the pedicels because the plants produced very few buds. Thus, patrolling by ants may be conditioned to nectar availability. Mutualism between ants and plants mediated by extrafloral nectaries is facultative, with several ant species visiting the plants and low fidelity to the association (Bronstein *et al.* 2006). Rudgers (2004) showed that ant visitations to the

species *Gossypium thurberi* (Malvaceae) diminished when the extrafloral nectaries were experimentally reduced and it resulted in herbivory increase. Calixto *et al.* (2015) showed after the leaves have expanded, the EFNs halt their nectar production in *Qualea multiflora* (Vochysiaceae) and ants no longer protected the plants against the herbivores. Plants are not always benefited by the interactions with ants. The benefits depend on several factors, such as the identity of the involved ant species, herbivores identity, the visited plant parts, if ants protect natural enemies of the plants (e.g. homopterans) and on other factors (Bronstein *et al.* 2006). For example, Vilela *et al.* (2014) showed that endophytic beetles from the family Curculionidae were not affected by ants on *Banisteriopsis laevifolia* (Malpighiaceae). Larval development on plant structures helps the beetles by ensuring, among other factors, shelter against predators and parasites. In the studied plants, *Cephalotes pusillus* was one of the most frequent ant species. This species forages on plants, including on the EFNs, but it offer no benefit to the plants (Byk & Del-Claro 2010).

Spiders abundance and frequency on plants increased over the months, probably in response to the increase in the number of herbivores and / or to the decrease in the number of ants. Although there are records of spiders feeding on nectar (Taylor & Foster 1996; Taylor & Pfannenstiel 2008, Chen *et al.* 2010) and it includes the observations involving the studied plants (Nahas pers. obs.), in both studied systems, the availability of potential preys and the ants overlap on plants seem to be more important than the availability of extrafloral nectar. Spiders use cues from both the prey and the substrate as satisfactory hunting site indicators (Chien & Morse 1998).

Spiders were affected by the presence of ants on both plant species, but the opposite did not happen. It is more likely that the effect was indirect, because we did not record predation events by ants over spiders. Other studies showed the negative effects of ants on spiders (Halaj *et al.* 1997; Faria & Lima 2008; Nahas *et al.* 2012). Moya-Laraño & Wise (2007) suggest that ants and spiders interact in an asymmetrical way, because ants - as they are social species - have high recruitment potential and are often more abundant than spiders on plants. Thus, ants can affect spiders more than spiders can affect them. Data analyses of each spider guild showed that ants affected the ‘Ambushers’ guild on *H. pteropetala* and the ‘Jumpers’ guild on *O. spectabilis*. We expected that wandering spiders, which do not build webs to catch their prey, would be more strongly affected by the presence of ants, since they have straight contact with the plants. Halaj *et al.* (1997) suggest that there is an interference competition between ants and wandering spiders.

Predators frequently have an impact on the density, spatial distribution and diversity of herbivores in systems involving three (or more) trophic levels, such as plants, herbivores and predators; thereby they alter herbivory patterns and levels in the plants (Del-Claro 2004). The effect caused by the presence of multiple predators can strongly affect herbivores and plants. Such impact can be greater than the caused by the sum of their individual effects (Cardinale *et al.* 2003). The presence of multiple predators may also reduce the trophic cascades strength due to the antagonistic interactions (e.g. intraguild competition and predation) among predators (Finke & Denno 2003). Multiple predator guilds are often found in natural systems; therefore, it is necessary to know their impact on herbivores and plants together, since we could not predict the outcomes from the trophic cascades just by knowing the effects of one or another predator guild. The outcomes from the interactions involving multiple species are highly complex and context-dependent.

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Appendix 1. Ant species, spider species and herbivore families observed foraging in *Heteropterys pteropetala* (Malpighiaceae) and *Ouratea spectabilis* (Ocnaceae) between September 2013 and February 2014 in cerrado reserve.

		<i>Heteropterys pteropetala</i>	<i>Ouratea spectabilis</i>	
Ants				
Subfamily	Species			
Dolichoderinae	<i>Azteca</i> sp1	X		
	<i>Tapinoma</i> sp1		X	
Ectatomminae	<i>Ectatomma tuberculatum</i> (Olivier, 1792)	X	X	
	<i>Gnamptogenys semiferox</i> Brown, 1958		X	
Formicinae	<i>Brachymyrmex</i> sp1	X		
	<i>Brachymyrmex</i> sp2	X	X	
	<i>Brachymyrmex</i> sp3	X		
	<i>Camponotus blandus</i> (Smith, 1858)	X	X	
	<i>Camponotus crassus</i> Mayr, 1862	X	X	
	<i>Camponotus bonariensis</i> Mayr, 1868		X	
	<i>Camponotus trapeziceps</i> Forel, 1908		X	
	<i>Camponotus</i> sp1		X	
Myrmicinae	<i>Atta</i> sp1	X		
	<i>Cephalotes pusillus</i> (Klug, 1824)	X	X	
	<i>Crematogaster quadriformis</i> Roger, 1863		X	
	<i>Crematogaster</i> sp1	X		
	<i>Ochetomyrmex semipolitus</i> Mayr, 1878	X	X	
	<i>Pheidole oxyops</i> Forel, 1908	X	X	
	<i>Pheidole</i> sp1	X		
Ponerinae	<i>Solenopsis</i> sp1	X		
	<i>Neoponera villosa</i> (Fabricius, 1804)		X	
Pseudomyrmecinae	<i>Pseudomyrmex</i> gr. <i>elongatus</i> sp. 1	X		
	<i>Pseudomyrmex flavidulus</i> (Smith, 1858)	X	X	
Spiders				
Family	Species			
Anyphaenidae	<i>Aysha</i> sp1		X	
	<i>Aysha</i> sp2	X		
Araneidae	<i>Sanogasta</i> sp1		X	
	<i>Alpaida</i> sp1	X	X	
	<i>Araneus venatrix</i> (C. L. Koch, 1838)	X	X	
	<i>Araneus</i> sp1	X		
	<i>Cyclosa</i> sp1	X		
	<i>Eustala</i> sp1		X	
	<i>Hypognatha</i> sp1		X	
	<i>Manogea porracea</i> (C. L. Koch, 1838)	X	X	
	<i>Parawixia</i> sp1		X	
	<i>Verrucosa</i> sp1		X	
Eutichuridae	<i>Wagneriana</i> sp1		X	
	<i>Wagneriana</i> sp2	X	X	
Eutichuridae	<i>Cheiracanthium inclusum</i> (Hentz, 1847)		X	
	Oxyopidae	<i>Hamataliwa</i> sp1	X	X
		<i>Oxyopes macroscelides</i> Mello-Leitao, 1929	X	X
Pisauridae	<i>Oxyopes salticus</i> Hentz, 1845		X	
	Pisauridae sp1	X		
Pisauridae sp2	X			

