

UNIVERSIDADE DE SÃO PAULO
FACULDADE DE FILOSOFIA, CIÊNCIAS E LETRAS DE RIBEIRÃO PRETO
PROGRAMA DE PÓS-GRADUAÇÃO EM ENTOMOLOGIA

“Ecologia das interações e diversidade de insetos em espécies de Fabaceae no Cerrado”
“Ecological interactions and diversity of insects on Fabaceae species in the Brazilian Cerrado”



Bruno de Sousa Lopes

Tese apresentada à Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto da Universidade de São Paulo, como parte das exigências para obtenção do título de Doutor em Ciências, obtido no Programa de Pós-Graduação em Entomologia.

RIBEIRÃO PRETO - SP
2020

UNIVERSIDADE DE SÃO PAULO
FACULDADE DE FILOSOFIA, CIÊNCIAS E LETRAS DE RIBEIRÃO PRETO
PROGRAMA DE PÓS-GRADUAÇÃO EM ENTOMOLOGIA

“Ecologia das interações e diversidade de insetos em espécies de Fabaceae no Cerrado”

“Ecological interactions and diversity of insects on Fabaceae species in the Brazilian
Cerrado”

“Versão corrigida”

Bruno de Sousa Lopes

Tese apresentada à Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto da Universidade de São Paulo, como parte das exigências para obtenção do título de Doutor em Ciências, obtido no Programa de Pós-Graduação em Entomologia.

RIBEIRÃO PRETO - SP

2020

UNIVERSIDADE DE SÃO PAULO
FACULDADE DE FILOSOFIA, CIÊNCIAS E LETRAS DE RIBEIRÃO PRETO
PROGRAMA DE PÓS-GRADUAÇÃO EM ENTOMOLOGIA

“Ecologia das interações e diversidade de insetos em espécies de Fabaceae no Cerrado”

“Ecological interactions and diversity of insects on Fabaceae species in the Brazilian
Cerrado”

“Versão corrigida”

Bruno de Sousa Lopes

Tese apresentada à Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto da Universidade de São Paulo, como parte das exigências para obtenção do título de Doutor em Ciências, obtido no Programa de Pós-Graduação em Entomologia.

Orientador: Prof. Dr. Kleber Del Claro

RIBEIRÃO PRETO - SP

2020

Autorizo a reprodução e divulgação total ou parcial deste trabalho, por qualquer meio convencional ou eletrônico, para fins de estudo e pesquisa, desde que citada a fonte.

FICHA CATALOGRÁFICA

Sousa-Lopes, Bruno de

Ecologia das interações e diversidade de insetos em espécies de Fabaceae no Cerrado.

Ribeirão Preto, 2020.

138 p.: il.; 30 cm

Tese de doutoramento apresentada à Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto da Universidade de São Paulo.

Orientador: Del-Claro, Kleber

Palavras-chave: 1. Escala espaço-temporal. 2. Força *bottom-up*. 3. Força *top-down*. 4. Herbivoria. 5. História natural. 6. Insetos endofíticos. 7. Interações tri-tróficas.



ATA DE DEFESA

Aluno: 59131 - 9145360 - 2 / Página 1 de 1

Ata de defesa de Tese do(a) Senhor(a) Bruno de Sousa Lopes no Programa: Entomologia, do(a) Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto da Universidade de São Paulo.

Aos 27 dias do mês de julho de 2020, no(a) Auditório da Entomologia realizou-se a Defesa da Tese do(a) Senhor(a) Bruno de Sousa Lopes, apresentada para a obtenção do título de Doutor intitulada:

"Ecologia das interações e diversidade de insetos em espécies de Fabaceae no Cerrado"

Após declarada aberta a sessão, o(a) Sr(a) Presidente passa a palavra ao candidato para exposição e a seguir aos examinadores para as devidas arguições que se desenvolvem nos termos regimentais. Em seguida, a Comissão Julgadora proclama o resultado:

Nome dos Participantes da Banca	Função	Sigla da CPG	Resultado
Kleber Del Claro	Presidente	UFU(FFCLRP)	Não Votante
Rodrigo Augusto Santinelo Pereira	Titular	FFCLRP - USP	<u>Aprovado</u>
Marcelo Nogueira Rossi	Suplente	UNIFESP - Externo	<u>Aprovado</u>
Cibele Stramare Ribeiro Costa	Suplente	UFPR - Externo	<u>Aprovado</u>

Resultado Final: Aprovado

Parecer da Comissão Julgadora *

A defesa se iniciou às 8:00 horas na sala de forma mista, com /gtd-ppp-ma e ocorreu tudo bem com a apresentação do candidato e arguições. A avaliação final ocorreu apenas entre os membros da comissão julgadora sem a presença do candidato ao público. Sendo aprovada a tese.

Eu, Cesar Pereira Brites _____, lavrei a presente ata, que assino juntamente com os(as) Senhores(as) _____, Ribeirão Preto, aos 27 dias do mês de julho de 2020.


Rodrigo Augusto Santinelo Pereira


Marcelo Nogueira Rossi


Cibele Stramare Ribeiro Costa


Kleber Del Claro
Presidente da Comissão Julgadora

* Obs: Se o candidato for reprovado por algum dos membros, o preenchimento do parecer é obrigatório.

A defesa foi homologada pela Comissão de Pós-Graduação em _____ e, portanto, o(a) aluno(a) _____, jus ao título de Doutor em Ciências obtido no Programa Entomologia.

Presidente da Comissão de Pós-Graduação

Aos meus amores Nayane Alves, Melissa Alves e Benjamin Lopes, dedico.

Agradecimentos

Esta é uma parte muito prazerosa e marcante de escrever. Aqui, como já dizem muitos, não preciso me preocupar com análises estatísticas, uso de termos, figuras, referências, etc. Aqui posso ser apenas eu, aquele “caipira” do interior do Triângulo Mineiro, de Prata! Por outro lado, esta parte também representa uma grande responsabilidade, pois esquecer pessoas que foram importantes em minha vida, durante o momento mais árduo em relação à formação acadêmica, representaria muita injustiça e, até, ingratidão. Tentarei falar de todos que por mim passaram e contribuíram para que eu me tornasse uma pessoa melhor durante o desenvolvimento desta tese (as lições ficarão!). Mas, como vivemos em uma época carregada de tarefas e responsabilidades, pode ser que ocorram lapsos de memória. Sendo assim, de antemão já peço desculpas se esquecer de alguém.

Na tese abordo assuntos relacionados a interações entre insetos, plantas e predadores, mas, para que tudo isso fosse possível, eu precisei de muitas outras interações com diferentes pessoas que me ajudaram a enxergar melhor o mundo. Afinal, ninguém faz nada sozinho na vida! Bom, a **minha rede de interações de vida** é constituída pelas seguintes pessoas, cada uma com as suas particularidades:

Foram eles que me mostraram que a educação pode mudar o mundo, que me incentivaram a estudar, que não mediram esforços (financeiro, psicológico, etc.) para que eu chegasse até aqui, que me ensinaram a argumentar (ah, pai e mãe muito obrigado pelas nossas conversas sobre a vida!). Eles que me perguntavam: “como está o doutorado?” e ouviam-me falar incessantemente sobre as dificuldades e possibilidades. Eles representam grande parte do que sou e o amor que sinto por eles é incondicional. Obrigado pai, **Heli Lopes Pires**, e mãe, **Silvana Aparecida de Sousa Lopes**! Vocês representam a minha base!

Depois que formamos uma família conseguimos ver o mundo com outros olhos. Eu, por exemplo, comecei a chorar ao ver um filme (lembra-se de “Viva, a vida é uma festa?”), a ajudar mais outras pessoas, enfim, a minha família me fez um humano melhor. Um humano que se emocionou quando a sua “neguinha” falou a primeira palavra, deu os primeiros passos, escreveu pela primeira vez, fez sua primeira pintura, entre tantas outras proezas que ela fez e faz. Ah, e quantas vezes a “neguinha” ajudou o papai a cuidar dos insetos? Quantas ideias ela sugeriu para melhorar meus trabalhos? Inúmeras! Essa minha filhota é fonte de entusiasmo e criatividade. Eu me tornei também um humano que sorriu mais intensamente, vibrou e se emocionou quando a “Branca” passou no vestibular e, principalmente, quando se tornou mãe. Ah, “Branca”! Foi você a minha principal coautora: ajudou-me em campo, leu e criticou minha escrita, sugeriu novos experimentos... Sem você o meu pessoal e profissional não seriam os mesmos. Já o Benjamin, meu caçulinha sorridente, representa a felicidade e minha maior motivação nos últimos anos para concluir esta tese. Resumindo, se há pessoas que sabem o quanto foi difícil e trabalhoso chegar até aqui e me ajudaram, foram eles. Eu não tenho palavras para agradecer por tudo, mas em especial pela paciência, o amor, o apoio e a união. Eu amo vocês, **Nayane Alves da Silva, Melissa Alves Lopes e Benjamin Alves Lopes**. Muito obrigado por tudo! Vocês representam amor, inspiração e união!

Pessoas que fazem de tudo para tentar nos ajudar são raras, assim como meu irmão, **Heli Lopes Pires Junior**. Muito obrigado por tudo que fez e faz por mim. Te amo, querido!

Há pessoas que marcam nossas vidas dando exemplos de amor, cuidado e estilo de vida. Podemos até perdê-las, mas as suas lições ficam! Muito obrigado por tudo que me

ensinaram enquanto estiveram por aqui, meu querido vô “Lope” e vô “Zica”, **Anor Lopes Alves** e **Maria das Graças de Moraes**.

Obrigado a toda minha família pela presença (mesmo que esporádica) e apoio, em especial vô **Maria Neli**, tio **Edmar**, tia **Mariluce**, tia **Cláudia**, tio **Nicolau**, tio **Marcelo**, **Adelmo**, primos **Ian**, **Isa**, **Maycon**, **Allyfer**, dona **Marta Helena**, **Bruno Alves**, **Nayara Alves**, dona **Eurípedes**, Sr. **Joaquim** e **José Lázaro**.

Pessoas que te aceitam e te ajudam em sua jornada devem ser consideradas especiais. Essas pessoas merecem o respeito e admiração, como é o caso do meu orientador, **Kleber Del Claro**. Além dos exemplos de vida, ele me mostrou com grande versatilidade como posso ser um pesquisador, professor e gestor dos recursos públicos. Muito obrigado pelos ensinamentos e pela inspiração dada, mestre!

Obrigado aos professores e meus colaboradores, **Cibele Stramare Ribeiro-Costa** e **James Bryan Whitfield**. Vocês são exemplos de humanidade no meio acadêmico. Foi um prazer conhecer vocês, que são meus ídolos! Quem diria que um dia eu escreveria um (na verdade, dois! Por enquanto!) trabalho junto com uma das mais proeminentes autoras de um livro texto base para as aulas práticas de zoologia dos invertebrados, lá da graduação? Ou que eu descreveria uma nova espécie de vespa parasitoide com um dos mais renomados entre os pesquisadores desses animais?

Obrigado doutora **Fernanda Alves Martins** pela enorme ajuda com as análises estatísticas, conselhos sobre a tese e conversas. Você é uma excelente colaboradora! Admiro você e sua garra!

Obrigado doutor **Rubens Queiroz** pela identificação de algumas plantas utilizadas nesta tese. Sem tal ajuda com os “produtores” não seria possível entender as interações!

Obrigado aos **editores e revisores das revistas** cujos capítulos desta tese foram submetidos. Seus questionamentos e sugestões foram extremamente valiosos para aprimorar os manuscritos.

Obrigado **Geraldo Salgado** por me dar motivação para descrever uma espécie nova de inseto. Era um dos meus sonhos!

Obrigado aos colegas **Élida Ferreira, Vera Farinelli, Lúcia, Danielle Freire, Patrícia, “caxibremas” e “probremas”** por todas as conversas que me motivaram ao longo desses anos.

Obrigado aos grandes professores, **Odair Aparecido Fernandes, Fábio Santos Nascimento, Jean Carlos Santos e Dalton de Souza Amorim** pelas valiosas lições. Sou admirador do trabalho de vocês!

Obrigado aos colegas do **Laboratório de Ecologia Comportamental e de Interações, Eduardo Calixto, Diego Anjos, Fernando Valdivia e Diogo Vilela** pelas conversas e/ou ajuda com as revisões de capítulos desta tese. Obrigado **Isamara Mendes, Italo Aleixo, Drielly Queiroga e Vitor Posterare** pela valiosa ajuda na plantação de 80 mudas nativas de jatobá. Obrigado também **Thayná do Carmo Vieira** por durante sua IC, que tive o privilégio de coorientar, me ajudar a coletar parte dos dados utilizados nesta tese.

Obrigado à pesquisadora **Helena Onody** e ao Prof. **Carlos Brandão** por me ajudarem no depósito do material tipo na coleção de Hymenoptera do **MZSP**.

Obrigado **Aluska Tavares** pela parceria que fizemos ao estudar um pouco da história de vida dos bruquíneos. Vamos prosseguir!

Agradeço à **Renata Andrade e Vera Cicilini**, vocês são exemplos de profissionalismo e me ajudaram como "mães", sempre que precisei. Sem vocês os programas de pós-graduação da **Biologia da FFCLRP** não seriam os mesmos. Serei eternamente grato!

Obrigado ao **Programa de Pós-graduação em Entomologia da USP-RP** pela oportunidade de estudar em uma das melhores instituições de ensino superior da América Latina. Serei um eterno “USPiano”, com orgulho!

Obrigado à **Universidade Federal de Uberlândia** por ceder espaço físico para que eu pudesse realizar o meu trabalho, participar de bancas, proferir palestras. A UFU sempre será a minha casa! Ah, obrigado também por, através de uma arrogante professora de minha esposa na graduação, me mostrar que mesmo sendo doutor não é demais ter humildade e que existe vida fora da academia.

Agradeço ao **Clube Caça e Pesca Itororó de Uberlândia** por disponibilizar a reserva de patrimônio particular para estudo. Espero que as próximas gerações possam ver essa área ainda protegida!

Agradeço ao **Instituto Nacional de Meteorologia (INMET)** por fornecer os dados climatológicos utilizados nesta tese.

Agradeço aos membros de minha banca, que mesmo que ainda por vir, tenho certeza que contribuirão para o aprimoramento deste trabalho.

Finalmente, agradeço ao **CNPq** pelo financiamento desta tese (Processo: 142280/2016-0).

Sinceramente, muito obrigado a todos!

RESUMO

SOUSA-LOPES, Bruno de. **Ecologia das interações e diversidade de insetos em espécies de Fabaceae no Cerrado**. 2020. 138 p. Tese (Doutorado) - Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto-SP, 2020.

As interações inseto-planta estão entre as relações bióticas mais antigas, diversas e complexas, variando de antagônicas (*e.g.*, herbivoria) a cooperativas (*e.g.*, mutualismo de proteção). Com relação à herbivoria, sabe-se que ambas as forças *bottom-up* e *top-down* podem afetar a ocorrência dos insetos herbívoros e, por conseguinte, a estrutura das comunidades terrestres. Contudo, ainda há muito a ser elucidado sobre como essas forças seletivas variam entre os diferentes tipos de ambientes e guildas alimentares, especialmente em regiões tropicais, como é o caso do Cerrado. Além de este bioma ser um dos mais ameaçados do planeta, devido à sua rápida transformação em pastagens e/ou agricultura comercial, grandes famílias botânicas presentes nele, como Fabaceae, carecem de informações básicas sobre suas interações com os insetos. Sendo assim, os objetivos gerais desta tese foram: (1) caracterizar a comunidade de artrópodes, insetos herbívoros e seus inimigos naturais, associados às fabáceas comumente encontradas no Cerrado: *Andira humilis*, *Bauhinia rufa*, *Chamaecrista cathartica*, *Mimosa setosa* var. *paludosa* e *Stryphnodendron polyphyllum*; e (2) avaliar os efeitos das forças *bottom-up* (traços das plantas) e *top-down* (aranhas, formigas e vespas) sobre a ocorrência destes insetos herbívoros. Esta tese foi dividida em cinco capítulos. No Capítulo 1 foram descritos os insetos herbívoros, seus inimigos naturais, a rede Fabaceae-herbívoros e métricas da comunidade (índice de diversidade de Shannon, especialização, modularidade e robustez). Ao todo foram encontrados 1623 insetos herbívoros pertencentes a cinco ordens, 23 famílias e 87 espécies. Seus principais inimigos naturais foram espécies de formigas ($n= 11$), aranhas ($n= 01$), hemípteros ($n= 01$) e vespas parasitóides ($n= 03$). A rede Fabaceae-herbívoros foi especializada e modular, com apenas seis espécies de insetos se alimentando em mais de uma espécie de planta hospedeira. No capítulo 2, foi mostrado que os dois insetos herbívoros mais abundantes deste estudo, *Acanthoscelides winderi* e *A. quadridentatus*, foram responsáveis pela predação de 15% das sementes produzidas por *M. setosa* var. *paludosa*. As sementes atacadas por eles não germinaram, e mesmo as sementes saudáveis de frutos infestados tiveram pior taxa de germinação que sementes saudáveis de frutos não infestados, o que sugere que as plantas reduzem a alocação de recursos em frutos atacados. *Acanthoscelides winderi* e *A. quadridentatus* foram sincronizados com a frutificação de sua hospedeira, porém com uma partição temporal em sua ocorrência, o que sugere a evitação da competição a fim de manter a sua coexistência. No Capítulo 3 foi mostrado que os traços das sementes utilizadas por estes besouros, tais como tamanho, peso, dureza e teor de água variaram em uma escala espacial (~240 km, quatro populações de *M. setosa* var. *paludosa* de Minas Gerais a Goiás) e temporal ao longo do período de frutificação. O peso do corpo de *A. quadridentatus* foi positivamente correlacionado com o peso, o tamanho e teor de água das sementes. Sementes maiores e mais pesadas foram mais protegidas (maior dureza), mas indivíduos de *A. quadridentatus* que superaram esta proteção obtiveram maiores tamanhos, sendo machos e fêmeas 15 e 25% maiores, respectivamente. No Capítulo 4 foi mostrado que um dos principais inimigos naturais encontrados, a aranha *Peuceetia flava*, reduziu 3.3 vezes o dano causado pelos insetos herbívoros em folhas de *M. setosa* var. *paludosa*. Esta aranha, além de diminuir o número de herbívoros sobre a planta, também se alimentou de carcaças de insetos

capturados pelos tricomas glandulares da planta, o que sugere um mutualismo facultativo. Mas, se por um lado a aranha foi eficiente em proteger a planta contra seus herbívoros exofíticos foliares, por outro ela foi inábil em proteger a planta contra os endofíticos das sementes (*e.g.*, *A. winderi*). No último caso, os besouros foram eficientes em se defender da aranha ao esconderem as partes vulneráveis de seus corpos sob os seus élitros. Consequentemente, a presença de aranhas não reduziu a proporção de sementes predadas pelos besouros. Finalmente, uma nova espécie de vespa parasitoide foi encontrada no sistema. Assim, no Capítulo 5, *Cotesia itororensis* foi descrita e notas acerca de seu impacto sobre o herbívoro hospedeiro, *Oospila pallidaria*, são apresentadas. Portanto, esta tese mostra que as espécies mais abundantes de insetos herbívoros associados às fabáceas apresentaram um padrão de alta especificidade e sincronia fenológica com suas plantas hospedeiras. Tanto os traços das plantas quanto os inimigos naturais afetaram os resultados das interações, os primeiros modificando traços da história de vida dos herbívoros (*e.g.*, tamanho) e os segundos restringindo sua ocorrência. Além disso, os dados desta tese sugerem condicionalidade nos resultados das interações, os quais podem variar espaço-temporalmente e depender da guilda e/ou táxon dos insetos herbívoros envolvidos. Tendo em vista a alta especificidade de plantas hospedeiras por parte dos herbívoros, sugere-se que é crucial a conservação de áreas com grande diversidade de plantas para manter a ocorrência desses insetos e, assim, seus importantes papéis ecológicos na estruturação das comunidades terrestres do Cerrado brasileiro. Assim o fazendo, oportunamente novas espécies de insetos também serão descritas, o que ampliará nosso conhecimento sobre a biodiversidade brasileira.

PALAVRAS-CHAVE: Escala espaço-temporal, Força *bottom-up*, Força *top-down*, Herbivoria, História natural, Insetos endofíticos, Interações tri-tróficas.

ABSTRACT

SOUSA-LOPES, Bruno de. **Ecological interactions and diversity of insects on Fabaceae species in the Brazilian Cerrado**. 2020. 138 p. Thesis (Doctorate degree) - Faculty of Philosophy, Sciences and Letters of Ribeirão Preto, University of São Paulo, Ribeirão Preto-SP, Brazil, 2020.

Insect-plant interactions are among the oldest, most diverse and complex biotic relationships, ranging from antagonisms (*e.g.*, herbivory) to mutualisms (*e.g.*, protection mutualism). Regarding the herbivory, we know that both bottom-up and top-down forces affect the occurrence of insect herbivores and the structure of terrestrial communities. However, it is not well understood how these selective forces change among herbivore guilds and environments, especially in tropical areas such as the Cerrado. In addition to being one of the most threatened biomes on the planet due to its rapid transformation into pasture and/or cash-crop agriculture, large Cerrado's botanical families such as Fabaceae has few information in relation to their interactions with insects. Thus, the general aims of this thesis were to: (1) characterize the community of arthropods, insect herbivores and their natural enemies, associated with the Fabaceae species commonly found in the Cerrado: *Andira humilis*, *Bauhinia rufa*, *Chamaecrista cathartica*, *Mimosa setosa* var. *paludosa*, and *Stryphnodendron polyphyllum*; and (2) evaluate the effects of bottom-up (plant traits) and top-down (spiders, ants and wasps) forces on the occurrence of insect herbivores. This thesis was divided in five chapters. In the Chapter 1, we described the insect herbivores, natural enemies, Fabaceae-herbivore network and community metrics such as Shannon-diversity index, specialization, modularity and robustness. We found 1623 insect herbivores belonging to five orders, 23 families and 87 species. Their main natural enemies were species of ants (n= 11), spiders (n= 01), hemipterans (n= 01), and parasitoid wasps (n= 03). The Fabaceae-herbivore network was modular, with only six species of insects feeding on more than one host plant species. In the Chapter 2, we chose the two most abundant insect herbivores as models, *Acanthoscelides winderi* and *A. quadridentatus*, and found that these beetles were responsible for the predation of about 15% of seeds produced by *M. setosa* var. *paludosa*. The attacked seeds did not germinate, and even the healthy seeds from infested fruits had worse germination rate than healthy seeds from non-infested fruits, suggesting a decrease in resource allocation by plants in attacked structures. *Acanthoscelides winderi* and *A. quadridentatus* were synchronized with the fruiting, but with a temporal partitioning in their occurrence, suggesting the avoidance of competition in order to maintain their coexistence. In the Chapter 3, we found that seed traits such as size, weight, hardness, and water content varied in a spatial (~240 km, four populations of *M. setosa* var. *paludosa* from Minas Gerais to Goiás) and temporal (fruiting period) scale, affecting the occurrence of these beetles. The body weight of *A. quadridentatus* was positively correlated with seed weight, length and water content. Larger and heavier seeds were most protected (hardness), but individuals of *A. quadridentatus* that had overcome seed hardness obtained larger sizes, being males and females 15 and 25% larger, respectively. In the Chapter 5, we showed that one of the main natural enemies found, *Peucetia flava*, decreased 3.3-fold the damage inflicted by insect herbivores on leaves of *M. setosa* var. *paludosa*. In addition, this spider also fed on insect carrion provided by the plant's glandular trichomes, which suggests a facultative mutualism. If on the one hand the spider was effective in protecting the plant against exophytic leaf herbivores, on the other hand it was unable to protect the plant against endophytic seed herbivores (*e.g.*, *A. winderi*) since these beetles

were able to defend themselves against the spider by hiding vulnerable body parts under their elytra. Consequently, the presence of spiders did not reduce the proportion of seeds taken by those beetles. Finally, we found a new species of parasitoid wasp in this system. Thus in the Chapter 5 we described *Cotesia itororensis* and notes on its impact on the host, *Oospila pallidaria*. The results of this thesis highlighted that the most abundant species of insect herbivores presented a pattern of high specificity and phenological synchronicity with their host plants. Both plant traits and natural enemies affected the results of interactions, the former changing traits of herbivores' life history (e.g., size) and the latter decreasing their occurrence. We suggest conditionality in the interaction outcomes, which may vary spatiotemporally and depend on the guild and/or taxa of the insect herbivores. Furthermore, due to the high host plant specificity by insect herbivores, we suggest that is crucial the conservation of areas with huge plant diversity to maintain insects' occurrence and their important roles in structuring terrestrial communities in Brazilian Cerrado. Making that it is also possible that more new species will be described, which will increases our knowledge about the Brazilian biodiversity.

KEYWORDS: Bottom-up force, Endophytic insects, Herbivory, Natural history, Spatio-temporal scale, Top-down force, Tritrophic interactions.

SUMÁRIO

INTRODUÇÃO GERAL	01
Objetivos gerais.....	09
Referências.....	11
Capítulo 1. Plant phenology and natural enemies shape the structure of a Fabaceae-insect herbivore community in a Neotropical savanna	18
Abstract.....	19
Introduction.....	20
Material and methods.....	23
Results.....	27
Discussion.....	29
References.....	33
Capítulo 2. Temporal distribution, seed damage and notes on the natural history of <i>Acanthoscelides quadridentatus</i> and <i>Acanthoscelides winderi</i> (Coleoptera: Chrysomelidae: Bruchinae) on their host plant, <i>Mimosa setosa</i> var. <i>paludosa</i> (Fabaceae: Mimosoideae), in the Brazilian Cerrado	51
Abstract.....	52
Introduction.....	53
Material and methods.....	56
Results.....	59
Discussion.....	61
References.....	65
Capítulo 3. Spatio-temporal variation in seed traits affects the occurrence and body-size pattern of a seed-feeding beetle (Chrysomelidae: Bruchinae) in Brazilian Cerrado	72
Abstract.....	73
Introduction.....	74
Material and methods.....	78
Results.....	82
Discussion.....	83
References.....	87
Capítulo 4. Antiherbivore protection and plant selection by the lynx spider <i>Peucetia flava</i> (Araneae: Oxyopidae) in the Brazilian Cerrado	98
Abstract.....	99
Introduction.....	101
Material and methods.....	103
Results.....	106
Discussion.....	107
References.....	109
Capítulo 5. <i>Cotesia itororensis</i> sp. nov. from Brazilian savanna: a new reared microgastrine wasp (Hymenoptera: Braconidae) described using an integrative taxonomic approach	116
Abstract.....	117

Introduction.....	117
Material and methods.....	119
Results.....	120
Discussion.....	128
References.....	130
CONSIDERAÇÕES FINAIS.....	136
ANEXOS.....	138

LISTA DE FIGURAS

Introdução Geral

Figura 1. Área de cerrado, em Uberlândia, no Triângulo Mineiro (MG), onde esta tese foi desenvolvida (com exceção a uma parte dos dados do Cap. 4, os quais foram obtidos em plantas de beira de estradas dos cerrados de MG e GO). Os estudos foram feitos nas fitofisionomias de campo cerrado, cerrado sensu stricto e vereda..... 12

Capítulo 1. Plant phenology and natural enemies shape the structure of a Fabaceae-insect herbivore community in a Neotropical savanna

Figure 1. Climatogram, according to Walter & Lieth 1960, for Uberlândia, Minas Gerais, Brazil, from October 2016 to September 2017. Black areas higher than the striped ones above the temperature line represent rainfall over 100 mm per month in the wet season. The dotted areas under the temperature line show the dry season. Annual precipitation: 1426 mm. Annual mean temperature: 23.2°C..... 46

Figure 2. Fabaceae-insect herbivore network in Brazilian Cerrado. Host plants are in the left side and insect herbivores are in the right side. Host plants from top-down are: *Mimosa setosa* var. *paludosa*, *Stryphnodendron polyphyllum*, *Chamaecrista cathartica*, *Andira humilis* and *Bauhinia rufa*. Main insect herbivores from top-down are: *Acanthoscelides winderi*, Crambidae sp. 1, *Pachybrachis* sp., Tettigoniidae sp. 6 and *Gibbobruchus* cf. *scurra*. The thickness of the branches represents the number of interactions. Note that few species of insect herbivores interact with more than one plant species, displaying a modular pattern in the network. Note also that *A. humilis* (fourth from top-down) and *S. polyphyllum* (second from top-down) had higher insect herbivores' richness and *M. setosa* var. *paludosa* (first from top-down) the higher frequency of interactions..... 47

Figure 3. Annual phenology of *Andira humilis*, *Bauhinia rufa*, *Chamaecrista cathartica*, *Mimosa setosa* var. *paludosa* and *Stryphnodendron polyphyllum*. Colors light green, dark green, blue, yellow and dark red represent new leaves, mature leaves, flower buds, flowers and fruits, respectively. Numbers within circles are the monthly means for each plant phenophase, and arrows are the mean months of occurrence..... 49

Figure 4. Relationship between the abundance of insect herbivores and their main natural enemies: (a) insect herbivores and ant abundance on extrafloral-nectaried plants, (b) insect herbivores and spider abundance on glandular plants, (c) simple linear regression between insect herbivores and ant abundance, and (d) simple linear regression between insect herbivores and spider abundance. Dashed lines in figures c and d represent intervals of confidence at 95%..... 50

Capítulo 2. Temporal distribution, seed damage and notes on the natural history of *Acanthoscelides quadridentatus* and *Acanthoscelides winderi* (Coleoptera: Chrysomelidae: Bruchinae) on their host plant, *Mimosa setosa* var. *paludosa* (Fabaceae: Mimosoideae), in the Brazilian Cerrado

Figure 1. The host plant of the seed beetles, *Mimosa setosa* var. *paludosa*, in the fruiting phase in the Brazilian Cerrado. The arrow indicates some fruits..... 69

Figure 2. (a) Annual distribution of fruit availability of *M. setosa* var. *paludosa*, (b) abundance of *A. winderi*, and (c) abundance of *A. quadridentatus*. Numbers within circles are the monthly means of fruits (a) and the absolute frequency of seed beetles (b, c).

Arrows are the mean month of fruits or the occurrence of <i>Acanthoscelides</i> beetles.....	69
Figure 3. Seed beetles (Bruchinae) associated with <i>M. setosa</i> var. <i>paludosa</i> : (a) <i>A. winderi</i> , (b) <i>A. quadridentatus</i> , (c) seed beetle egg on the fruit surface, and (d) attacked seeds. The arrow indicates a single <i>Acanthoscelides</i> beetle inside a seed. Scale: 1 mm.....	70
Figure 4. Cumulative percentage of germination of healthy seeds from noninfested and infested fruits, and attacked seeds of <i>M. setosa</i> var. <i>paludosa</i> . Letters indicate significant statistical difference among treatments by Bonferroni's post hoc test at $p < 0.0001$	71

Capítulo 3. Spatio-temporal variation in seed traits affects the occurrence and body-size pattern of a seed-feeding beetle (Chrysomelidae: Bruchinae) in Brazilian Cerrado

Figure 1. Spatial distribution of the populations (P1 = Morrinhos-GO, P2 = Goiatuba-GO, P3 = Tupaciguara-MG and P4 = Uberlândia-MG) of <i>Mimosa setosa</i> var. <i>paludosa</i> (Fabaceae: Mimosoideae) from which <i>Acanthoscelides quadridentatus</i> (Chrysomelidae: Bruchinae) individuals were collected. Dark and light grey colors indicate the Brazilian states Goiás and Minas Gerais, respectively.....	93
Figure 2. Body traits of <i>Acanthoscelides quadridentatus</i> (Chrysomelidae: Bruchinae) taken to estimate its body-size pattern among populations (P1 = Morrinhos-GO, P2 = Goiatuba-GO, P3 = Tupaciguara-MG and P4 = Uberlândia-MG). The arrows indicate: (a) pronotum, (b) left elytra, and (c) right elytra.....	94
Figure 3. Correlation matrix between traits of the host plant, <i>Mimosa setosa</i> var. <i>paludosa</i> (seed weight, hardness, size, and water content), and <i>Acanthoscelides quadridentatus</i> (female weight and body size, male weight and body size). Data are presented in bars, scatter plots and with Spearman correlation values for each relationship. Analyses must be performed taking into account lines versus columns. * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$...	95
Figure 4. Number of unhatched and hatched eggs of <i>Acanthoscelides</i> beetles (Chrysomelidae: Bruchinae) in the end (September 2017) and beginning of fruiting phase (April 2018) on fruits of <i>Mimosa setosa</i> var. <i>paludosa</i> (Fabaceae: Mimosoideae).....	96