Salticidae	<i>Chira lanei</i> Soares & Camargo, 1948	X	X
	<i>Cylistella</i> sp		X
	<i>Cyllodania</i> sp1		X
	<i>Helvetia</i> sp1	X	X
	<i>Lyssomanes</i> sp1	X	X
	<i>Synemosyna</i> sp1		X
	<i>Thiodina</i> sp1		X
	Euophryinae sp1	X	X
	Dendryphantinae sp1		X
	Dendryphantinae sp2	X	X
	Dendryphantinae sp3		X
	Salticidae sp1	X	X
	Salticidae sp2		X
Scytodidae	<i>Scytodes</i> sp1		X
Senoculidae	<i>Senoculus</i> sp1		X
Sparassidae	Sparassidae sp2	X	
Tetragnathidae	<i>Leucauge</i> sp1		X
	<i>Tetragnatha</i> sp	X	X
Theridiidae	<i>Anelosimus</i> sp1		X
	<i>Cryptachaea hirta</i> Taczanowski, 1873	X	X
	<i>Theridion</i> sp1	X	X
	Theridiidae sp1		X
	Theridiidae sp2	X	
Thomisidae	<i>Synema</i> sp1	X	X
	<i>Tmarus</i> sp1	X	X
	<i>Tmarus</i> sp2	X	X
	<i>Tmarus</i> sp3	X	X

Herbivores

Order	Family		
Chewing herbivores			
Coleoptera	Alleculidae	X	
	Cerambycidae	X	
	Chrysomelidae	X	X
	Curculionidae	X	X
	Lampyridae		X
	Tenebrionidae	X	X
	Coleoptera Larvae	X	X
Lepidoptera	Geometridae larvae	X	X
	Other Lepidoptera larvae	X	X
Orthoptera	Acrididae	X	X
	Gryllidae	X	X
	Proscopiidae	X	X
	Tettigoniidae	X	X
Sucking herbivores			
Hemiptera	Alydidae	X	X
	Cicadellidae	X	X
	Cicadidae	X	
	Coccidae		X
	Coreidae	X	X
	Dictyopharidae		X
	Flatidae	X	X
	Fulgoridae	X	
	Lygaeidae	X	

Membracidae		X
Miridae		X
Nogodinidae	X	X
Pentatomidae	X	X
Pyrrhocoridae		X
Rhopalidae	X	X
Scutelleridae	X	X

CAPÍTULO 3

CONSUMO DE NÉCTAR POR ARANHAS ERRANTES E CONSTRUTORAS DE TEIA EM NECTÁRIOS EXTRAFLORAIS NA SAVANA NEOTROPICAL

Wandering and web spiders feeding on the nectar from extrafloral nectaries in neotropical savanna

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Resumo

Aranhas podem suplementar suas dietas com outras fontes alimentares, como néctar extrafloral, apesar de seus hábitos essencialmente carnívoros. Nós investigamos a presença de frutose e sua concentração em aranhas errantes e construtoras de teia coletadas em plantas com nectários extraflorais em uma área de savana neotropical por meio de testes utilizando o reagente Antrona. As aranhas testadas pertenceram a sete famílias: Anyphaenidae, Araneidae, Oxyopidae, Pisauridae, Salticidae, Theridiidae e Thomisidae. Um total de 265 (88.04%) de todos os indivíduos testados foram positivos para a ingestão de frutose. Não houve diferença na porcentagem de aranhas positivas entre espécies errantes e construtoras de teia ou entre indivíduos adultos e imaturos. Os resultados encontrados no presente estudo corroboram resultados de outros estudos que sugerem que o consumo de néctar é uma atividade comum entre aranhas e inclui uma nova família (Theridiidae) na lista de aranhas nectarívoras.

Palavras-chave: antrona, frutose, nutrição.

Abstract

Despite their essentially carnivorous habits, spiders can supplement their diets with other food sources, such as extrafloral nectar. We investigate the presence of fructose and its concentration in the bodies of wandering and web weaver spiders collected on extrafloral nectary-bearing plants from a neotropical savanna area by means of cold anthrone test. The tested spiders belonged to seven families: Anyphaenidae, Araneidae, Oxyopidae, Pisauridae, Salticidae, Theridiidae and Thomisidae. A total of 265 (88.04%) of all tested individuals were fructose intake positive. There was no difference in the percentage of positive spiders between wandering and web weaver spider species or between adult and immature individuals. Results found in the present study corroborate those from other studies that suggest that nectar feeding is a common activity among spiders and it includes a new family (Theridiidae) in the nectivory spiders list.

Key words: anthrone, fructose, nutrition.

Introduction

Spiders are essentially terrestrial carnivores (Coddington & Levi 1991). The diet of most spider species includes a wide variety of prey items (Nentwig 1987; Pekár *et al.* 2012) and only a few spider species are restricted to a single prey group, such as *Zodarion* spp. (Zodariidae) which feeds exclusively on ants (Pékar 2004), or *Mastophora* spp. (Araneidae), which preys exclusively on lepidopterans. Some spiders are able to capture and eat insects or even vertebrates with body length and weight greater than their own (Rypstra & Tirey 1991; Nyffeler & Pusey 2014).

Despite their predatory habits, spiders can supplement their diets with other food sources. Volegei & Greissl (1989) demonstrated that pollen and nectar could be nutrition components in the diet of spiders for a long period of time. Pollard *et al.* (1995) showed that nectar feeding is important for the longevity of *Misumenoides formosipes* (Thomisidae) male individuals. Actually, several other studies have recorded nectar consumption by spiders (e.g. Jackson *et al.* 2001; Taylor & Bradley 2009). Meehan *et al.* (2009), for instance, showed the extreme case of a predominantly herbivorous jumping spider (*Bagheera kiplingi*). Based on behavioral field observations and stable-isotope analyses, these authors showed that the main

item in the diet of this spider comprises specialized leaf tips (Beltian food bodies) produced by *Vachellia* spp. (Fabaceae).

Spiders can find extrafloral nectaries (glands that secrete nectar but are not involved with pollination) when they forage on plants. The extrafloral nectar is rich in mono and disaccharides, but it can also present other compounds, such as proteins, lipids, phenols, alkaloids and volatile organic compounds (Gonzalez-Teuber & Heil 2009). Thus, it can constitute an excellent complementary energy source for spiders (e.g. Taylor & Bradley 2009; Nahas *et al.* 2012; Patt *et al.* 2012) and other arthropods (e.g. Byk & Del-Claro 2011). However, some questions about herbivory among spiders need to be better investigated, for instance: does herbivory only occur in certain life stages and in few spider species or is it a general phenomenon that has been largely ignored? If this is a general phenomenon, many spiders may be considered to be omnivores rather than carnivores. Another question to be investigated is whether herbivory among spiders may be associated with specific hunting strategies (Sanders 2013).

Extrafloral nectary-bearing plants are found in at least 28% of all plant species and in 33% of all plant individuals in Cerrado (Neotropical savanna) (Rico-Gray & Oliveira 2007). Our previous studies have shown several spiders - mainly members of the families Anyphaenidae, Oxyopidae, Salticidae and Thomisidae - resting near the nectaries and sometimes feeding on them (Nahas *et al.* 2012). As for the current study, we investigated the fructose presence and its concentration in the bodies of wandering and web weaver spiders collected on extrafloral nectary-bearing plants. Different fructose, sucrose and glucose ratios can be found in the nectar (Nicolson & Thornburg 2007). Clearly, the presence of fructose in spider bodies is nectar consumption indication. Glucose, on the other hand, is a nectar component but it is also found in some spiders' blood and lymphatic elements (Barron *et al.* 1999). We tested the following hypotheses: (1) Spiders foraging on extrafloral nectary-bearing plants in Cerrado feed on nectar; (2) the wandering spiders eat bigger nectar proportions than the web weaver spiders, because they are in permanent contact with the vegetation; (3) immature spiders feed on bigger nectar proportions than the adult ones, since early-instar spiders are more vulnerable to starvation than their later-instar counterparts. Sugar meals may be important to spiderlings because they are a nutrition source during the time spiders are not able to capture prey (Anderson 1974; Kuja *et al.* 2012).

Methods

Spider collection

We manually collected spiders from extrafloral nectary-bearing plants during 32 mornings times (07:00-11:00 hours) and in one night (19:00–22:00 hours) between October 2013 and February 2014 at Clube Caça e Pesca Itororó Cerrado reserve (18°56'21.04"S, 48°16'14.33"W), in Uberlândia County, Minas Gerais State, Brazil. We searched for spiders on the following plant species: *Banisteriopsis malifolia*, *Heteropterys pteropetala* (Malpighiaceae), *Ouratea spectabilis* (Ochnaceae), *Qualea multiflora*, *Q. grandiflora*, *Q. parviflora* (Vochysiaceae), *Plathymenia reticulata* and *Stryphnodendron polyphyllum* (Fabaceae). The extrafloral nectaries of these species were active in this time of the year. We classified the collected spiders as “Wandering” (Anyphaenidae, Oxyopidae, Pisauridae, Salticidae and Thomisidae) or “Web weavers” (Araneidae and Theridiidae) according to their prey capturing strategies (Souza 2007). Sampling efforts do not necessarily reflect the relative abundance of plants and spiders.

We immediately killed spiders collected in the field by liquid nitrogen immersion. Samples were stored in a freezer at -80 °C. Within one or two days, they were heat-treated at 85 °C for 15 min, using water bath to drive off all moisture. They were then stored for further tests. Our results should be a conservative nectar consumption measure, because some digestion has most likely occurred between collection and the complete enzymatic activity arrestment (see Chen *et al.* 2010).

Fructose detection in spiders

We used the cold anthrone-sulphuric acid method described by Van Handel (1972) to test spiders for nectar feeding. This test just detects monosaccharide fructose when it appears alone or as half (with glucose) of the disaccharide sucrose. The anthrone reagent was prepared by adding 380 ml concentrated sulphuric acid to 150 ml distilled water (diluted sulphuric acid), and then by mixing 150 mg anthrone and 100 ml diluted sulphuric acid.

Spiders' anthrone test preparation consisted of placing each spider within a 5-ml test tube and on moistening it with two drops of 1:1 chloroform-methanol solution to cuticular wax removal. The spiders were gently crushed with glass stirring rod after 20 min. The anthrone reagent (0.5 ml) was added to the tubes, the tubes were agitated in vortex mixer and then held

in 26 °C water bath for one hour. A fructose-free specimen is a clear yellow. Otherwise, when the tubes are held for 1h at 26°C, the anthrone reagent turns from green to green-blue and finally to blue, depending on the amount of fructose found in the samples.

We created a series of standard sucrose solutions corresponding to 1, 2, 4, 8, 16, 32, 64, 128, and 256 µg/µl (0.1- 25.6% solutions) to set fructose concentration in the samples by dissolving 25.6 g reagent-grade sucrose in 50 ml distilled water, and added water enough to make 100 ml solution and eight two-fold serial dilutions (Haramis & Foster 1983). Next, we pipetted 200 µl of each spider sample on a plate as well as each standard sucrose solution; and read the absorbance in a spectrophotometer at 620 nm. We developed a graph of the fructose concentration values in standard solutions and their respective absorbance values. We determine the fructose concentration in all spider samples by straight-line equation.

Fructose consumption by adults / immature spiders and wandering / web weaver spiders was compared using the binomial proportion (χ^2) test in the Biostat 5.3 software (Instituto de Desenvolvimento Sustentável Mamirauá). Significance was established at $p \leq 0.05$.