Capítulo 4. Antiherbivore protection and plant selection by the lynx spider *Peucetia flava* (Araneae: Oxyopidae) in the Brazilian Cerrado

Figure 1. Lynx spider–herbivore interactions on <i>Mimosa setosa</i> var. <i>paludosa</i> in the Brazilian Cerrado. (a) The lynx spider, <i>Peucetia flava</i> Keyserling 1877; (b) <i>Mimosa setosa</i> var. <i>paludosa</i> ; (c) glandular trichomes with a hymenopteran entrapped; (d) glandular trichomes with an isopteran entrapped; (e) <i>P. flava</i> with egg sac (black arrow) on fruit surface, offering parental care; (f) juveniles of <i>P. flava</i> dispersing (white arrow); (g) the endophytic seed beetle, <i>Acanthoscelides winderi</i> Kingsolver, 1984; (h) <i>P. flava</i> preying on the exophytic herbivore, <i>Naupactus</i> sp; (i) <i>P. flava</i> preying on an unidentified hymenopteran entrapped on the host plant. Scale bar: 1 mm, except in (b) with 1 m.....	113
Figure 2. Mean proportion (\pm SE) of foliolules damaged in spiders-absent and spiders-present treatments on <i>Mimosa setosa</i> var. <i>paludosa</i> . Note a significant statistical difference at unpaired t -test ($P < 0.0001$, $n = 10$ plants). The total number of foliolules damaged on spiders absent and spiders present were 1012 (30%) and 305 (10%), respectively.....	114
Figure 3. Mean proportion (\pm SE) of seeds damaged on <i>Mimosa setosa</i> var. <i>paludosa</i> by <i>Acanthoscelides winderi</i> in <i>Peucetia flava</i> absence or presence. Note a non-significant statistical difference at paired t -test ($P = 0.34$, $n = 13$ plants, 26 samples). The total number of seeds damaged on spiders absent and spiders present were 54 (19%) and 43 (15%), respectively.....	115

Capítulo 5. *Cotesia itororensis* sp. nov. from Brazilian savanna: a new reared microgastrine wasp (Hymenoptera: Braconidae) described using an integrative taxonomic approach

Figure 1. (a) The host plant, *Mimosa setosa* var. *paludosa* (Fabaceae), and (b) the second instar larva of *Oospila pallidaria* (Geometridae) in the Brazilian savanna..... 133

Figure 2. (a) Lateral habitus of *Cotesia itororensis* (Braconidae) female, (b) Lateral habitus of *C. itororensis* male, (c) frontal view of head of *C. itororensis* female, (d) dorsal view of head of *C. itororensis* female, (e) mesosoma and tergites of *C. itororensis* female, (f) wings of *C. itororensis* female, and (g) lateral view of posterior end of metasoma of *C. itororensis* female, showing hypopygium and ovipositor sheaths..... 134

Figure 3. (a) Parasitized larva of *Oospila pallidaria* (Geometridae) in the fourth instar, with a swollen body in the posterior half, (b) parasitized larva in the abaxial side of the leaf, and (c) dead larva and yellow cocoon of *Cotesia itororensis* (Braconidae)..... 135

LISTA DE TABELAS

Capítulo 1. Plant phenology and natural enemies shape the structure of a Fabaceae-insect herbivore community in a Neotropical savanna

Table 1. Richness (Rich.), Shannon diversity index (H), robustness (R), and Strength of Interactions (SI) of the Fabaceae-insect herbivore network in the Brazilian Cerrado.....	38
Table 2. Host plant, prey, and absolute abundance of natural enemies of insect herbivores associated with Fabaceae species in the Brazilian Cerrado.....	39
Table S1. Host plant, guild, strength of interactions, and abundance of insect herbivores sampled on Fabaceae species in the Brazilian Cerrado. Herbivorous guilds were adapted from Novotny et al. (2010). Ex. = external (exophytic) and In. = internal (endophytic).....	41

Capítulo 2. Temporal distribution, seed damage and notes on the natural history of *Acanthoscelides quadridentatus* and *Acanthoscelides winderi* (Coleoptera: Chrysomelidae: Bruchinae) on their host plant, *Mimosa setosa* var. *paludosa* (Fabaceae: Mimosoideae), in the Brazilian Cerrado

Table 1. Circular statistical analysis of the seasonality of <i>Acanthoscelides</i> beetles abundance together with fruits of their host plant, <i>M. setosa</i> var. <i>paludosa</i>	68
--	----

Capítulo 3. Spatio-temporal variation in seed traits affects the occurrence and body-size pattern of a seed-feeding beetle (Chrysomelidae: Bruchinae) in Brazilian Cerrado

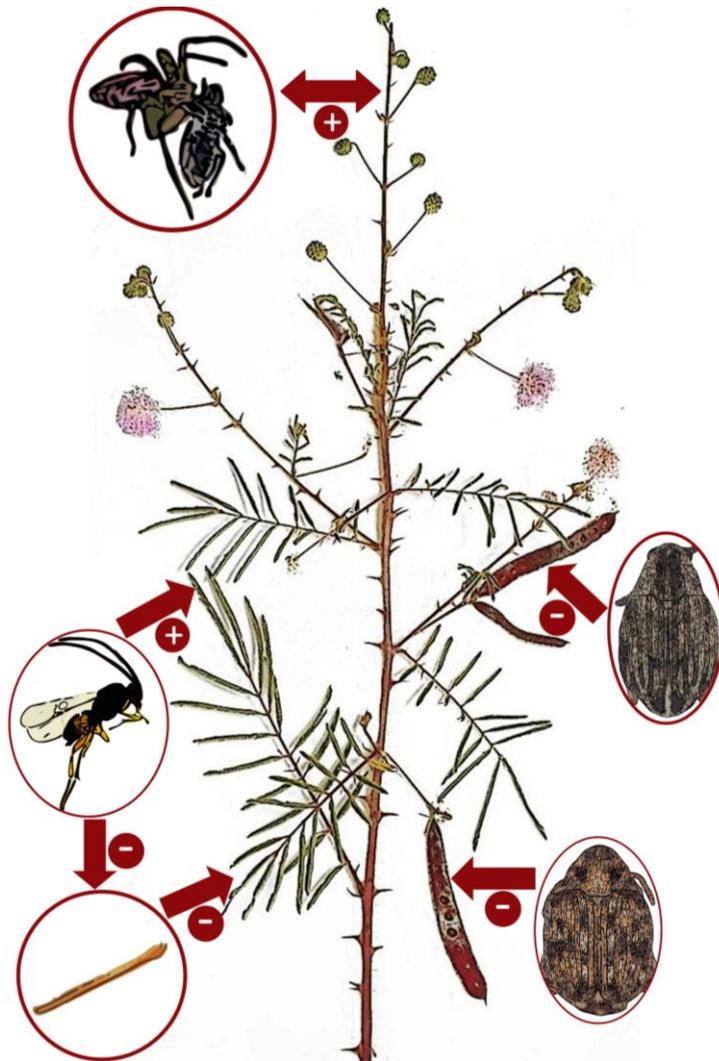
Table 1. Damaged and undamaged seeds (total number and percentage) for each population of <i>Mimosa setosa</i> var. <i>paludosa</i> (Fabaceae: Mimosoideae) in roadsides from Brazilian Cerrado. P1= Morrinhos-GO, P2 = Goiatuba-GO, P3 = Tupaciguara-MG and P4 = Uberlândia-MG. Observed frequencies differed statistically from the expected frequencies by the Chi-square test at $P < 0.0001$	91
Table 2. Seed traits (mean \pm SD) from each population of <i>Mimosa setosa</i> var. <i>paludosa</i> (Fabaceae: Mimosoideae) in roadsides from Brazilian Cerrado. P1= Morrinhos-GO, P2 = Goiatuba-GO, P3 = Tupaciguara-MG and P4 = Uberlândia-MG. Different letters represent that means differed statistically by Tukey's post-hoc test at $P < 0.05$	92
Table 3. Weight (mean \pm SD) and body size (mean \pm SD) of <i>Acanthoscelides quadridentatus</i> (Chrysomelidae: Bruchinae) according to the population of <i>Mimosa setosa</i> var. <i>paludosa</i> (Fabaceae: Mimosoideae) where they were collected. P1= Morrinhos-GO, P2 = Goiatuba-GO, P3 = Tupaciguara-MG and P4 = Uberlândia-MG; M = male and F = female. Body size is the sum of left elytra, pronotum, and right elytra. Different letters represent that means differed statistically by Tukey's post-hoc test at $P < 0.05$	92

Capítulo 4. Antiherbivore protection and plant selection by the lynx spider *Peucetia flava* (Araneae: Oxyopidae) in the Brazilian Cerrado

Table 1. Hurdle regression model between the presence and abundance of <i>P. flava</i> and plant size, carrion abundance and abundance of live insects on <i>Mimosa setosa</i> var. <i>paludosa</i> . The zero hurdle model (above) accounts for the effect of the predictors (first column) on the presence of <i>P. flava</i> . The count model (below) accounts for the effect of the	
---	--

predictors (first column) on the abundance of *P. flava*. In bold, significant result.
Marginally significant results are underlined..... 112

INTRODUÇÃO GERAL



Introdução Geral

Interações inseto-planta: aspectos gerais e sua importância na atual conjectura de declínio das populações de insetos

As interações inseto-planta estão entre as relações bióticas mais antigas, diversas e complexas (Labandeira *et al.* 1994; Grimaldi & Engel 2005; Schoonhoven *et al.* 2005). Elas são datadas em pelo menos 403 milhões de anos (herbivoria, Labandeira 2007) e variam de antagônicas a cooperativas (Price *et al.* 2011; Del-Claro 2012). Sendo assim, seus resultados podem ser condicionais, dependendo da identidade, comportamento e história de vida das espécies envolvidas (Bronstein 1994; Thompson & Cunningham 2002; Del-Claro 2004; Chamberlain *et al.* 2014; Del-Claro *et al.* 2016).

Entre as interações antagônicas, a herbivoria tem especial destaque, pois ela está amplamente difundida nas ordens de insetos com maior riqueza (Coleoptera, Diptera, Hymenoptera e Lepidoptera; Grimaldi & Engel 2005). Entretanto, a herbivoria se desenvolveu mais marcadamente em apenas oito das 30 ordens de insetos recentes (Dermaptera, Hemiptera, Orthoptera e Thysanoptera, além das outras quatro acima mencionadas; Norris 1991), o que indica que este tipo de alimentação não representa uma condição fácil de ser atingida (Vanin 2012). Isso ocorre principalmente devido ao fato dos tecidos vegetais serem pobres em nutrientes como proteínas e, além disso, altamente protegidos por defesas físicas (*e.g.*, tricomas) e químicas (*e.g.*, taninos), tornando-os impalatáveis e/ou tóxicos para os herbívoros (Schoonhoven *et al.* 2005).

Além das defesas das plantas, os insetos herbívoros também enfrentam forte pressão de seleção por parte dos predadores, os quais podem eliminá-los e, a partir de um efeito em cascata, contribuir positivamente com a *performance* e aptidão das plantas (Price *et al.* 1980; Schoonhoven *et al.* 2005). Portanto, para melhor entender a ecologia das interações inseto-planta geralmente são elucidados pelo menos três níveis tróficos:

as plantas (produtores), os insetos herbívoros (consumidores) e os inimigos naturais dos últimos (consumidores/carnívoros) (Price *et al.* 1980, 2011; Vidal & Murphy 2018).

Nestas interações tri ou multitróficas, as influências que as espécies exercem umas sobre as outras são classificadas como forças *bottom-up* (*e.g.*, plantas sobre os herbívoros, as primeiras reduzindo a densidade dos últimos) ou *top-down* (*e.g.*, herbívoros sobre as plantas ou predadores/parasitoides sobre os herbívoros, os primeiros reduzindo a densidade dos últimos) (Ehrlich & Raven 1964; Paine 1980; Carpenter *et al.* 1985; Schmitz 1994; Singer & Stireman 2005; Price *et al.* 2011; Vidal & Murphy 2018). Tais forças regulam as populações tanto de plantas quanto de animais e estruturam as comunidades terrestres (Vidal & Murphy 2018), dando também arcabouço teórico para o avanço de diversas áreas da biologia (*e.g.*, evolução) e também para fomentar programas de manejo e conservação (Thompson 2005; Price *et al.* 2011; Blüthgen 2012).

Desde o seminal estudo de Hairston *et al.* (1960), no qual foi delineada a hipótese de que o mundo é verde devido à forte pressão de seleção exercida pelos predadores sobre os insetos herbívoros, tem havido um embate em que alguns pesquisadores defendem que as forças *top-down* exercem maior pressão seletiva que as forças *bottom-up* sobre as populações de herbívoros (*e.g.*, Bernays & Graham 1998; Hoog & Daane 2015). Enquanto que, por outro lado e principalmente mais recente, vários pesquisadores têm defendido que ambas as forças *bottom-up* e *top-down* influenciam a evolução, distribuição e dinâmica populacional dos insetos herbívoros em diferentes contextos que devem ser analisados com cautela (Price *et al.* 1980; Paine 1980; Carpenter *et al.* 1985; Denno *et al.* 2005; Singer & Stireman 2005; Mooney *et al.* 2012). Outros estudos também têm mostrado que as comunidades de herbívoros podem ser moldadas por interações que ocorrem no mesmo nível trófico (*e.g.*, Kaplan & Denno

2007; Cornelissen *et al.* 2013). Contudo, ainda não é bem elucidado como essas forças seletivas variam entre os diferentes tipos de ambientes, especialmente tropicais, amplitudes de dieta (*e.g.*, especialista ou generalista) e guildas alimentares dos herbívoros (*e.g.*, endofítico ou exofítico) (Vidal & Murphy 2018). Assim, mais estudos são necessários para conhecer o efeito de ambas as forças seletivas em diferentes comunidades, principalmente envolvendo grupos taxonômicos e guildas menos estudadas (*e.g.*, endofíticos).

Devido à atual conjectura da perda de biodiversidade em escala global (*e.g.*, Hallmann *et al.* 2017; Sanchez-Bayo & Wyckhuys 2019; mas ver Simmons *et al.* 2019; Willig *et al.* 2019), em especial no Cerrado, um dos biomas mais ameaçados do mundo (ver Oliveira & Marquis 2002; Klink & Machado 2005; Strassburg *et al.* 2017), o conhecimento das interações inseto-planta está entre os mais importantes desafios para a pesquisa ecológica (Lewinsohn *et al.* 2012) e taxonômica. Afinal, as populações de insetos podem estar declinando (Sanchez-Bayo & Wyckhuys 2019), e com elas importantes papéis ecológicos, como a herbivoria. No caso do Cerrado isso seria ainda mais marcante, pois os insetos representam o principal grupo de herbívoros (Dias 1992), haja vista a discreta diversidade de herbívoros vertebrados neste bioma.

Entretanto, não se pode afirmar com certeza que as populações de insetos estão declinando nas regiões tropicais, tendo em vista que os estudos sobre esse tema são escassos nessas regiões e, ainda, os poucos resultados disponíveis sobre a diversidade e dinâmica populacional dos insetos têm mostrado resultados contrastantes nesses ambientes (Lamarre *et al.* 2020). Neste contexto, sugere-se que são necessários mais estudos de longa duração para monitorar as populações de insetos das regiões tropicais a fim de que seja confirmado ou refutado o declínio de suas populações (*e.g.*, causados pelas mudanças climáticas e outras pressões antrópicas; ver Lamarre *et al.* 2020).

Portanto, conhecer a biodiversidade interativa, da qual os insetos herbívoros fazem parte, representa o primeiro passo para compreender quais são os papéis ecológicos de cada espécie em determinada comunidade, o que possibilitará entender como os sistemas biológicos saudáveis funcionam e se conectam (Thompson 2005; Fornoff *et al.* 2019). Logo, seria possível definir como ser rápido e eficiente na resolução do provável problema supracitado (perda da biodiversidade). Mas, para tanto, ainda é necessário conhecer aspectos básicos dessas relações, como a identidade e a história natural das espécies (Del-Claro *et al.* 2013; Tewksbury *et al.* 2014).