Results

We collected and tested the ingested fructose from 301 spiders belonging to seven families and 39 species (considering adults and subadults). Most species and most individuals were positive for fructose (Table 1). A total of 265 (88.04%) of all tested individuals were positive. The fructose contents ranged from 0.054 to 44.532 µg/µl. Fructose concentration was above 2 µg/µl in 175 positive individuals (58.14% of all collected individuals). Literature often considers such value as being the standard limit above which it is possible to be sure about the presence of fructose in the samples even without the use of a spectrophotometer (Taylor & Pfannenstiel 2008; Chen *et al.* 2010). The highest values of fructose concentration were found in two samples of two species: an adult *Araneus venatrix* (44,53 µg/µl) collected at night on *Q. grandiflora* and an adult *Tmarus* sp2 (32 µg/µl) collected in the morning on *H. pteropetala*. The highest percentage of positive individuals was observed in Pisauridae (100%), Salticidae (92.96%) and Thomisidae (90.76%). The family Anyphaenidae presented the lowest percentage of positive individuals (76%) (Table 1). We observed several individuals quite close to the extrafloral nectaries. They were sometimes feeding on these nectaries (Figure 1).

Table 1. Percentage of fructose-positive individuals and average concentration from seven families of spiders in cerrado.

Spiders	Percent (n° fructose positive / n° spiders)	Average concentration (minimum concentration –maximum concentration)
Anyphaenidae	76% (19/25)	1.806 (0.054-11.015)
<i>Aysha</i> sp.1	100% (3/3)	
Anyphaenidae sp.1	100% (1/1)	
Anyphaenidae sp.2	100% (2/2)	
Immature specimens	68.42% (13/19)	
Araneidae	87.88% (29/33)	7.588 (0.437-44.532)
<i>Alpaida</i> sp.1	100% (3/3)	
<i>Araneus venatrix</i>	100% (6/6)	
<i>Araneus</i> sp.1	100% (4/4)	
<i>Cyclosa</i> sp.1	100% (2/2)	
Araneidae sp.1	100% (1/1)	
Araneidae sp.2	100% (1/1)	
Araneidae sp.3	100% (1/1)	
Araneidae sp.4	100% (1/1)	
Immature specimens	71.43% (10/14)	
Oxyopidae	79.17% (19/24)	3.786 (0.146-22.312)
<i>Hamataliwa</i> sp.1	100% (3/3)	
<i>Oxyopes macroscelides</i>	70.59% (12/17)	
<i>Oxyopes salticus</i>	100% (4/4)	
Pisauridae	100% (2/2)	4.722 (4.209-5.236)
<i>Architis</i> sp.1	100% (1/1)	
Pisauridae sp.1	100% (1/1)	
Salticidae	92.96% (66/71)	4.642 (0.058-17.416)
<i>Chira lanei</i> sp.1	100% (9/9)	
<i>Helvetia</i> sp.1	100% (5/5)	
<i>Lyssomanes</i> sp.1	100% (5/5)	
<i>Thiodina</i> sp.1	100% (2/2)	
Dendryphantinae sp.1	100% (2/2)	
Euophryinae sp.1	100% (5/5)	
Salticidae sp.1	100% (1/1)	
Salticidae sp.2	100% (3/3)	
Salticidae sp.3	100% (3/3)	
Salticidae sp.4	100% (1/1)	
Immature specimens	85.71% (30/35)	
Theridiidae	81.48% (22/27)	4.129 (0.222-15.371)
<i>Cryptachaea hirta</i>	77.78% (7/9)	
<i>Theridion</i> sp1	100% (1/1)	
Therididae sp1	100% (1/1)	
Therididae sp2	100% (1/1)	
Therididae sp3	100% (1/1)	
Therididae sp4	100% (1/1)	

Theridiidae sp5	100% (1/1)	
Imaturos	75% (9/12)	
Thomisidae	90.76% (108/119)	4.294 (0.118-32.000)
<i>Tmarus</i> sp.1	100% (2/2)	
<i>Tmarus</i> sp.2	97.08% (49/51)	
<i>Tmarus</i> sp.3	57.14% (4/7)	
Thomisidae sp.1	100% (1/1)	
Thomisidae sp.2	100% (1/1)	
Thomisidae sp.3	0% (0/1)	
Immature specimens	91.07% (51/56)	

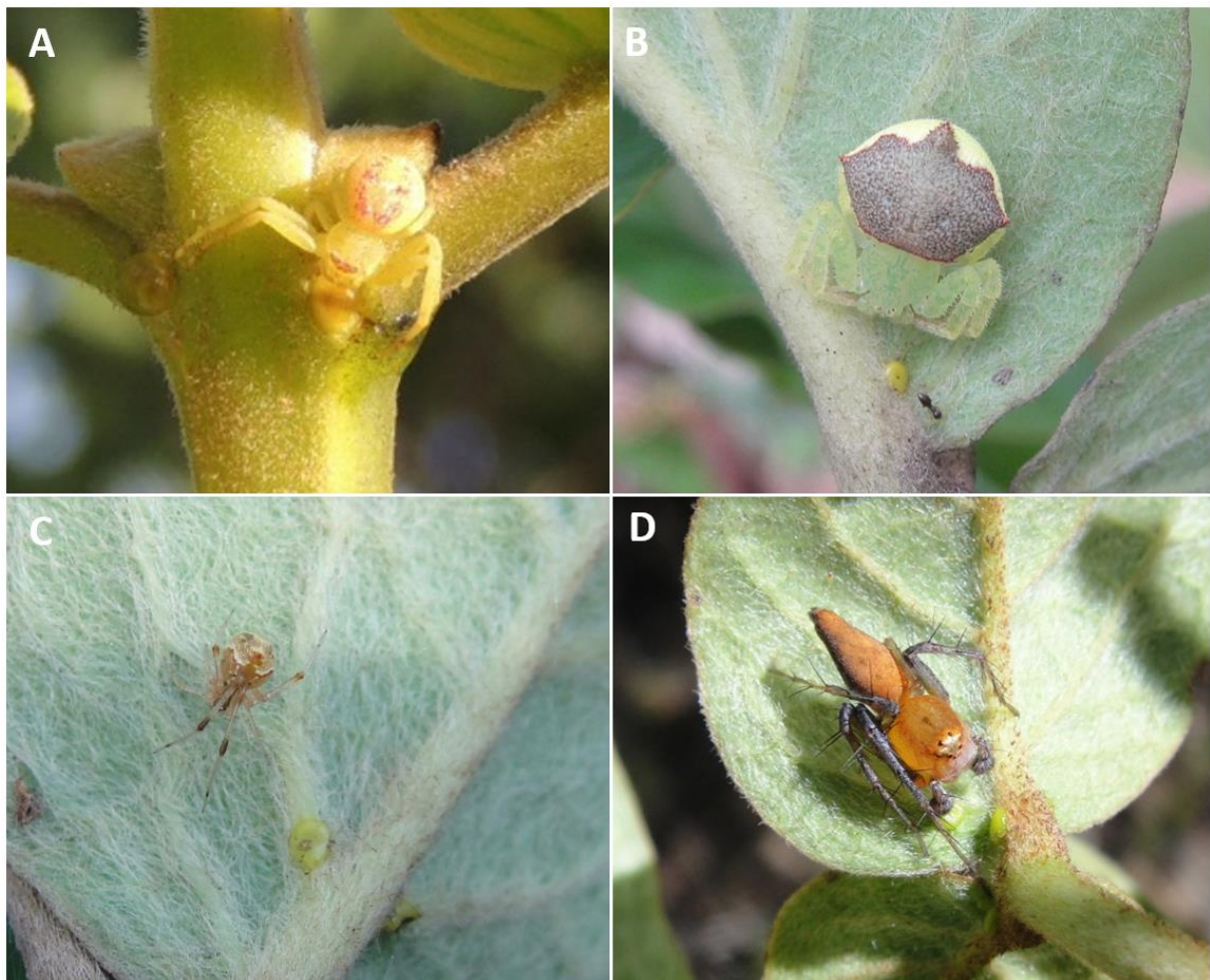


Figure 1. (A) Photograph of an individual of Thomisidae feeding on extrafloral nectary of *Qualea grandiflora* (Vochysiaceae) Photo: M.S. Pires. (B) Photograph of an individual of Thomisidae near to extrafloral nectary of *Heteropterys pteropetala*. (C) Photograph of an individual of Theridiidae near to extrafloral nectary of *Heteropterys pteropetala*. (D) *Oxyopes macroscelides* (Oxyopidae) with pedipalps on extrafloral nectary of *Heteropterys pteropetala*.

Wandering and web weaver spiders presented positive individuals rates (Table 2) and in both cases spiders were mainly collected on *H. pteropetala*. There was no difference in the percentage of positive spiders between wandering and web weaver species (Figure 2). When we analyzed each plant species there was significantly more positive wandering spider individuals on *O. spectabilis* and on *S. polyphyllum*, and significantly more web spider positive individuals on *Q. parviflora* (Table 2). However, the number of records regarding these plants was very low to lead for a reliable conclusion.

Table 2. Guild comparisons of the numbers of spiders positive for fructose in each plant species sampled. $p < 0.05$ indicates significant statistical difference between samples.

Plant species	Wandering spiders positive for fructose	Web spiders positive for fructose	χ^2 Test for independence
<i>Banisteriopsis malifolia</i>			
All concentrations	15/16 (93.75%)	5 / 6 (83.33%)	$\chi^2 = 0.613$ p=0.4790
Above 2 $\mu\text{g}/\mu\text{l}$	11 / 16 (68.75%)	4 / 6 (66.66%)	$\chi^2 = 0.032$ p=0.9254
<i>Heteropterys pteropetala</i>			
All concentrations	115 / 126 (91.27%)	17 / 22 (77.27%)	$\chi^2 = 1.163$ p=0.3167
Above 2 $\mu\text{g}/\mu\text{l}$	68 / 126 (53.97%)	13 / 22 (59.09%)	$\chi^2 = 0.232$ p=0.6984
<i>Ouratea spectabilis</i>			
All concentrations	21 / 26 (80.77%)	1 / 2 (50%)	$\chi^2 = 0.724$ p=0.0092
Above 2 $\mu\text{g}/\mu\text{l}$	13 / 26 (50%)	0 / 2 (0%)	$\chi^2 = 0.50$ p<0.0001
<i>Qualea multiflora</i>			
All concentrations	17 / 18 (94.44)	9 / 11 (81.82%)	$\chi^2 = 0.904$ p=0.3814
Above 2 $\mu\text{g}/\mu\text{l}$	12 / 18 (66.67%)	7 / 11 (63.64%)	$\chi^2 = 0.07$ p=0.8589
<i>Qualea grandiflora</i>			
All concentrations	42 / 44 (95.45%)	17 / 17 (100%)	$\chi^2 = 0.106$ p=0.7996
Above 2 $\mu\text{g}/\mu\text{l}$	40 / 44 (90.91%)	13 / 17 (76.47%)	$\chi^2 = 1.246$ p=0.2989
<i>Qualea parviflora</i>			
All concentrations	2 / 2 (100%)	1 / 1 (100%)	$\chi^2 = 0.000$ p=1.000
Above 2 $\mu\text{g}/\mu\text{l}$	1 / 2 (50%)	1 / 1 (100%)	$\chi^2 = 16.667$ p<0.0001
<i>Plathymania reticulata</i>			
All concentrations	3 / 3 (100%)		
Above 2 $\mu\text{g}/\mu\text{l}$	1 / 3 (33.33%)		
<i>Stryphnodendron polyphyllum</i>			
All concentrations	3 / 5 (60%)	1 / 1 (100%)	$\chi^2 = 10.000$ p=0.002
Above 2 $\mu\text{g}/\mu\text{l}$	2 / 5 (40%)	0 / 1 (0%)	$\chi^2 = 40.000$ p<0.0001

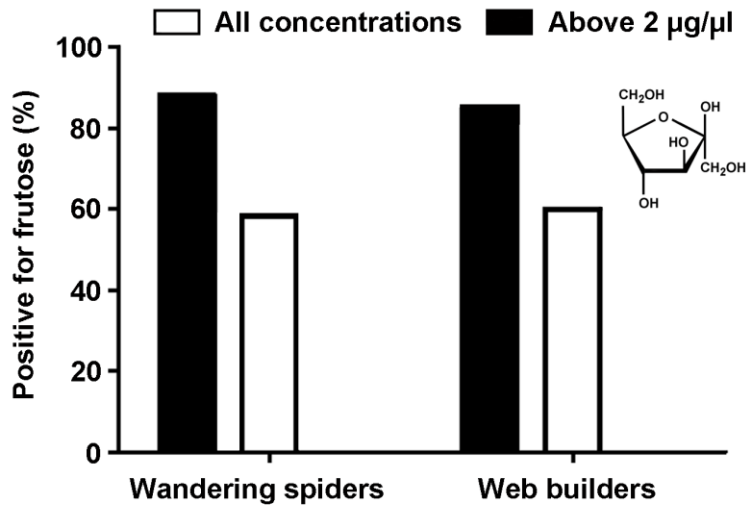


Figure 2. Percentage of positive individuals of wandering and web spiders tested for ingested fructose.