Os insetos: uma breve contextualização sobre sua diversidade e ecologia

Desde o famoso e polêmico estudo de Terry Erwin (1982), no qual ele estimou haver mais de 30 milhões de espécies de insetos, ainda há um grande debate sobre quantas espécies de insetos devem realmente existir. Diferentes estimativas têm sugerido que esse número pode variar entre cinco e 100 milhões (Adler & Footitt 2009; Berenbaum 2009; Stork 2018); porém, o que se sabe hoje é que mais de um milhão de espécies de insetos já foram descritas, mas representando um percentual pequeno, que varia entre 10 e 20% das espécies que devem existir (Lewinsohn *et al.* 2012; Stork 2018).

Uma das maneiras de conhecer a diversidade de insetos é fazendo inventários em diferentes plantas e escalas espaço-temporais, pois muitos insetos possuem alta especificidade de plantas hospedeiras (Lewinsohn *et al.* 2005, 2012; Lewinsohn & Roslin 2008) e, além disso, são fortemente sincronizados com determinadas fenofases das plantas, as quais podem ser efêmeras (*e.g.*, frutos, Bernays & Chapman 1994). Devido à alta especificidade de dieta dos insetos herbívoros, especialmente nos trópicos, é possível que sejam encontrados padrões modulares nas redes de interação planta-herbívoro, ou seja, compartimentos de interações dentro de uma comunidade, com

nenhuma ou poucas espécies se interagindo com mais de uma espécie de planta hospedeira (Prado & Lewinsohn 2004). Sendo assim, além de amostragens rápidas na folhagem, a criação de insetos em laboratório também é necessária, pois assim se confirma com segurança se o inseto coletado é mesmo um herbívoro daquela planta ou se sua coleta foi meramente fortuita (Lewinsohn *et al.* 2012).

A coleta de partes das plantas (*e.g.*, frutos) também possibilita que insetos endofíticos sejam amostrados e criados, aumentando o conhecimento da biodiversidade (Lewinsohn *et al.* 2001, 2005). Ainda, ao mensurar os diferentes traços das plantas, como tamanho e qualidade nutricional, podem ser indicados os melhores recursos e oportunidades de sobrevivência para os insetos herbívoros (*e.g.*, Canto *et al.* 2019). Atrélendo estas informações com estudos ecológicos envolvendo manipulações experimentais é possível elucidar o papel de cada uma das espécies nas interações em que estão envolvidas (ver Del-Claro 2012), assim fornecendo informações para um melhor entendimento das interações inseto-planta, especialmente sobre guildas menos exploradas como a dos endofíticos.

Entre os insetos endofíticos, os bruquíneos (Coleoptera: Chrysomelidae: Bruchinae) representam um dos grupos mais notáveis de herbívoros que se alimentam de sementes (Southgate 1979), com cerca de 1700 espécies conhecidas (Alvarez *et al.* 2006; Ribeiro-Costa & Almeida 2012; Ribeiro-Costa *et al.* 2014). É estimado que mais de 80% dessas espécies se alimentam das sementes de Fabaceae, o que reforça a importância desta família botânica para esses besouros (Ribeiro-Costa & Almeida 2012; Romero 2017). Geralmente os bruquíneos têm dietas restritas (oligofagia ou monofagia; Kergoat *et al.* 2005) e cerca de dois terços deles se alimentam em uma única semente durante todo o seu estágio larval (Center & Johnson 1974; Ribeiro-Costa & Almeida 2012). Assim, durante sua fase imatura, os bruquíneos são na maioria das vezes sésseis

e, conseqüentemente, fáceis de serem amostrados e criados a partir da coleta de frutos. Portanto, eles são considerados excelentes modelos para estudos de ampla escala geográfica, os quais são impraticáveis com muitos outros organismos (Fox *et al.* 2007). Contudo, muitas espécies de bruquíneos, em especial várias daquelas pertencentes ao gênero *Acanthoscelides* Schilsky, 1905, ainda são desconhecidas quanto à sua história natural e também ao impacto causado em suas plantas hospedeiras (mas, ver Rossi *et al.* 2011; English & Olckers 2014; Haga & Rossi 2016; Escobar-Domínguez *et al.* 2018). Tal fato fomenta a busca de respostas sobre como ambos os níveis tróficos (produtor e consumidor endofítico) podem exercer influências recíprocas. Por exemplo, como esses besouros podem afetar a germinação das sementes? Esses besouros seriam facilitadores da germinação ou predadores delas? Perguntas como essas são relativamente pouco exploradas e as respostas são contrastantes, inclusive ao analisar a mesma espécie de besouro, como é o caso de *Acanthoscelides macrophthalmus* (Schaeffer 1907) (vide Haga & Rossi 2016; Silva & Rossi, 2019). Mas existem exceções, ou seja, casos em que se conhece bem a ecologia das interações dos bruquíneos e suas plantas hospedeiras. Esse tipo de situação ocorre com espécies de importância econômica devido à sua alimentação em grãos armazenados, como é o caso de *Acanthoscelides obtectus* Say, 1831 em *Phaseolus vulgaris* L., cujos aspectos ecológicos são bem conhecidos desde o século passado (Baier & Webster 1992).

Por outro lado, amostrar espécies de insetos com tamanhos diminutos (*i.e.*, dois milímetros ou menos) e com hábito parasitário também pode trazer significativos resultados no conhecimento da biodiversidade, especialmente em alguns táxons pouco conhecidos (Lewinsohn *et al.* 2001). Por exemplo, mesmo em Hymenoptera, um táxon amplamente estudado, há grupos de vespas parasitoides diminutas, como os Braconidae: Microgastrinae, cujas espécies são pouco estudadas na América do Sul (ver Whitfield

1997; Fernandez-Triana *et al.* 2020). Essas vespas são altamente hospedeiro-específicas e, assim sendo, têm enorme importância ecológica por meio das forças *top-down* que exercem sobre os seus hospedeiros. Por isso elas também são consideradas importantes agentes de controle biológico (Smith *et al.* 2008; Whitfield *et al.* 2009), o que pode estar sendo subexplorado na América do Sul, tendo em vista a falta de conhecimento sobre as espécies locais (Sousa-Lopes *et al.* 2019a). Por exemplo, *Cotesia* Cameron, 1891 é o segundo maior gênero de Braconidae, mas apenas 21 espécies são descritas para toda a região Neotropical (Sousa-Lopes *et al.* 2019a), mas com potencial muito maior (Rodriguez *et al.* 2012; Fernandez-Triana *et al.* 2020). Sendo assim, reforça-se que a coleta e a criação de insetos associados a espécies de plantas pouco estudadas, como é o caso de muitas Fabaceae, podem aprimorar nosso conhecimento sobre a biodiversidade (ver Sousa-Lopes *et al.* 2019a).

As fabáceas e suas relações com os insetos herbívoros e seus predadores no Cerrado

As espécies pertencentes à família Fabaceae geralmente são descritas como facilitadoras, visto que fixam nitrogênio no solo e, por isso, permitem a colonização do habitat por outras plantas de diferentes grupos taxonômicos (McKey 1994; Montesinos-Navarro *et al.* 2017). Mas, há também, espécies de Fabaceae, como *Mimosa setosa* var. *paludosa* (Benth.) Barneby, as quais podem se tornar importantes invasoras, trazendo prejuízos econômicos e ecológicos. No caso da planta citada, ela pode colonizar áreas de pastagem, onde seus grandes acúleos podem causar graves injúrias no gado (Lorenzi 2000; Pott *et al.* 2006). Devido a essas importantes funções ecológicas, as fabáceas são consideradas prioritárias em programas de recuperação de áreas degradadas (Citadini-Zanette *et al.* 2017) ou foco de programas de controle de espécies de plantas invasoras (Williams & Hoagland, 2007; Tuda *et al.* 2009). Portanto, conhecer suas interações ecológicas é de suma relevância (ver Sousa-Lopes *et al.* 2016, 2019a, b, c).

No Cerrado, Fabaceae é a família botânica mais representativa, com 1.174 espécies (Mendonça *et al.* 2008). Entre as espécies de fabáceas há elevado destaque para *Mimosa* L. (Mimosoideae), pois é o segundo maior gênero de plantas desta família no Cerrado, com 214 espécies descritas (Mendonça *et al.* 2008). Contudo, plantas dos gêneros *Andira* Lam. (Papilionoideae), *Bauhinia* L. (Cesalpinioideae), *Chamaecrista* Moench (Cesalpinioideae) e *Stryphnodendron* Mart. (Mimosoideae) também são comumente encontradas no Cerrado (Medeiros 2011).

Entre esses gêneros há espécies de plantas com tricomas glandulares, os quais podem atrair as aranhas do gênero *Peucetia* Thorell, 1869 (Araneae: Oxyopidae), as quais interagem com as plantas e com diferentes insetos herbívoros (Romero *et al.* 2008; Sousa-Lopes *et al.* 2019b). No entanto, poucos dados empíricos estão disponíveis na literatura sobre interações específicas entre plantas e aranhas, e elucidando quais e como as características das plantas afetam as populações de aranhas e estas dos herbívoros (Vasconcellos-Neto *et al.* 2017).

Por outro lado, há também fabáceas com Nectários Extraflorais (NEFs), ou seja, plantas que possuem glândulas que secretam substâncias ricas em carboidratos e aminoácidos (Shenoy *et al.* 2012), porém, sem ter relação direta com a polinização (Fiala & Maschwitz 1991). Essas glândulas atraem grande diversidade de insetos, especialmente formigas (Beattie 1985; Rico-Gray & Oliveira 2007; Del-Claro *et al.* 2018), as quais utilizam esse recurso e são favorecidas por um melhor desenvolvimento e crescimento das colônias (Byk & Del-Claro 2011). Como contrapartida, muitas vezes as plantas recebem proteção das formigas contra seus herbívoros, sendo este processo considerado um tipo de defesa biótica das plantas, o qual pode reduzir significativamente os danos aos tecidos vegetais (Del-Claro *et al.* 1996; Kost & Heil 2005; Nascimento & Del-Claro 2010; Fagundes *et al.* 2017).

Neste amplo contexto, para elucidar as interações fabáceas-insetos-predadores e avaliar os resultados destas relações no Cerrado, foram escolhidas duas plantas glandulares, *Mimosa setosa* var. *paludosa* (Benth.) Barneby e *Chamaecrista cathartica* (Mart.) H.S. Irwin & Barneby, e três plantas com NEFs comumente encontradas no Cerrado, *Andira humilis* Mart. ex. Benth, *Bauhinia rufa* (Bong.) Steud e *Strypnodendron polyphyllum* (Mart.) (vide detalhes dessas plantas no Capítulo 1). Os

Objetivos gerais desta tese são:

- Caracterizar a comunidade de artrópodes, insetos herbívoros e seus inimigos naturais, associados às fabáceas acima listadas;
- Avaliar os efeitos das forças *bottom-up* (traços das plantas) e *top-down* (aranhas, formigas e vespas) sobre a ocorrência dos insetos herbívoros no Cerrado.

Especificamente, tivemos como objetivos fazer um levantamento dos insetos herbívoros e seus inimigos naturais associados a todas as plantas supracitadas, bem como calcular as métricas da rede Fabaceae-herbívoros (diversidade, especialização, modularidade e robustez) e a correlação entre a ocorrência de herbívoros e a fenologia das plantas e inimigos naturais (Cap.1). Posteriormente, foram escolhidos dois insetos herbívoros: *Acanthoscelides quadridentatus* (Schaeffer, 1907) e *Acanthoscelides winderi* Kingsolver, 1984, os quais tiveram maior abundância em campo, fácil observação, coleta e manipulação, para estudos mais aprofundados, onde foi avaliado: (i) como os insetos herbívoros afetam o valor adaptativo das plantas (efeito *top-down*; taxas de predação e germinação das sementes; Cap. 2), e (ii) como e quais os traços das plantas (peso, tamanho, conteúdo de água, dureza) afetam a ocorrência desses insetos herbívoros (efeito *bottom-up*; Cap. 3). Por também ser muito abundante, a aranha

Peucetia flava Keyserling (1877) foi selecionada para avaliar como os predadores afetam a densidade dos herbívoros e, conseqüentemente, as taxas de herbivoria na planta hospedeira (efeito *top-down*; Cap. 4). Adicionalmente, uma nova espécie de vespa parasitoide foi descoberta e descrita, *Cotesia itororensis* Sousa-Lopes & Whitfield 2019. Notas acerca de seu impacto sobre o herbívoro hospedeiro, *Oospila pallidaria* (Schaus, 1897), em *M. setosa* var. *paludosa*, são brevemente apresentadas (efeito *top-down*; Cap. 5).

Cada um dos capítulos desta tese tem suas particularidades, porém, todos estão interligados por: (1) terem a família Fabaceae como base do sistema, (2) testarem a influência das forças *bottom-up* e/ou *top-down* sobre os insetos herbívoros, e (3) ocorrerem no bioma Cerrado (Fig. 1). A tese está organizada nos seguintes capítulos:

1. Plant phenology and natural enemies shape the structure of a Fabaceae-insect herbivore community in a Neotropical savanna
2. Temporal distribution, seed damage and notes on the natural history of *Acanthoscelides quadridentatus* and *Acanthoscelides winderi* (Coleoptera: Chrysomelidae: Bruchinae) on their host plant, *Mimosa setosa* var. *paludosa*, in the Brazilian Cerrado (**Publicado no Journal of Natural History**)
3. Spatio-temporal variation in seed traits affects the occurrence and body size pattern of a seed-feeding beetle (Chrysomelidae: Bruchinae) in Brazilian Cerrado (**Publicado em Acta Oecologica**)
4. Antiherbivore protection and plant selection by the lynx spider *Peucetia flava* (Araneae: Oxyopidae) in the Brazilian Cerrado (**Publicado no Journal of Zoology**)

5. *Cotesia itororensis* sp. nov. from Brazilian savanna: a new reared microgastrine wasp (Hymenoptera: Braconidae) described using an integrative taxonomic approach (**Publicado na revista Zootaxa**)



Figura 1. Área de cerrado, em Uberlândia, no Triângulo Mineiro (MG), onde esta tese foi desenvolvida (com exceção a uma parte dos dados do Cap. 4, os quais foram obtidos em plantas de beira de estradas dos cerrados de MG e GO). Os estudos foram feitos nas fitofisionomias de campo cerrado, cerrado sensu stricto e vereda.

Referências

- Adler, P.H. & Foottit, R.G. 2009. Introduction, p. 1-6. In Foottit, R. G. & P. H. Adler (Eds.). 2009. Insect Biodiversity: Science and Society. Wiley-Blackwell, West Sussex, UK.
- Alvarez N, Romero-Napoles J, Anton KW, Benrey B, Hossaert-McKey M. 2006. Phylogenetic relationships in the Neotropical bruchid genus *Acanthoscelides* (Bruchinae, Bruchidae, Coleoptera). *J Zool Syst Evol Res.* 44:63–74.
- Baier AH & Webster BD. 1992. Control of *Acanthoscelides obtectus* Say (Coleoptera:Bruchidae) in *Phaseolus vulgaris* L. seed stored on small farms I. Evaluation of damage. *J. Stored Prod. Res.* 28:289–293.
- Beattie, A.J. 1985. The evolutionary ecology of ant-plant mutualism, Cambridge University Press, Cambridge.
- Berenbaum, M. 2009. Insect biodiversity – millions and millions, p.561-574. In Foottit, R. G. & P. H. Adler (Eds.). 2009. Insect Biodiversity: Science and Society. Wiley-Blackwell, West Sussex, UK.

- Bernays EA & Chapman RF. 1994. Host-plant selection by phytophagous insects. New York (NY): Chapman & Hall Press.
- Bernays, E. & Graham, M. 1988. On the evolution of host specificity in phytophagous arthropods. *Ecology* 69: 886–892.
- Blüthgen, N. 2012. Interação planta-animal e a importância funcional da biodiversidade. In Del-Claro, K. & Torezan-Silingardi, H.M. (eds.). *Ecologia das Interações Plantas Animais: uma abordagem ecológico-evolutiva*. Rio de Janeiro, Technical Books.
- Bronstein, J.L. 1994. Conditional outcomes in mutualistic interactions. *Trends in Ecology & Evolution*, 9, 214–217.
- Byk, J. & Del-Claro, K. 2011. Ant-plant interaction in the Neotropical savanna: direct beneficial effects of extrafloral nectar on ant colony fitness. *Population Ecology* 53 (2): 327–332.
- Canto, A., Rodríguez, R. and Reyes-Novelo, E. 2019. Relationship between the weights of seed beetles of the genus *Megacerus* Fähræus, 1839 (Coleoptera: Chrysomelidae: Bruchinae) and their host seeds of the family Convolvulaceae. *Sci Rep.* 9:8438.
- Carpenter, SR; Kitchell, JF; Hodgson, JR. 1985. Cascading Trophic Interactions and Lake Productivity. *BioScience*, v. 35, n. 10, 634–639.
- Center TD & Johnson CD. 1974. Coevolution of some seed beetles (Coleoptera: Bruchidae) and their hosts. *Ecology*. 55:1096–1103.
- Chamberlain, S.A., Bronstein, J.L. & Rudgers, J.A. 2014. How context dependent are species interactions? *Ecology Letters*, 17: 881–890.
- Citadini-Zanette, V., Negrelle, R.B.R., Leal-Filho, L.S., Remor, R., Elias, G.A. & Santos, R. *Mimosa scabrella* Benth. (Fabaceae) enhances the restoration in coal mining areas in the Atlantic rainforest. *Cerne*, 23(1), 2017.
- Cornelissen T., Guimarães C.D.C., Viana J.P.R. & Silva B. 2013. Interspecific competition influences the organization of a diverse sessile insect community. *Acta Oecol.* 52: 15–18
- Del-Claro, K. 2004. Multitrophic Relationships, Conditional Mutualisms, and the Study of Interaction Biodiversity in Tropical Savannas. *Neotropical Entomology*, 33(6): 665–672.
- Del-Claro, K. 2012. Origens e importância das relações plantas-animais para a ecologia e conservação. In Del-Claro, K. & Torezan-Silingardi, H.M. (eds.). *Ecologia das Interações Plantas Animais: uma abordagem ecológico-evolutiva*. Rio de Janeiro, Technical Books.
- Del-Claro, K., Berto, V. & Réu, W. 1996. Herbivore deterrence by visiting ants increases fruit-set in an extrafloral nectary plant *Qualea multiflora* (Vochysiaceae) in cerrado vegetation. *J. Trop. Ecol.* 12: 887–892.
- Del-Claro, K., Lange, D., Torezan-Silingardi, H.M., Anjos, D.V., Calixto, E.S., Dáttilo, W. & Rico-Gray, V. 2018. The complex ant-plant relationship within tropical ecological networks. In Dáttilo, W. & Rico-Gray, V. (eds.). *Ecological networks in the tropics: an integrative overview of species interactions from some of the most species-rich habitats on Earth*. Springer, DOI:10.1007/978-3-319-68228-0.
- Del-Claro, K., Rico-Gray, V., Torezan-Silingardi, H.M., Alves-Silva, E., Fagundes, R., Lange, D., Dáttilo, W., Vilela, A.A., Aguirre, A. & Rodriguez-Morales, D. 2016. Loss and gains in ant-plant interactions mediated by extrafloral nectar: Fidelity, cheats, and lies. *Insectes Sociaux*. doi: 10.1007/s00040-016-0466-2
- Del-Claro, K., Stefani, V., Lange, D., Vilela, A.A., Nahas, L., Velasques, M. & Torezan-Silingardi, H.M. 2013. The importance of natural history studies for a

- better comprehension of animal-plant interactions networks. *Bioscience Journal*, 29(2): 439–448.
- Denno, R.F., Lewis, D. & Gratton, C. 2005. Spatial variation in the relative strength of top-down and forces: causes phytophagous insect populations and consequences for. *Ann. Zool. Fennici*, 42: 295–311.
- Dias, B.F.S. 1992. Alternativas de desenvolvimento dos Cerrados: manejo e conservação dos recursos naturais renováveis. Brasília, DF: Funatura. 97p.
- Ehrlich, P.R. & Raven, P.H. 1964. Butterflies and plants: a study in coevolution. *Evolution*, 18: 586–608.
- English, K.F. & Olckers, T. 2014. Does the size of the seeds and seed pods of the invasive tree *Leucaena leucocephala* (Fabaceae) affect their utilization by the biological control agent *Acanthoscelides macrophtalmus* (Chrysomelidae: Bruchinae). *African Entomology*, 22: 872–879.
- Erwin, T.L. 1982. Tropical forests: Their richness in Coleoptera and other arthropod species. *The Coleopt Bull*, 36 (1): 74–75.
- Escobar-Domínguez, A.A., Romero-Nápoles, J., Equihua-Martínez, A., Carrilo-Sánchez, J.L., & Ramírez-Alarcón, S. 2018. Bruchids (Coleoptera: Bruchidae) associated with seeds of species of *Mimosa* L. genus (Leguminosae: Mimosoideae). *Acta Zoológica Mexicana*, 34: 1–17.
- Fagundes, R., Dattilo, W., Ribeiro, S.P., Rico-Gray, V., Jordano, P. & Del-Claro, K. 2017. Differences among ant species in plant protection are related to production of extrafloral nectar and degree of leaf herbivory. *Biol J Linn Soc*, XX: 1–13.
- Fernandez-Triana, J. Shaw, M.R., Boudreault C., Beaudin, M., & Broad, G.R. 2020. Annotated and illustrated world checklist of Microgastrinae parasitoid wasps (Hymenoptera, Braconidae). *Zookeys*, 920: 1–1089. <https://doi.org/10.3897/zookeys.920.39128>
- Fiala, B. & Maschwitz, U. 1991. Extrafloral nectaries in the genus *Macaranga* (Euphorbiaceae) in Malaysia; comparative studies of their possible significance as predispositions for myrmecophitism. *Biol. J. Linnean Soc.* 44: 287–305.
- Foelix, R.F. 1996. *Biology of Spiders*. Second Edition. Oxford, UK: Oxford University Press, 330 pp.
- Fornoff, F., Klein, A-M., Blüthgen, N. & Staab, M. 2019. Trees diversity increases robustness of multi-trophic interactions. *Proc Biol Sci*, doi: 10.1098/rspb.2018.2399.
- Fox C.W., Stillwell R.C. & Moya-Laraño J. 2007. Variation in selection, phenotypic plasticity, and ecology of sexual size dimorphism in two seed-feeding beetles. Pp. 88–96 in Fairbairn DJ, Blanckenhorn WU, Szekely T. eds. *Sex, size and gender roles: evolutionary studies of sexual size dimorphism*. Oxford University Press, Oxford, NY.
- Grimaldi, D. & Engel, M.S. 2005. *Evolution of the Insects*. Cambridge University Press, Cambridge.
- Hairston, N.G., Smith, F.E. & Slobodkin, L.B. 1960. Community Structure, Population Control, and Competition. *Amer Nat*, 94 (879): 421–425.
- Hallmann, C.A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stenmans, W., Müller, A., Sumser, H., Hörren, T., Goulson, D. & Kro, H. de. 2017. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLoS ONE* 12(10): e0185809. <https://doi.org/10.1371/journal.pone.0185809>.

- Hoog B. & Daane K. 2015. Impacts of exotic spider spillover on resident arthropod communities in a natural habitat. *Ecol. Entomol.* 40: 69–77.
- Kaplan I. & Denno R.F. 2007. Interspecific interactions in phytophagous insects revisited: a quantitative assessment of competition theory. *Ecol. Lett.* 10: 977–994.
- Kergoat GJ, Delobel A, Fédière G, Rü BL, Silvain J-F. 2005. Both host-plant phylogeny and chemistry have shaped the African seed-beetle radiation. *Mol Phylogenetics Evol.* 35(3):602–611.
- Klink, C.A. & Machado, R.B. 2005. A conservação do cerrado brasileiro. *Megadiversidade*, 1(1): 147–155.
- Kost, C., & Heil, M. 2005. Increased availability of extrafloral nectar reduces herbivory in lima beans (*Phaseolus lunatus*, Fabaceae). *Basic Appl. Ecol* 6:237–248.
- Labandeira, C.C. 2007. The origin of herbivory on land: Initial patterns of plant tissue consumption by arthropods. *Insect Sci*, 14(4): 259–275.
- Labandeira, C.C., Dilcher, D.L., Davis, D.R. & Wagner D.L. 1994. Ninety-seven million years of angiosperm-insect association: paleobiological insights into the meaning of coevolution. *Proc Natl Acad Sci*, 91: 12278–12282.
- Lamarre, G.P.A, Fayle, T.M., Segar, S.T. et al. 2020. Monitoring tropical insects in the 21st century. *Advances in Ecol. Res.*, 62:295–330.
- Lewinsohn, T. M. & Roslin, T. 2008. Four ways toward tropical herbivore megadiversity. *Ecology Letters*, 11: 398–416.
- Lewinsohn, T. M., P. I. Prado, & A. M. Almeida. 2001. Inventários bióticos centrados em recursos: insetos fitófagos e plantas hospedeiras. p174–189 in I. Garay & B. F. S. Dias, editors. *Conservação da biodiversidade em ecossistemas tropicais*. Editora Vozes, Petrópolis, Brazil.
- Lewinsohn, T.N., Freitas, A.V.L. & Prado, P.I. 2005. Conservação de invertebrados terrestre e seus habitats no Brasil. *Megadiversidade*, 1(1).
- Lewinsohn, T.N., Jorge, L.R. & Prado, P.R. 2012. In Del-Claro, K. & Torezan-Silingardi, H.M. (eds.). *Ecologia das Interações Plantas Animais: uma abordagem ecológico-evolutiva*. Rio de Janeiro, Technical Books.
- Lorenzi H. 2000. *Plantas daninhas do Brasil: terrestres, aquáticas, parasitas e tóxicas*. Nova Odessa: Instituto Plantarum, SP
- McKey, D. 1994. Legumes and nitrogen: the evolutionary ecology of a nitrogen-demanding lifestyle. Pages 211–228. in J. I. Sprent and D. McKey. *Advances in legume systematics. Part 5. The nitrogen factor*. Royal Botanic Gardens. Kew, UK.
- Medeiros, J. D. 2011. *Guia de campo: vegetação do Cerrado 500 espécies*. Brasília: MMA/SBF.
- Mendonça, R.C.; Felfili, J.M.; Walter, B.M.T.; Júnior, M.C.S.; Rezende, A.S.; Filgueira, T.S.; Nogueira, P.E. & Fagg, C.W. 2008. Flora vascular do bioma Cerrado: checklist com 12356 espécies. In: Sano, S.M.; Almeida, S.P. & Ribeiro, J.F. (eds.). *Cerrado: ecologia e flora*. 2 vols. Embrapa Cerrados, Embrapa Informações Tecnológicas, Brasília. p. 151–199.
- Montesinos-Navarro, A., Verdú, M., Querejeta, J.I. & Valiente-Banuet, A. 2017. Nurse plants transfer more nitrogen to distantly related species. *Ecology*, 98(5): 1300–1310.
- Mooney, K.A., Pratt, R.T. & Singer, M.S. 2012. The tri-trophic interactions hypothesis: Interactive effects of host plant quality, diet breadth and natural enemies on herbivores. *PLoS ONE*, 7(4).

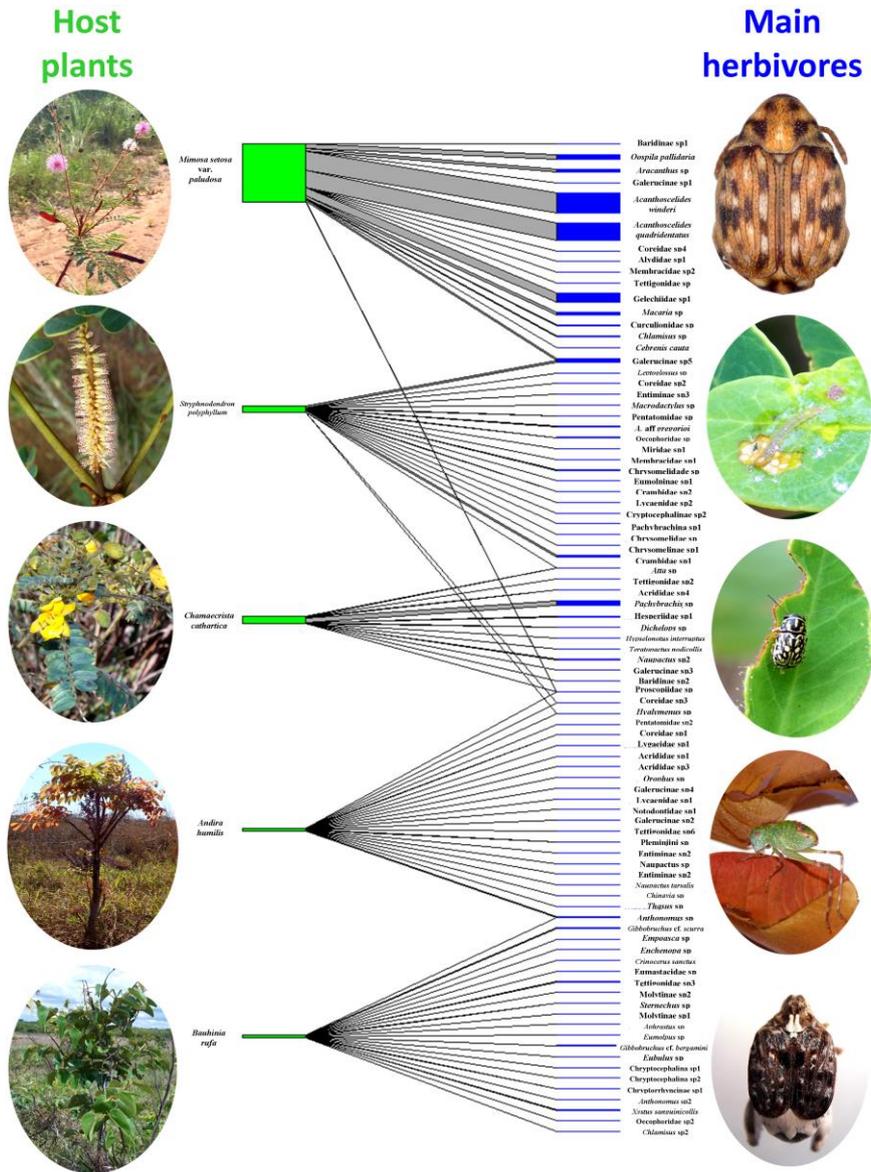
- Nascimento, E.A. & Del-Claro, K. 2010. Ant visitation to extrafloral nectaries decreases herbivory and increases fruit set in *Chamaecrista debilis* (Fabaceae) in a Neotropical savanna. *Flora*, 205: 754–756.
- Norris, K.R. 1991. General biology, p.68-108. In Naumann I.D., Carne P.B., Lawrence, J.F., Nilsen E.S., Spradberry, J.P., Taylor, R.W., Whitten M.J. & Littlejohn M.J. (eds.). *The insect of Australia: a textbook for students and research workers*, second edition. Melbourne, CSIRO, Melbourne University Press, 2 vol. 1137p.
- Oliveira, P.S. & R.J. Marquis (eds.). 2002. *The Cerrados of Brazil. Ecology and natural history of a neotropical savanna*. Columbia University Press, New York.
- Paine, R.T. 1980. Food webs: Linkage, interaction strength and community infrastructure. *Journal of Animal Ecology* 49, 667–685.
- Pott A, Pott VJ, Souza TW. 2006. *Plantas daninhas de pastagem na região dos Cerrados*. Campo Grande, MS: EMBRAPA Gado de Corte.
- Prado PI, Lewinsohn TM. 2004. Compartments in insect–plant associations and their consequences for community structure. *Journal of Animal Ecology* 73, 1168–1178.
- Price, P.W., Bouton, C.E., Gross, P. McPherson, B.A., Thompson, J.N. & Weis, A.E. 1980. Interaction between three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Ann. Rev. Ecol Syst.* 11: 41–65.
- Price, P.W., Denno, R.F., Eubanks, M.D., Finke, D.L. & Kaplan. I. 2011. *Insect Ecology: behavior, population and communities*. Cambridge University Press.
- Ribeiro, J.F. & Walter, B.M.T. 2008. As principais fitofisionomias do Bioma Cerrado. In Sano, S.M, Almeida, S.P; Ribeiro, J.F. *Ecologia e flora*. Brasília: EMBRAPA, 1: 152–212.
- Ribeiro-Costa CS & Almeida LM. 2012. Seed-Chewing Beetles (Coleoptera: Chrysomelidae, Bruchinae). In: Panizzi AR, Parra JRP (orgs.). *Insect Bioecology and Nutrition for Integrated Pest Management*. 1ed. Boca Raton: CRC Press, 2012, v. 1, p. 325–352.
- Ribeiro-Costa, CS, Vieira, M.K., Manfio, D. & Kergoat, G.G. 2014. A remarkable new species group of green seed beetles from genus *Amblycerus* Thunberg (Coleoptera, Chrysomelidae, Bruchinae), with description of a new Brazilian species. *ZooKeys*, 401: 31–44.
- Rico-Gray, V. & Oliveira, P.S. 2007. *The ecology and evolution of ant-plant interactions*. University of Chicago Press, Chicago.
- Rodriguez, J.J., Fernandez-Triana, J., Smith, M.A., Janzen, D.H., Hallwachs, W., Erwin, T.L. & Whitfield, J.B. 2012. Extrapolations from field studies and known faunas converge on dramatically increased estimates of global microgastrine parasitoid wasp species richness (Hymenoptera: Braconidae). *Insect Conservation and Diversity*, 6, 530–536.
- Romero, G.Q., Souza, J.C. & Vasconcellos-Neto, J. (2008). Anti-herbivore protection by mutualistic spiders and the role of plant glandular trichomes. *Ecology* 89, 3105–3115.
- Romero, N.J. 2017. Bruchidae, p 311–321. In Cibrián D.T. (ed.). *Fundamentos de Entomología Florestal*. Publicación de la Red de Salud Florestal Redes temáticas e La Comisión Nacional de Ciencia y Tecnología (CONACYT).
- Root RB (1973) Organization of a plant-arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). *Ecol Monogr*, 43:95-124