Adult and immature spiders presented high positive individual rates for fructose (Table 3). A bigger number of adults and immatures were collected on *H. pteropetala*. Overall, there was no significant difference in fructose consumption between adult and immature spiders ($X^2=0.929$; $p=0.387$). However, when we analyzed each spider family, we observed that adult Anyphaenidae and Araneidae consumed significantly more nectar than juveniles (Figure 3). When we analyzed each plant species, we observed that adults on *B. malifolia*, *H. pteropetala* and *O. spectabilis* also consumed significantly more nectar than juveniles (Table 3). There were significantly more adult positive individuals presenting fructose concentration above 2 µg/µl (Wandering spiders: $X^2=4.39$ $p=0.045$ / Web spiders: $X^2=4.973$ $p=0.032$) (Table 4 presents the percentage of positive adults and immatures in each fructose concentration class).

Table 3. Comparison between adult and immature spiders positive for fructose in each plant species sampled. p < 0.05 indicates significant statistical difference between samples.

Plant species	Immature spiders positive for fructose	Adults + subadults spiders positive for fructose	χ^2 Test for independence
<i>Banisteriopsis malifolia</i>			
All concentrations	11 / 12 (91.67%)	8 / 9 (88.89%)	$X^2= 0.043$ p=0.8946
Above 2 $\mu\text{g}/\mu\text{l}$	6 / 12 (50%)	8 / 9 (88.89%)	$X^2= 10.889$ p= 0.0013
<i>Heteropterys pteropetala</i>			
All concentrations	61 / 74 (82.43%)	72 / 76 (94.74%)	$X^2= 0.855$ p=0.3955
Above 2 $\mu\text{g}/\mu\text{l}$	32 / 74 (43.24%)	56 / 76 (73.68%)	$X^2= 7.925$ p= 0.0065
<i>Ouratea spectabilis</i>			
All concentrations	6 / 9 (66.67%)	16 / 19 (84.21%)	$X^2= 2.039$ p=0.1781
Above 2 $\mu\text{g}/\mu\text{l}$	0 / 9	13 / 19 (68.42%)	$X^2= 68.42$ p< 0.0001
<i>Qualea multiflora</i>			
All concentrations	11 / 14 (78.57%)	17 / 17 (100%)	$X^2= 2.572$ p=0.1263
Above 2 $\mu\text{g}/\mu\text{l}$	8 / 14 (57.14%)	13 / 17 (76.47%)	$X^2= 2.797$ p=0.1128
<i>Qualea grandiflora</i>			
All concentrations	34 / 36 (94.44%)	25 / 25 (100%)	$X^2= 0.159$ p=0.7437
Above 2 $\mu\text{g}/\mu\text{l}$	28 / 36 (77.78%)	23 / 25 (92%)	$X^2= 1.191$ p=0.3103
<i>Qualea parviflora</i>			
All concentrations	1 / 1 (100%)	2 / 2 (100%)	$X^2= 0.000$ p=1.000
Above 2 $\mu\text{g}/\mu\text{l}$	0 / 1 (100%)	2 / 2 (100%)	$X^2= 0.000$ p=1.000
<i>Plathymania reticulata</i>			
All concentrations	2 / 2 (100%)	1 / 1 (100%)	$X^2= 0.000$ p=1.000
Above 2 $\mu\text{g}/\mu\text{l}$	1 / 2 (50%)	0 / 1 (50%)	$X^2= 0.000$ p=1.000
<i>Stryphnodendron polyphyllum</i>			
All concentrations	2 / 3 (66.67%)	2 / 3 (66.67%)	$X^2= 0.000$ p=1.000
Above 2 $\mu\text{g}/\mu\text{l}$	2 / 3 (66.67%)	2 / 3 (66.67%)	$X^2= 0.000$ p=1.000

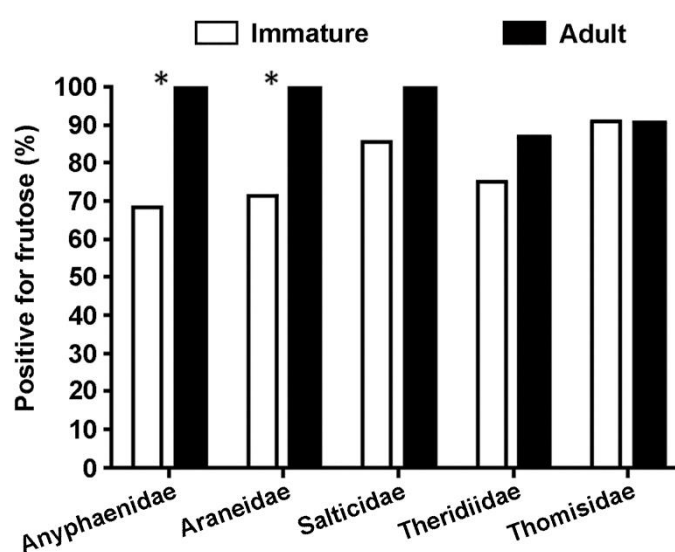


Figure 3. Percentage of individuals of five spider families tested positive (adults and immatures) for fructose ingestion. * represents significant statistical difference between adults and immatures (p<0.05).

Table 4. Percentage of adult and immature for wandering and web spiders in each class of fructose concentration.