- Rossi MN, Rodrigues LMS, Ishino MN & Kestring D. 2011. Oviposition pattern and within-season spatial and temporal variation of pre-dispersal seed predation in a population of *Mimosa bimucronata* trees. *Arthropod Plant Interact.* 5:209–217.
- Sánchez-Bayo, F. & Wyckhuys, K.A.G. 2019. Worldwide decline of the entomofauna: A review of its drivers. *Biol conserv.* 232: 8–27.
- Schmitz, O.J. 1994. Resource edibility and trophic exploitation in an old-field food web. *Proc Natl Acad Sci U S A.* 91(12): 5364–5367.
- Schoonhoven, L.M., van Loon, J.J.A. & Dicke, M. 2005. *Insect Plant Biology.* Oxford University Press, Oxford, 421 p.
- Shenoy SS, Nanda H & Losche M. 2012. Membrane association of the PTEN tumor suppressor: Electrostatic interaction with phosphatidylserine-containing bilayers and regulatory role of the C-terminal tail. *J Struct Biol.* 180:394–408.
- Simmons, BI, Balmford, A, Bladon AJ. et al. 2019. Worldwide insect decline: an important message, but interpret with caution. *Eco. Evol.* 9:3678–3680.
- Singer, M.S. & Stireman, J.O. 2005. The tri-trophic niche concept and adaptive radiation of phytophagous insects. *Ecol. Lett.*, 8: 1247–1255.
- Smith, M.A., Rodriguez, J.J., Whitfield, J.B., Deans, A.R., Janzen, D.H., Hallwachs, W. & Hebert, P.D.N. (2008) Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology, and collections. *Proc Natl Acad Sci U S A*, 105(35), 12359–12364.
- Sousa-Lopes B, Alves-da-Silva N, Alves-Martins F & Del-Claro K. 2019b. Antiherbivore protection and plant selection by the lyx spider *Peuceitia flava* (Araneae: Oxyopidae) in the Brazilian Cerrado. *J. Zool.* 308(2): 121–127.
- Sousa-Lopes B, Alves-da-Silva N, Ribeiro-Costa CS, Del-Claro K. 2019c. Temporal distribution, seed damage and notes on the natural history of *Acanthoscelides quadridentatus* and *Acanthoscelides winderi* (Coleoptera: Chrysomelidae: Bruchinae) on their host plant, *Mimosa setosa* var. *paludosa* (Fabaceae), in the Brazilian Cerrado. *J Nat Hist.* 53(9-10):611–623.
- Sousa-Lopes B, Bächtold A & Del-Claro K. 2016. Biology, natural history and temporal fluctuation of the geometrid *Oospila pallidaria* associated with host plant phenology. *Stud Neotrop Fauna Environ.* 51:135–143.
- Sousa-Lopes B, Whitfield, J.B., Salgado-Neto, G. & Del-Claro, K. 2019a. *Cotesia itororensis* sp Nov. from Brazilian savanna: a new reared microgastrinae wasp described using an integrative taxonomic approach. *Zootaxa*, 4544 (3): 437–445.
- Southgate, B.J. 1979. Biology of the Bruchidae. *Annu. Rev. Entomol.* 24:449–73.
- Stork, N.E. 2018. How many species of insects and other terrestrial arthropods are there on Earth? *Ann Rev.* 63: 31–45.
- Strassburg, B., Brooks, T., Feltran-Barbieri, R., Iribarrem, A., Crouzeilles, R., Loyola, R., Latawiec, A.E., Oliveira Filho, F.J.B., Scaramuzza, C.A.M., Scarano, F.R., Soares-Filho, B. & Balmford, B. 2017. Moment of truth for the Cerrado hotspot. *Nat Ecol Evol.* 1(4): 1–3.
- Tewksbury, J.J., Anderson, J.G.T., Bakker, J.D., Dunwiddie, P.W., Groom, M.J., Hampton, S.E., Herman, S.G., Levey, D.J., Machnicki, N.J., Del-Rio, C.M., Power, M.E., Rowell, K., Salomon, A.K., Stacey, L., Trombulak, S.C. & Wheeler, T.A. 2014. Natural history's place in science and society. *Biosc.* 64(4): 300–314.
- Thompson, J.N. & Cunningham, B.M. 2002. Geographic structure and dynamics of coevolutionary selection. *Nature*, 417, 735–738.

- Thompson, J.N. 1988. Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. *Entomol. Exp. Appl.*, 47: 3–14.
- Thompson, J.N. 2005. *The geographic mosaic of coevolution*. The University of Chicago Press, 439p.
- Tuda, M.; Wu, L.H.; Tateishi, Y.; Niyomdham, C.; Buranapanichpan, S.; Morimoto, K. A novel host shift and invaded range of a seed predator, *Acanthoscelides macrophthalmus* (Coleoptera: Chrysomelidae: Bruchinae), of an invasive weed, *Leucaena leucocephala*. *Entomol. Sci.*, 12:1– 8, 2009.
- Vanin, S.A. Filogenia e classificação, p.81-109. In Rafael J.A., Melo G.A.R., Carvalho C.J.B., Casari, S. & Constantino R. *Insetos do Brasil: diversidade e taxonomia*. Ribeirão Preto-SP, Holos Editora. 810p.
- Vasconcellos-Neto, J., Messas, Y.F., Souza, H.S., Villanueva-Bonila, G.A. & Romero, G.Q. (2017). Spider–plant interactions: An Ecological Approach. In *Behaviour and Ecology of spiders: contribution from the Neotropical region*. pp.165–214. Viera, C. & Gonzaga, M.O. (Ed.). Gewerbestrasse: Springer.
- Vidal, M.C. & Murphy, S.M. 2018. Bottom-up vs. top-down effects on terrestrial insect herbivores: a meta-analysis. *Ecology Letters*, 21(1): 138–150.
- Williams, R.D.; Hoagland, R.E. Phytotoxicity of mimosine and albizziine on seed germination and seedling growth of crops and weeds. *Allelopathy J.*, 19:423–430, 2007.
- Whitfield, J.B. 1997. Subfamily Microgastrinae. In: Wharton, R.A., Marsh, P.M., Sharkey, M.J. editors. *Identification Manual to the New World Genera of the Family Braconidae (Hymenoptera)*, pp. 333-364. International Society of Hymenopterists Special Publication.
- Whitfield, J.B., Rodriguez, J.J. & Masonick, P.K. 2009. Reared microgastrine wasps (Hymenoptera: Braconidae) from Yanayacu Biological Station and environs (Napo Province, Ecuador): diversity and host specialization. *J Insect Sci*, 9(1), article 31. <https://doi.org/10.1673/031.009.3101>
- Willig, M.R., Woolbright, L., Presley, S.J., Schowalter, T.D. et al. 2019. Populations are not declining and food webs are not collapsing at the Luquillo Experimental Forest. *PNAS*, 116:12143–12144.

Capítulo 1

Plant phenology and natural enemies shape the structure of a Fabaceae-insect herbivore community in a Neotropical savanna



Plant phenology and natural enemies shape the structure of a Fabaceae-insect herbivore community in a Neotropical savanna

Bruno de Sousa-Lopes and Kleber Del-Claro

Abstract

Plant-herbivore networks are the main driver of terrestrial communities' dynamics and structure. However, information on how plant phenology (bottom-up) and predators (top-down) affect these communities is still incipient, especially in Neotropical environments such as Brazilian Cerrado. Our main aims were to: (1) describe a Fabaceae-insect herbivore network through the metrics richness, Shannon diversity index, strength of interactions, specialization, modularity and robustness, and (2) investigate what the influence is of bottom-up and top-down forces on the occurrence of these insects. We chose five Fabaceae species commonly found in Brazilian Cerrado and performed 582h of observation on 97 individuals of them in order to assess plant phenology, insect herbivores and natural enemies. We reared insects in laboratory to confirm whether they were fortuitous or true herbivores of the sampled plants. We found 1623 insect herbivores from five orders, 23 families and 87 species, being beetles of the genus *Acanthoscelides* the most abundant. Only six insect herbivore species fed on more than one host plant, highlighting a specialized and modular network. The network also presented low robustness which suggests high possibility of secondary extinction in disturbance events. We found a positive relationship between the number of fruits and the abundance of endophytic seed chewers. We also found a trend in decreasing the abundance of exophytic herbivores when increasing spider abundance on plants. Therefore, we showed that both bottom-up and top-down forces affect the structure of the Fabaceae-insect herbivore community. Due to the high specialization

found here we suggest the choice of areas with huge plant diversity for future conservation efforts in Brazilian Cerrado.

Keywords: Bottom-up force, food web, insect-plant interaction, modular pattern, top-down force.

INTRODUCTION

Plant-herbivore networks represent more than 40% of global terrestrial diversity, and are the main driver of communities' dynamics and structure (Price 2002a, b; Futuyma and Agrawal 2009). Despite the insects are the main herbivores, they are also restricted to a few plant genus or families because plant traits (i.e. physical and/or chemical defenses) are generally similar among related plants, which constrain their usage (Janzen 1983; Strong et al. 1984; Basset 1992; Price et al. 2011). In this sense, several studies have shown that many insect herbivores have narrow diet breadth (monophagy or oligophagy), especially in high biodiversity environments such as in the tropics (e.g. Diniz and Morais 1997; Novotny and Basset 2005). Therefore, one can expect a modular pattern (i.e. aggregated subsets of interacting species within a community; Dormann and Strauss 2014) and an increased strength of interactions (i.e. high frequency) by few specialist species when analyzing a plant-insect herbivore network in a tropical environment (Prado and Lewinsohn 2004). However, the use of network approach on antagonistic interactions is less common than in mutualistic interactions then further studies are needed to evaluate this pattern (but see Lewinsohn et al. 2006; Novotny et al. 2010; Pinho et al. 2017).

The Brazilian tropical savanna, i.e. Cerrado, is the second largest biome of this country, after Amazon forest (Klink and Machado 2005; Ribeiro and Walter 2008). Cerrado covers approximately 22% of Brazil's area, representing 2 million km² of

extension (Goodland 1971; Oliveira and Marquis 2002). This biome has very impressive species richness, encompassing more than 30% of Brazil's and 15% of Earth's biodiversity (Lewinsohn and Prado 2002; Aguiar and Camargo 2004). Further, Cerrado is also known by its high endemism (Myers et al. 2000; Silva and Bates 2002; Strassburg et al. 2017). For example, it was estimated 7,000 endemic vascular plants (Klink and Machado 2005; Mendonça et al. 2008), and also 90,000 species of insects, many of them interacting with those endemic plants through the herbivory (Dias 1992).

Studies on Cerrado plant-herbivore interactions have shown high host plant specificity and low densities of insect herbivores (Marquis et al. 2002). For instance, the classical study performed by Diniz and Morais (1997) in four Cerrado *sensu stricto* areas near Brasília (DF, Brazil) highlighted low similarity, low abundance, and a large proportion (74%) of lepidopterans occurring on only one host plant family among the sampled plants of the genus *Byrsonima* Rich. ex Kunth (Malpighiaceae), *Erythroxylum* P. Browne (Erythroxylaceae), and *Qualea* Aubl. (Vochysiaceae). However, there is a plethora of underestimated attributes such as the relative importance of environmental factors, bottom-up (phenology and plant traits) and top-down forces (natural enemies) in structuring Cerrado communities (Marquis et al. 2002), despite the increasing in studies on these subjects in recent years (e.g. Madeira et al. 2013; Vilela et al. 2014; Del-Claro et al. 2016; Sousa-Lopes et al. 2016, 2019a; Kuchenbecker and Fagundes 2018).

Environmental factors such as temperature and rainfall are known for affecting Cerrado plant-herbivore interactions as the seasonal variation between the wet and dry season changes plant phenology and consequently the availability of food resource to herbivores (Silva and Neves 2014; Vilela et al. 2014; Sousa-Lopes et al. 2016). A common pattern in Cerrado plants is a peak of leaf flushing in the beginning of the wet

season and then the high herbivore abundance on plants, especially those exophytic folivores from the chewing guild (Morais et al. 1995; Marquis et al. 2002; Novais et al. 2019). However, this pattern must be seen with caution since some herbivores may have a delayed occurrence regarding to plant phenology or occur throughout the year since some plant species can produce/maintain leaves all year round (Bächtold et al. 2014; Sousa-Lopes et al. 2016). On the other hand, since several plants have a peak of flowering and/or fruiting in the dry season, we can also expect high florivore/frugivore abundance in this period (e.g. Vilela et al. 2014; Sousa-Lopes et al. 2019b).

In Cerrado plants it has been shown that ants, spiders and wasps are among the main predators of insect herbivores, responsible for decreasing their abundances on extrafloral-nectaried plants (Nahas et al. 2012; Stefani et al. 2015; Del-Claro et al. 2016, 2017) as well as in glandular trichome-bearing plants (Sousa-Lopes et al. 2019a). If on the one hand, extrafloral nectar is rich in carbohydrates, lipids, enzymes, amino acids, phenols, alkaloids and volatile organic compounds, which attract especially ants and spiders that feed on them and in return act as a complementary biotic defense on these plants (González-Teuber & Heil 2009; Nahas et al. 2012; Del-Claro et al. 2017). On the other hand, glandular trichomes entrap some herbivores (carrion) which are eaten by spiders that, in return to this benefit given by the host plant, decrease the abundance of live herbivores on them (Romero et al. 2008; Sousa-Lopes et al. 2019a). Thus, natural enemies (top-down force) can affect the occurrence and distribution of insect herbivores which, as a response to this selective pressure, can synchronize their occurrence on plants when natural enemies are absent or in low abundances (Morais et al. 1999; Diniz et al. 2012). Nonetheless, some herbivores can occur at the same time with natural enemies as they overcome enemies' attack strategies (Alves-Silva et al. 2014; Linz et al.

2016; Sousa-Lopes et al. 2019a). Therefore, the outcomes of herbivore-predator interactions may be context-dependent (Del-Claro et al. 2016).

Fabaceae Lindl. is one of the richest angiosperm families in Cerrado (Mendonça et al. 2008), but ecological information on their interactions remain lacking for several species. Using five Fabaceae species commonly found in Cerrado, *Andira humilis* Mart. ex. Benth (Papilionoideae), *Bauhinia rufa* (Bong.) Steud. (Caesalpinioideae), *Chamaecrista cathartica* (Mart.) H.S. Irwin & Barneby (Caesalpinioideae), *Mimosa setosa* var. *paludosa* (Benth.) Barneby (Mimosoideae) and *Stryphnodendron polyphyllum* Mart. (Mimosoideae), our main aims were to: (a) describe the insect herbivores associated to these plant species using the network approach (richness and abundance); and (b) investigate what the influence is of bottom-up (plant phenology) and top-down forces (natural enemies) on the occurrence of these insects.

Specifically, we hypothesized that: (i) the plant-herbivore network is modular and there is an increased strength of interactions by few species due to the high specificity of insect herbivores on the host plants in tropical environments, including the Cerrado (e.g. Diniz and Morais 1997; Prado and Lewinsohn 2004; Novotny and Basset 2005); (ii) insect herbivores are closely related with the peak of the host plant phenophase which they feed (e.g. Bernays and Chapman 1994; Vilela et al. 2014); and (iii) the abundance of insect herbivores is negatively affected by increases in the abundance of natural enemies (e.g. Morais et al. 1999; Diniz et al. 2012; Del-Claro et al. 2016).

MATERIAL AND METHODS

Study area and plant species

This study was performed in a Brazilian cerrado area of the Clube Caça e Pesca Itororó (CCPIU) in Uberlândia city, southeastern Brazil (18°59'S, 48°17'W). Local vegetation

is dominated by herbaceous plants with some trees ranging between 2 and 8 m tall. The climate in the region is markedly seasonal, characterized by a wet (October to March) and a dry season (April to September) (see Sousa-Lopes et al. 2016). The monthly mean temperature during the study period, from October 2016 to September 2017, ranged from 19°C (July) to 24.9°C (October), and 94% of the precipitation was concentrated in the wet season (INMET 2019; Fig. 1). We used preexistent trails of CCPIU, in a predominantly cerrado stricto sensu area, to locate all plants evaluated in this study.

Andira humilis is an extrafloral-nectaried shrub endemic from Brazil that ranges from 80cm to 2.5m tall in study area (Sousa-Lopes pers. obs.). The plant was described as having allelopathic effect against the germination and growth of lettuce and radish (Periotto et al. 2004). In addition, Diniz et al. (2001) recorded two species of Elachistidae (Lepidoptera), *Cerconota tabida* (Butler, 1877) and *Loxotoma elegans* Zeller, 1854, feeding on *A. humilis* in Distrito Federal, Brazil. *Bauhinia rufa* is an extrafloral-nectaried shrub/tree native from Brazilian Cerrado (Flora do Brasil 2019; Machado et al. 2008). In this study area this species ranged from 1 to 2m tall (Sousa-Lopes pers. obs.). *Bauhinia rufa* was recorded as host plant of *Urbanus proteus proteus* (Linnaeus, 1758) (Lepidoptera) in Distrito Federal, Brazil (Diniz et al. 2001). *Chamaecrista cathartica* is a glandular-trichome bearing shrub endemic from Brazil (Flora do Brasil 2019). In this study area this plant species ranged from 30cm to 1m tall (Sousa-Lopes pers. obs.). *Mimosa setosa* var. *paludosa* is a glandular-trichome bearing shrub endemic from Brazil and Paraguay that ranges from 1 to 3m tall (Pott et al. 2006; Dutra and Garcia 2014). This plant species is important for apiarian activity and the restoration of wetlands, but it is also an important weed in pasture areas as its large thorny branches can injure cattle (Lorenzi 2000; Pott et al. 2006). Insect herbivores from different taxa, spiders and parasitoid wasps were found interacting on this plant

species (Sousa-Lopes et al. 2016, 2019a, b, c). *Stryphnodendron polyphyllum* is an extrafloral-nectaried tree endemic from Brazil (Flora do Brasil 2019). In the study area this plant ranged from 85cm to 2.5m tall (Sousa-Lopes pers. obs.). This plant species was recorded attracting a rich ant fauna on its extrafloral nectaries (Lange et al. 2017).

Assessment of insect herbivores, natural enemies and plant phenology

Visual inspections were carried out monthly from October 2016 to September 2017 on 97 randomly tagged individuals of *A. humilis* (n= 20), *B. rufa* (n= 20), *C. cathartica* (n= 17), *M. setosa* var. *paludosa* (n= 20) and *S. polyphyllum* (n= 20). From 08 am to 18 pm we carefully inspected all plant parts (i.e. leaves, flower buds, flowers and fruits) searching for insect herbivores (richness and abundance) and their natural enemies, during 30 minutes divided into two samplings per month (totaling 582h of observation in the year). As soon we found insects, they were carefully collected with a brush or voile net and then placed individually into transparent plastic pots (500 ml) which were then labeled with information of the host plant, plant part and date. Pots were taken to the Behavioral Ecology and Interactions Laboratory at the Federal University of Uberlândia (LECI-UFU), where insects were reared in laboratory conditions (12-h light and 20–30°C) and with the same plant part where they were found. When available we removed leaves, flowers, and fruits and conditioned them in transparent plastic pots that were conditioned in the LECI to await the emergence of internal insect herbivores (n= 20 plant parts, monthly). Insects seen feeding on plant parts in field or laboratory were considered herbivores and the others fortuitous. After rearing, insects were identified by specific taxonomic keys at the lower taxonomic level possible (e.g. Rafael et al. 2012 for family and subfamilies) and with the help of specialists (e.g. for some Bruchinae, Dr Cibele Stramare Ribeiro Costa, and for Braconidae, Dr James Bryan Whitfield). Specimens of insect herbivores were deposited

at the LECI-UFU, Coleção Entomológica Padre Jesus Santiago Moure (DZUP), Departamento de Zoologia, Universidade Federal do Paraná, and Museu de Zoologia da Universidade de São Paulo (MZSP). Finally, we build a matrix with the richness and abundance of the insect herbivores reared in this system. The number of new (not fully expanded) and mature leaves (fully expanded), flower buds, flowers and fruits were monthly recorded and computed for each of the 97 previously tagged individual plants after searching for insects.

Data analysis

We used the package *bipartite* from R 3.5.3 (R Development Core Team, 2019) to build the network through a matrix with values of the absolute frequencies for each herbivore on its host plant throughout the year. In that, strength of interactions (SI), calculated by frequencies of insect herbivores, indicate weak (the lines of the graph are narrower) or strong relationships (the lines of the graph are thicker) (Tylianakis et al. 2010). We calculated the degree of specialization of the network (H_2'), which values range from 0 (no specialization) to 1 (perfect specialization; Blüthgen et al. 2006). Modularity (Q) was calculated through the QuanBiMo method (Dormann and Strauss 2014). This metric is characterized by the presence of species subsets more densely connected among themselves than with the other species in the network, also named modules/compartments (Dormann et al. 2009). The number of steps taken for the modularity analysis was 10×10^5 . The significance of metrics (H_2' and Q) was estimated through the comparison with 1000 null networks generated using the Patefield algorithm (Patefield 1981). The metric robustness of the network (R) was calculated considering the secondary extinction of herbivores resulting in the primary extinction of the host plants. This metric is based on the cumulative removal of species from a network, where values closer to 1 indicate more stable network against secondary

extinction (Burgos et al. 2007; Rocha-Filho et al. 2019). To obtain the robustness we run one network for each of plant species.

The circular analysis from Oriana (Kovach Computing Services, Pentraeth, Isle of Anglesey, UK) was used to describe the phenology and calculate the mean month in which each phenophase (i.e. new and mature leaves, flower buds, flowers, and fruits) reached the peak in each of the all plant species. The months were converted into angles (30° intervals) and were combined with the respective values of each phenophase for each plant species (Sousa-Lopes et al. 2016, 2019b). In addition, we sum the occurrence of each phenophase in all plant species together to describe their peaks in the community throughout the year. Pearson correlations were used to test the influence of the number of leaves and fruits on leaf chewers and seed chewers. These assemblages of herbivores were chose as they were the most abundant in this study. Simple linear regressions were performed to test whether the abundance of the main predators, ants and spiders, affected the abundance of insect herbivores. Data were previously checked as to its normality and variance with Shapiro-Wilk and Levene test, respectively. Data were also Log 10 transformed when necessary to improve analyses. Graph Pad Prism 5 (GraphPad Company, San Diego, CA, USA) was also used to perform graphics.

RESULTS

Plant-insect herbivore network

Altogether, 1623 individuals of insect herbivores from five orders, 23 families and 87 species were sampled on plants (Table S1). The network was highly specialized ($H_2' = 0.94$, $p < 0.001$) and modular ($Q = 0.39$, $p < 0.001$). Indeed, only six species of insect herbivores fed on more than one host plant, highlighting the modular pattern (Fig. 2). *Mimosa setosa* var. *paludosa* had the higher frequency of interactions, *A. humilis* and *S. polyphyllum* had the higher insect herbivore's richness, *A. humilis* had higher diversity

and strength of interactions, and *C. cathartica* presented the higher robustness (Table 1, Fig. 2).

About 50% of the sampled insect herbivores were found only one time throughout the year (Table S1). The most abundant insect herbivores were those from the endophytic guild (internal feeders; Table S1, Fig. 2), of which only nine species accounted for 63% of the abundance of all sampled insects. The species most abundant were Tettigoniidae sp. 6 (Orthoptera; n= 09; Fig. 2) and *Hyalymenus* sp. (Hemiptera: Alydidae; n= 07) on *A. humilis*, *Gibbobruchus* cf. *scurra* Boheman, 1833 (Coleoptera: Chrysomelidae; n= 17; Fig. 2) and *Gibbobruchus* cf. *bergamini* Manfio & Ribeiro Costa, 2014 (Coleoptera: Chrysomelidae; n= 10) on *B. rufa*, *Pachybrachis* sp. (Coleoptera: Chrysomelidae; n= 105; Fig. 2) and Tettigoniidae sp. 2 (Orthoptera; n= 09) on *C. cathartica*, *Acanthoscelides winderi* Kingsolver, 1984 (Coleoptera: Chrysomelidae; n= 420; Fig. 2) and *A. quadridentatus* (Schaeffer, 1907) (Coleoptera: Chrysomelidae; n= 364) on *M. setosa* var. *paludosa*, and Galerucinae sp. 5 (Coleoptera: Chrysomelidae; n= 38) and Crambidae sp. 1 (Lepidoptera; n= 37; Fig. 2) on *S. polyphyllum*.

Plant phenology and its effects on insect herbivores' occurrence

All evaluated plants were evergreen since leaves (new and/or mature) were found all year round. *Chamaecrista cathartica*, *M. setosa* var. *paludosa* and *S. polyphyllum* produced new leaves throughout the year (Fig. 3). In general, the peak of new leaves occurred in September, when all plants together produced 13% of new leaves observed during the year. Mature leaves peaked in February, when 12% of them were found, although the mean months were May (*A. humilis*, *B. rufa* and *C. cathartica*), June (*S. polyphyllum*) and July (*M. setosa* var. *paludosa*). Flower buds peaked in August with 51% of occurrence (dry season), but the mean months also varied among plant species

(Fig. 1 and 3). Flowering occurred mainly in the wet season when 67% of flowers were found. Fruiting occurred mainly in the dry season when 87% of fruits were produced (Fig. 1 and 3).

We found a negative, although non-significant, relationship between the number of leaves and the abundance of leaf chewers (Pearson correlation: $r = -0.56$, $p = 0.060$). Indeed, these insects were found all year round and not synchronized in a specific period. On the other hand, we found a positive and significant relationship between the number of fruits and the abundance of seed chewers (Pearson correlation: $r = 0.78$, $p = 0.002$). Since fruits were found with higher abundance in the dry season (Fig. 3), frugivore insects synchronized their occurrence in this period, when 88% of them were found.

Effects of natural enemies on insect herbivores' occurrence

The main natural enemies found on plants were 11 ant species, one spider species, one hemipteran species and three species of parasitoid wasps (Table 2). Ants were found mainly on extrafloral-nectaried plants, whereas spiders, hemipterans and wasps were found in trichome-bearing plants throughout the year (Table 2, Fig. 4a, b). Simple linear regression showed that increases in the abundance of ants predicted increases in insect herbivores' abundance on extrafloral-nectaried plants ($R^2 = 0.34$, $p = 0.044$; Fig. 4c). On the other hand, we found a trend in decreasing insect herbivores' abundance when increasing spider abundance on trichome-bearing plants ($R^2 = 0.32$, $p = 0.054$; Fig. 4d).

DISCUSSION

Our hypothesis that the Fabaceae-herbivore network would be modular and would there an increased strength of interactions by few species was corroborated. Indeed, studies on antagonistic interactions have shown a modular pattern in ecological networks (Prado and Lewinsohn 2004; Pinho et al. 2017); and in community studies it is also

expected the occurrence of few abundant (high frequency in the network) and several rare species (low frequency in the network) (Krebs 1994). For instance, Novotny et al. (2010) evaluating a complex species-rich plant–herbivore network in a tropical rain forest in Papua New Guinea found that insect herbivores from different guilds, including leaf and fruit chewers, are highly specialized, with very little exchange of conspecific among different plant species, leading to highly modular network. In the Brazilian Cerrado, Diniz and Morais (1997) also found insect herbivores in low abundance, high specialization of host plants, and with low similarity among the sampled areas.

Our results plus to the studies aforementioned reinforces a trend of high specialization of herbivores in the tropics (see also Novotny and Basset 2005), and a low abundance of them on host plants in Cerrado (Diniz and Morais 1997; Marquis et al. 2002). Further, we highlight that even on abundant and phylogenetic related plants there is a low exchange of herbivores, which make us suggest these insects are singletons and then more sampling on specific plant-herbivore interactions are necessary to know these relationships and their influence on Cerrado communities. That is especially important because we also found low robustness in the network which suggests high possibility of secondary extinction in the case of deforestation, for example. Afterall, in the last 50 years, Cerrado has been transformed into pasture or cash-crop agriculture, and only 7.5% of its area is legally protected (Myers et al. 2000; Silva and Bates 2002; Klink and Machado 2005; Strassburg et al. 2017).

Among the insect herbivores found, those endophytic were the most abundant and they overweighed the network, especially Bruchinae from the genus *Acanthoscelides* (Coleoptera: Chrysomelidae). These insects are known for their high host specificity, with many species feeding in a single seed during all larval stage,

especially on Fabaceae species (Ribeiro-Costa and Almeida 2012; Sousa-Lopes et al. 2019b). It has been shown that larvae of endophytic herbivores have relatively higher early survival than exophytic herbivores as they are most protected against environmental factors and natural enemies inside plant tissues (Cornell and Hawkins 1995; Price et al. 2011). Cornell and Hawkins (1995) reviewed patterns of table life from 124 holometabolous insect herbivores and found that in overall exophytic feeders were exposed to 5-10% greater mortality than endophytic herbivores. Therefore, in a high biodiversity environment (including natural enemies) and with marked environmental changes throughout the year (wet and dry season) such as in Brazilian Cerrado, endophytic insects can have great advantages feeding inside plant tissues.

The hypothesis that the occurrence of insect herbivores would be closely related with the peak of the host plants phenophase which they feed was also corroborated (synchronicity). Although we did not find a significant relationship between the peak of leaf production and leaf chewers' abundance, we found that the abundance of seed chewers was highly correlated with fruit production. Since leaves were available all year round may be they are not the most important factor accounting for a peak in leaf chewers' occurrence. Indeed, other studies have shown that the occurrence of exophytic leaf herbivores in Brazilian Cerrado may not be only correlated with host plant phenology, but with natural enemies' occurrence (Morais et al. 1995, 1999). On the other hand, studies focused on seed chewers and fruiting have shown a close relationship between these insects and their host plants (Rossi et al. 2011; Sousa-Lopes et al. 2019b). Therefore, we present data that reinforces that when the food resources are ephemeral such as fruits, insect herbivores related to them are also restricted to a short period of occurrence, being their occurrence most predictable than that from other

herbivores (i.e. exophytic leaf chewers) which their resources are available all year round (see also Bernays and Chapman 1994).

Ants were the most abundant natural enemies of insect herbivores found on extrafloral-nectaried plants. However, increases in ant abundance did not decrease insect herbivores' abundance. Several studies have shown that ants in counterpart to feeding on extrafloral nectar prey on or chase away herbivores, benefitting the plant (Bronstein 1998; Oliveira and Freitas 2004; Rico-Gray and Oliveira 2007; Rosumek et al. 2009; Marazzi et al. 2013; Zhang et al. 2015). However, other studies have shown that ants can fail to protect the plant partners (Alves-Silva et al. 2014; Alves-Silva and Del-Claro 2016a, 2016b). For instance, Alves-Silva et al. (2014) showed that *Camponotus blandus* (Smith, 1858) was unable to protect *Banisteriopsis malifolia* (Nees and Martius) B. Gates (Malpighiaceae) against *Anthonomus* weevils (Curculionidae) in the Brazilian Cerrado. Like in that study, during fieldwork we also found ants biting some beetles which were immune to attacks and remained feeding on plants (Sousa-Lopes, pers. obs.). This data reinforces that the role of ants as plant bodyguards is taxon and/or context-dependent (see Del-Claro et al. 2016).