Fructose concentration (µg/µl)	Wandering spiders		Web weaver spiders	
	Immatures	Adults	Immatures	Adults
0-2	45 / 94 (47.87%)	29 / 120 (24.17%)	9 / 19 (47.37%)	7 / 32 (21.87%)
2.1 - 4	20 / 94 (21.28%)	30 / 120 (25%)	6 / 19 (31.58%)	7 / 32 (21.87%)
4.1 - 6	18 / 94 (19.15%)	35 / 120 (29.17%)	3 / 19 (15.79%)	6 / 32 (18.75%)
6.1 - 8	6 / 94 (6.38%)	8 / 120 (6.67%)	0 / 19	1 / 32 (3.12%)
8.1 - 10	1 / 94 (1.06%)	10 / 120 (8.33%)	1 / 19 (5.26%)	4 / 32 (12.5%)
10.1 - 16	1 / 94 (1.06%)	4 / 120 (3.33%)	0 / 19	3 / 32 (9.37%)
16.1 - 20	2 / 94 (2.13%)	2 / 120 (1.67%)	0 / 19	1 / 32 (3.12%)
20.1 - 30	1 / 94 (1.06%)	1 / 120 (0.83%)	0 / 19	2 / 32 (6.25%)
>30	0 / 94	1 / 120 (0.83%)	0 / 19	1 / 32 (3.12%)

Discussion

Our results showed that nectivory by spiders in Cerrado seems to be common, since a big number of the analyzed spiders was positive for fructose ingestion and it corroborates our first hypothesis. All the seven families tested in this study presented fructose positive individuals. Six of these families had already been described in other studies as being nectar consumers (Taylor & Pfannestiel 2008; Chen *et al.* 2010; Kuja *et al.* 2012). Theridiidae species had not been found feeding on nectar yet. Chen *et al.* (2010) tested four unidentified Theridiidae individuals for fructose presence by Anthrone method and they did not find positive results.

Carbohydrates can be more important to spiders that feed on nectar than water. Jackson *et al.* (2001) confirmed by field and laboratory observations that 90 jumping spider species (Salticidae) feed on nectar. They concluded by means of choice tests done with spiders that sugar, and not just water, is relevant to salticids because these spider species spent more time drinking from a simulated nectar source (30% sucrose solution) than from distilled water during the experiment. However, probably water is also important in Cerrado, since it is a relatively hot and dry environment and spiders are sensible to desiccation (Pulz 1987). Thus, nectar could help spiders' hydration within this environment, as well as be a complementary energy source, since it is mainly composed of carbohydrates. Nectar water content depends on nectary activity, on its removal by foragers and it is additionally affected by environment humidity balance (Pacini & Nicolson 2007).

Studies also show that the access to nectar might enhance spiders' longevity because of the amino acids, lipids, vitamins, and minerals normally found in it, besides the sugars. Taylor & Pfannestiel (2009) show that Miturgidae spider species feeding on nectar present better

fitness with enhanced survival, growth, and fecundity rates, particularly when prey density is inadequate or marginal. Taylor & Bradley (2009) showed that the extrafloral nectar significantly helped survival and molting in prey-deprived *Cheiracanthium mildei* (Miturgidae) and *Hibana velox* (Anyphaenidae) individuals. Therefore, nectar consume benefits spiders in several ways and these individuals may have developed mechanisms to locate suitable nectar sources such as the extrafloral nectaries. Patt & Pfannenstiel (2008) suggest that nectarivorous spiders have sensory capabilities and programmed behaviors that are necessary for the effective nectar source detection, recognition, and location. These authors showed that *Hibana futilis* (Anyphaenidae) juveniles can recognize and remember particular chemical stimuli associated with nectar.

In the present study, the fructose detected in spiders' bodies may originate from floral or extrafloral nectaries. There is also the possibility that nectarivorous insects have transferred some fructose to spiders - acquired by them short before being eaten by spiders. However, we often observed the presence of spiders quite close to the extrafloral nectaries and sometimes they had their mouthparts on them, thus indicating nectar feeding. During the studied period, all the plants spiders were collected on presented active extrafloral nectaries. In addition, this possibility seems unlikely because none of the collected spiders were found eating a prey at the time we sampled them. The fructose acquired by nectivory preys would be harder to detect, since the insects would have metabolized at least part of it and the test just detects newly acquired fructose. We believe that the fructose found in spiders in the present study has been directly acquired by nectar feeding, due to the aforementioned factors and to other reports in the literature as well as to evidences that cursorial spiders could detect and respond to nectar odours (Patt & Pfannenstiel 2008). Chen *et al.* (2010) also used the anthrone method to detect fructose in spiders living in three different habitats. These authors observed two *Ebrechtella tricuspidata* (Thomisidae) individuals feeding at EFNs that have tested positive for fructose and one individual (a clubionid) feeding on prey, which tested negative for fructose. They suggested that if nectar can offer energy to spiders that ingest fructose, they need fewer preys to maintain themselves. Spiders can avoid some risks and energy costs involved with stalking insects and other motile arthropods just by feeding on nectaries. In a study carried out by Taylor & Pfannenstiel (2008) all the spiders found directly over the extrafloral nectaries were fructose positive.

Contrary to what we expected, the fructose consumption rate was high for both the wandering and the web weaver spiders. Thus, our second hypothesis was refused. Therefore, there was no difference regarding nectar consumption between the two studied guilds. Other

studies suggest that wandering spiders are more likely to encounter EFNs due to their wandering lifestyle, which enables more contact with vegetation (Taylor & Pfannenstiel 2008). Chen *et al.* (2010), however, showed that members of web-building families also feed on nectar. Spiders from the families Tetragnathidae, Araneidae, Agelenidae and Nephilidae were fructose positive in their authors' study. However, the percentage of fructose-positive species in these four web-building families was lower than that of non-web-building families. In Cerrado, we observed that many web weavers build shelters on leaves during the day, probably to prevent heat stress. This behavior may help finding and accessing EFNs. For instance, *Araneus venatrix* (Araneidae) individuals spend the days within leaf shelters and at night they go out to the web. The highest fructose concentration was found in an individual of this species (44,53 µg/µl). We also observed that some spiders build their webs next to extrafloral nectaries. Such behavior was demonstrated by *Cryptachaea hirta* (Theridiidae) individuals, for example. When we tested an individual of this species for fructose it also presented a high concentration of it (15,371 µg/µl).

For some spider families and plant species, the rate of positive fructose adults and subadults was higher than that of juveniles and it goes against our third hypotheses. These results can show a straight relation between body size and fructose contents. Fructose can be faster metabolized by juveniles since their bodies are smaller than the bodies of adults. Thus, spiders should be collected immediately after nectar consumption to detect fructose presence, fact that was not necessarily the case. Immatures also presented fructose positive results at a lower rate than adults (26.5 % and 66.7 % respectively) in the study conducted by Chen *et al.* (2010). These authors suggest that immatures may feed on nectar less often than adults do or that they may feed on it just as often as adults but their fructose content is too low to be detected. Kuja *et al.* (2012) also used the Anthrone method and found that fructose positive *Evarcha culicivora* (Salticidae) juveniles were significantly more often than adults after they were housed on a plant cutt for 24 hours. These authors suggested that nectar meals are especially important to the smaller juveniles.

Results in the present study corroborate those of other studies that suggest nectar feeding as being a common spider activity and it includes a new family (Theridiidae) in the list of nectivory spiders. Spiders are among the most abundant arthropods on vegetation and they can significantly contribute to herbivores and herbivory decrease on plants. Several studies show that spiders presence significantly reduces herbivores and/or herbivory (e.g. Romero & Vasconcellos-Neto 2004; Nahas *et al.* 2012) and it can positively affect plant fitness (e.g. Ruhren & Handel 1999; Stefani *et al.* 2015). However, few studies showed mutualism - when

both partners are benefited by association (e.g. Whitney 2004). Sanders (2013) have suggested that there is a greater mutualistic interaction potential between plants and spiders if one considers that many spiders feed on nectar provided by plants, than it is currently described in the literature. In the present study, we show that spiders can indeed benefit from their interactions with extrafloral nectary-bearing plants and therefore, they can establish mutualisms with such plants in Cerrado, as it is common between plants and ants (e.g. Nascimento & Del-Claro 2010; Vilela *et al.* 2014). Sanders (2013) suggested that for plants, attracting spiders seems to be a good idea, since ants are often engaged in ant-aphid mutualism and it is certainly not beneficial to the plant (but see Moreira & Del-Claro 2005). Further studies should investigate the true role of nectar in the survival and fitness of spiders within Cerrado environment and reveal the rate of different food sources in spiders' diet, by studying stable isotopes, for instance.

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CONSIDERAÇÕES FINAIS

Os resultados aqui descritos elucidam atributos básicos das interações tri tróficas entre plantas com nectários extraflorais, herbívoros, aranhas e formigas: a) demonstram que há uma fauna diversa e abundante de predadores (aranhas e formigas) que visitam plantas com NEFs dia e noite em ambiente de Cerrado; b) as redes de interações entre a comunidade de plantas e os dois grupos de predadores possuem padrão semelhante, sendo aninhado e com baixa especialização; c) constatam ainda que apesar de haver mudanças nas espécies e suas posições específicas nas redes ao longo do dia, o padrão das interações se mantém constante. Uma implicação importante desse turnover de espécies entre dia e noite é que para formigas ele é mais expressivo do que para aranhas. A fauna de formigas do core generalista da rede muda completamente dependendo do período do dia, enquanto na fauna de aranhas do core generalista há poucas mudanças entre dia e noite. Vantagens podem emergir de ambas as situações. No caso de formigas, uma mudança nas espécies representa também uma mudança nas estratégias de forrageamento e a possibilidade de afetar maior diversidade de herbívoros. No caso de aranhas, a permanência das espécies por maior período na planta pode acarretar também maior efetividade na supressão de herbívoros mesmo quando não há predação, através do risco de predação. Nós ainda observamos que em geral, formigas foram registradas nas plantas mais frequentemente no período diurno, enquanto aranhas foram mais frequentes nas mesmas plantas no período noturno. Fatores fisiológicos e bióticos (como competição intraguilda) podem ser responsáveis por tais observações.

Com relação às espécies de plantas, de modo geral, há espécies chave que são importantes para a manutenção das interações com os dois grupos de predadores estudados. Exemplos dessas espécies são *Heteropterys pteropetala* (Malpighiaceae) e *Ouratea spectabilis* (Ochnaceae). Nessas espécies de plantas nós experimentalmente avaliamos os efeitos de aranhas e formigas (juntas e isoladamente) sobre herbívoros e herbivoria. Apesar de encontrarmos um efeito emergente de aranhas e formigas sobre abundância e riqueza de herbívoros, somente aranhas reduziram significativamente a herbivoria de ambas as espécies de plantas. Isso pode ser devido, entre outros fatores, à identidade das espécies envolvidas. Por exemplo, efeitos sobre herbívoros sugadores geralmente não são detectados em avaliações de herbivoria que levam em conta a porcentagem de área foliar perdida, como foi feita nesse estudo. Esse estudo contribui para realçar a importância de estudar interações em um sentido mais amplo, envolvendo múltiplas espécies, uma vez que essa é a realidade dos sistemas naturais e os resultados dessas interações são complexos e variáveis.

Finalmente, avaliamos em que medida o néctar pode mediar interações entre aranhas e plantas com NEFs. Por meio de testes de laboratório que detectam a presença de frutose no

corpo das aranhas, constatamos que cerca de 88% das aranhas coletadas em plantas com NEFs e testadas para presença de frutose consomem néctar. O consumo de néctar foi constatado tanto para adultos quanto para imaturos e tanto para aranhas errantes quanto para aranhas que constroem teias. Ao comparar adultos e imaturos, para algumas famílias de aranhas a proporção de adultos que consomem néctar foi maior que imaturos. Ao comparar aranhas errantes e construtoras de teia, constatamos que não há diferença na proporção de indivíduos que consomem néctar. Apesar de não ter sido comprovado, no presente estudo, se o néctar foi adquirido diretamente da planta ou indiretamente pelo consumo de presas que se alimentaram dele, observações de aranhas próximas aos nectários e algumas vezes se alimentando neles nos levam a crer que o néctar tenha sido adquirido diretamente das plantas. Além disso há o fato de que o teste utilizado detecta frutose recém adquirida, e a frutose adquirida por consumo de presas seria mais difícil de detectar uma vez que já poderia ter sido metabolizada pelo inseto que a ingeriu. Esses resultados reforçam o potencial de aranhas, assim como formigas, estabelecerem relações mutualísticas com plantas com nectários extraflorais e contribuir para diminuição de herbivoria e/ou aumento do sucesso reprodutivo das plantas visitadas, ao mesmo tempo em que se beneficiam de um recurso alimentar alternativo.

Com os resultados dos presentes estudos é possível compreender melhor a estrutura das interações entre predadores e plantas com nectários extraflorais em ambiente de Cerrado, bem como sua variação ao longo do dia e os efeitos desses predadores sobre a herbivoria e herbívoros de plantas abundantes na área de estudo. Além disso, esse estudo mostrou o potencial de aranhas, assim como formigas, agirem como mutualistas de plantas com nectários extraflorais, uma vez que foi comprovado que nesse ambiente, muitas espécies de aranhas consomem néctar.