On the other hand, on glandular trichome-bearing plants, we observed spiders, mainly *Peucetia flava* Keyserling, 1877, preying on several insects, although they failed against the endophytic *A. winderi* which hide its vulnerable body parts under the elytra (Sousa-Lopes et al. 2019a). But in general, our analyses indicated a trend in decreasing insect herbivores' abundance when increasing spider abundance. Therefore, our hypothesis that the abundance of insect herbivores would be negatively influenced by the abundance of natural enemies was also corroborated, at least for spiders against exophytic herbivores.

Our results on the Fabaceae-insect herbivore network suggest as following: (i) a specialized and modular ecological network, (ii) many rare and few abundant species of insect herbivores, (iii) a higher abundance of endophytic seed chewers, (iv) a close temporal relationship between seed chewers and fruiting, and (v) a trend in spiders decreasing the abundance of insect herbivores on glandular plants. Therefore, we showed that both bottom-up and top-down forces affect the structure of an insect herbivores community in different ways depending on insect taxon and/or guild. Since it was also found a low robustness in the networks, suggesting high possibility of secondary extinction in disturbance events, we suggest that is crucial the conservation of areas with huge plant diversity to maintain insects' occurrence and their important roles in structuring terrestrial communities in Brazilian Cerrado.

REFERENCES

- Aguiar LMS, Camargo AJA. (2004) Cerrado: ecologia e caracterização. Brasília: Embrapa Informação Tecnológica. 249 p.
- Alves-Silva E, Bächtold A, Barônio GJ, Torezan-Silingardi HM, Del-Claro K. (2014). Ant-herbivore interactions in an extrafloral nectaried plant: are ants good plant guards against curculionid beetles? *J Nat His.* <http://dx.doi.org/10.1080/00222933.2014.954020>
- Alves-Silva E, Del-Claro K. (2016a). On the inability of ants to protect their plant partners and the effect of herbivores on different stages of plant reproduction. *Austral Ecol.* doi:10.1111/aec.12307.
- Alves-Silva E, Del-Claro K. (2016b). Wasps are better plant-guards than ants in the extrafloral nectaried shrub *Ouratea Spectabilis* (Ochnaceae). *Sociobiology* 63(1): 705-711.
- Bächtold A, Lange D, Del-Claro K. (2014). Influence, or the lack thereof, of host phenology, architecture and climate on the occurrence of *Udranomia spitzi* (Hesperiidae: Lepidoptera). *Entomol Sci.* 17:66–74.
- Bascompte J, Jordano P. (2007). Plant-Animal Mutualistic Networks: The Architecture of Biodiversity *Annu. Rev. Ecol. Evol. Syst.* 38:567–93.
- Basset Y. (1992). Host specificity of arboreal and free-living insect herbivores in rain forests. *Biol. J. Linn. Soc.* 47, 115–133.
- Bernays EA, Chapman RF. (1994). *Host-plant selection by phytophagous insects.* New York (NY): Chapman & Hall Press.
- Blüthgen N. (2006). Measuring specialization in species interaction networks. *BMC Ecol.* 6: 1–12.
- Bronstein JL. (1998). The contribution of ant plant protection studies to our understanding of mutualism. *Biotropica* 30:150–161. doi:10. 1111/j.1744-7429.1998.tb00050.x.

- Burgos E, Ceva H, Perazzo RPJ, Devoto M, Medan D, Zimmermann M, Delbue AM. (2007). Why nestedness in mutualistic networks? *J Theor Biol*, 249(2): 307–313.
- Cornell HV, Hawkins BA. (1995). Survival patterns and mortality sources of herbivorous insects: some demographic trends. *Am. Nat.* 145:563–593.
- Del-Claro K, Rico-Gray V, Torezan-Silingardi HM, Alves-Silva E, Fagundes R, Lange D, Dattilo W, Vilela AA, Aguirre A, Rodriguez-Morales D. (2016). Loss and gains in ant–plant interactions mediated by extrafloral nectar: fidelity, cheats, and lies. *Insect. Soc.* 63:207–221.
- Del-Claro K, Stefani V, Nahas L, Torezan-Silingardi, HM. (2017). Spiders as plant partners: complementing ant services to plants with extrafloral nectaries. In *Behaviour and ecology of spiders: contribution from the Neotropical region: 215–226*. Viera, C. & Gonzaga, M.O. (Eds). Gewerbestrasse: Springer.
- Dias, BFS. (1992). Alternativas de desenvolvimento dos Cerrados: manejo e conservação dos recursos naturais renováveis. Brasília, DF: Funatura, 97p.
- Diniz IR, Hay JD, Rico-Gray V, Greeney HF, Morais HC. (2012). Shelter-building caterpillars in the cerrado: seasonal variation in relative abundance, parasitism, and the influence of extra-floral nectaries. *Anthropod-Plant Interactions*, 6, 583–589.
- Diniz IR, Morais HC. (1997). Lepidopteran caterpillar fauna of cerrado host plants. *Biodiversity and Conservation*, 6: 817–836.
- Diniz IR, Morais HC, Camargo AJA. (2001). Host plants of lepidopteran caterpillars in the cerrado of the Distrito Federal, Brazil. *Revista Brasileira de Entomologia* 45(2): 107-122.
- Dormann CF, Fründ J, Blüthgen N, Gruber B. (2009). Indices, graphs and null models: analyzing bipartite ecological networks. *The Open Ecology Journal*, 2:7–24.
- Dormann CF, Strauss R. (2014). Detecting modules in quantitative bipartite networks: the QuaBiMo algorithm. arXiv [q-bio.Qm], 1304.3218v.
- Dutra VF, Garcia FCP. (2014). *Mimosa* L.(Leguminosae-Mimosoideae) dos campos rupestres de Minas Gerais, Brasil. *Iheringia Serie Bot.* 69:49–88.
- Flora do Brasil 2020 em construção. Jardim Botânico do Rio de Janeiro. Disponível em: <<http://reflora.jbrj.gov.br/reflora/floradobrasil/FB82684>>. Acesso em: 20 Jul. 2019.
- Futuyma D, Agrawal AA. (2009). Macroevolution and the biological diversity of plants and herbivores. *Proc. Natl. Acad. Sci. U. S.* 84, 18054e18061.
- González-Teuber M, Heil M. (2009). The role of extrafloral nectar amino acids for the preferences of facultative and obligate ant mutualists. *J. Chem. Ecol.*, 35(4), 459-468.
- Goodland R. (1971). A physiognomic analysis of the “cerrado” vegetation of central Brazil. *J. Ecol.* 59, 411–419.
- INMET (Instituto Nacional de Meteorologia). 2019. Available from: <http://www.inmet.gov.br/portal>. Accessed 20 jul 2019.
- Janzen DH. (1983). Food webs: who eats what, why, how, and with what effects in a tropical forest? In *Tropical rain forest ecosystems* (ed. FBI Golley), pp. 167–182. Amsterdam: Elsevier.
- Klink CA, Machado RB. (2005). Conservation of the Brazilian Cerrado. *Conserv. Biol.* 19, 707–713.
- Kuchenbecker J, Fagundes M. (2018). Diversity of insects associated with two common plants of the Brazilian Cerrado: responses of two guilds of herbivores to bottom-up and top-down forces. *European Journal of Entomology*, 115, 354–363.

- Lange D, Calixto ES, Del-Claro K. (2017). Variation in Extrafloral Nectary Productivity Influences the Ant Foraging. *PLoS ONE* 12(1): e0169492. doi:10.1371/journal.pone.0169492.
- Lewinsohn TM, Prado PI, Jordano P, Bascompte J, Olesen JM. (2006). Structure in plant/animal interaction assemblages. *Oikos* 113:1, 174–184.
- Lewinsohn TM, Prado PI. (2002). Biodiversidade brasileira: síntese do estado atual do conhecimento. Contexto, São Paulo.
- Linz DM, Hu AW, Sitvarin MI, Tomoyasu Y. (2016). Functional value of elytra under various stresses in the red flour beetle, *Tribolium castaneum*. *Sci. Rep.* 6, 34813.
- Lorenzi H. (2000). Plantas daninhas do Brasil: terrestres, aquáticas, parasitas e tóxicas. Nova Odessa: Instituto Plantarum, SP.
- Machado SRL, Morellato PC, Sajo MG, Oliveira PS. (2008). Morphological patterns of extrafloral nectaries in woody plant species of the Brazilian cerrado. *Plant Biology* 10, 660–673.
- Madeira JA, Fernandes GW, González-Rodríguez A, Cuevas-Reyes P. (2013). Tri-trophic interactions among congeneric sympatric host plants of *Chamaecrista*, seed predators and parasitoids. *Arthropod-Plant Interac*, 7, 4, 403–413.
- Marazzi B, Bronstein JL, Koptur S. (2013). The diversity, ecology and evolution of extrafloral nectaries: current perspectives and future challenges. *Ann Botany*. 111:1243–1250. doi:10.1093/aob/mct109.
- Marquis RJ, Morais HC, Diniz IR. (2002). Interactions among cerrado plants and their herbivores: unique or typical? In PS Oliveira, RJ Marquis (Eds.). *The cerrados of Brazil: Ecology and natural history of a Neotropical savanna* (pp. 306–328). New York: Columbia University Press.
- Mendonça RC, Felfili JM, Walter BMT, Júnior MCS, Rezende AS, Filgueira TS, Nogueira PE, Fagg CW (2008). Flora vascular do bioma Cerrado: checklist com 12356 espécies. In: Sano, S.M.; Almeida, S.P. & Ribeiro, J.F. (eds.). *Cerrado: ecologia e flora*. 2 vols. Embrapa Cerrados, Embrapa Informações Tecnológicas, Brasília. p. 151–199.
- Morais HC, Diniz IR, Baumgarten LC. (1995). Padrões de produção de folhas e sua utilização por larvas de Lepidoptera em um cerrado de Brasília, DF, Brasil. *Revista Brasileira de Botânica*, 18, 2, 165–172.
- Morais HC, Diniz IR, Silva DMS. (1999). Caterpillar seasonality in a central Brazilian cerrado. *Revista de Biologia Tropical*, 47, 4, 1025–1033.
- Myers N, Mittermeier RA, Mittermeier CG, Fonseca GAB, Kent J. (2000). Biodiversity hotspots for conservation priorities. *Nature* 403, 853–858.
- Nahas L, Gonzaga MO, Del-Claro K. (2012). Emergent Impacts of Ant and Spider Interactions: Herbivory Reduction in a Tropical Savanna Tree. *Biotropica* 44(4): 498–505.
- Novais SMA de, Monteiro GF, Macedo-Reis LE, Leal CRO, Neves F.S. (2019). Changes in the insect herbivore fauna after the first rains in a tropical dry forest. *Oecologia Australis*, 23(2):381–387.
- Novotny V, Basset Y. (2005). Host specificity of insect herbivores in tropical forests. *Proc. R. Soc. B* (2005) 272, 1083–1090.
- Novotny V, Miller SE, Baje L, Balagawi S, Basset Y, Cizek L, Craft KJ, Dem F, Drew, RAI, Hulcr J, Leps J, Lewis OT, Pokon R, Stewart AJA, Samuelson GA, Weiblen GD. (2010). Guild-specific patterns of species richness and host specialization in plant-herbivore food webs from a tropical forest. *Journal of Animal Ecology*, 79(6), 1193–1203. doi:10.1111/j.1365-2656.2010.01728.x.

- Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner E. (2018). Vegan: community ecology package. R package version 2.5-2.
- Oliveira PS, Freitas AVL. (2004). Ant plant herbivore interactions in the neotropical cerrado savanna. *Naturwissenschaften* 91:557–570. doi:10.1007/s00114-004-0585-x.
- Oliveira PS, Marquis RJ. (2002). *The Cerrados of Brazil: Ecology and Natural History of a Neotropical Savanna*. Columbia University Press, New York, viii + 398 pp.
- Patefeld WM. (1981). Algorithm AS159. An efficient method of generating $r \times c$ tables with given row and column totals. *Appl Statistics* 30(1):91–97
- Periotto F, Perez SGA, Lima MIS. (2004). Allelopathic effect of *Andira humilis* Mart. ex Benth in the germination and growth of *Lactuca sativa* L. and *Raphanus sativus* L. *Acta Bot. Bras.* vol.18 no.3 São Paulo July/Sept.
- Pinho BX, Dáttilo W, Leal IR. (2017). Structural breakdown of specialized plant-herbivore interaction networks in tropical forest edges. *Global Ecology and Conservation*, 12, 1-8.
- Pott A, Pott VJ, Souza TW. (2006). *Plantas daninhas de pastagem na região dos Cerrados*. Campo Grande, MS: EMBRAPA Gado de Corte.
- Prado PI, Lewinsohn TM. (2004). Compartments in insect–plant associations and their consequences for community structure. *Journal of Animal Ecology* 73, 1168–1178.
- Price PW, Denno RF, Eubanks MD, Finke DL, Kaplan I. (2011). *Insect Ecology: behavior, populations and communities*. New York: Cambridge University Press. 764p.
- Price PW, Bouton CE, Gross P, McPherson BA, Thompson JN, Weiss AE. (1980). Interactions Among Three Trophic Levels: Influence of Plants on Interactions Between Insect Herbivores and Natural Enemies. *Ann Rev Ecol Syst*, 11, 41–65.
- Price PW. (2002a). Species interactions and the evolution of biodiversity. In: Herrera CM, Pellmyr O (eds) *Plant–animal interactions: an evolutionary approach*. Blackwell Science, Oxford, pp 3–25.
- Price PW. (2002b). Resource-driven terrestrial interaction webs. *Ecol. Res.* 17, 241–247.
- R Development Core Team. (2019). R: A language and environment for statistical computing. Available from: <http://www.R-project.org>. Accessed 20 jul 2019.
- Rafael JA, Melo GAR, Carvalho CJB, Casari AS, Constantino R. (2012). *Insetos do Brasil: diversidade e taxonomia*. Ribeirão Preto: Holos Editora. 795pp.
- Ribeiro JF, Walter BMT. (2008). As principais fitofisionomias do Bioma Cerrado. In: Sano, S. M, Almeida, SP, Ribeiro, JF. *Ecologia e flora*. Brasília: EMBRAPA. v. 1, p. 152-212.
- Ribeiro-Costa CS, Almeida LM. (2012). Seed-Chewing Beetles (Coleoptera: Chrysomelidae, Bruchinae). In: Panizzi AR, Parra JRP, editors. *Insect bioecology and nutrition for integrated pest management*. 1st ed. Vol. 1. Boca Raton: CRC Press; p. 325–352.
- Rico-Gray V, Oliveira PS. (2007). *The ecology and evolution of ant– plant interactions*. The University of Chicago Press, Chicago.
- Rocha-Filho, LC, Moure-Oliveira, D, Carvalho, SM, Frantine-Silva, W, Augusto, SC. 2019. Diversity and host-parasite interactions of cavity-nesting Hymenoptera communities in the Brazilian Savannah. *J Insect Cons.* <https://doi.org.10.1007/s10841-019-00157-7>

- Romero GQ, Souza, JC, Vasconcellos-Neto J. (2008). Anti-herbivore protection by mutualistic spiders and the role of plant glandular trichomes. *Ecology* 89, 3105–3115.
- Rossi MN, Rodrigues LMS, Ishino MN, Kestring D. (2011). Oviposition pattern and within-season spatial and temporal variation of pre-dispersal seed predation in a population of *Mimosa bimucronata* trees. *Arthropod Plant Interact.* 5:209 – 217. doi:10.1007/s11829-011-9130-y
- Rosumek FB, Silveira FAO, Neves FS, Barbosa NP, Diniz L, Oki Y, Pezzini F, Fernandez WG, Cornelissen T. (2009). Ants on plants: a meta-analysis of the role of ants as plant biotic defenses. *Oecologia* 160:537–549. doi:10.1007/s00442-009-1309-x
- Silva JMC, Bates JM. (2002). Biogeographic patterns and conservation in the south American cerrado: a tropical savanna hotspot. *Bioscience*, 52, 3, 225–234.
- Silva JO, Neves FS. (2014) Insect herbivores associated with an evergreen tree *Goniorrhachis marginata* Taub. (Leguminosae: Caesalpinioideae) in a tropical dry forest. *Brazilian Journal of Biology*, 74, 3, 623–631.
- Sousa-Lopes B, Alves-da-Silva N, Alves-Martins F, Del-Claro K. (2019a). Antiherbivore protection and plant selection by the lynx spider *Peucetia flava* (Araneae: Oxyopidae) in the Brazilian Cerrado. *J. Zool*, 308(2): 121–127.
- Sousa-Lopes B, Alves-da-Silva N, Ribeiro-Costa CS, Del-Claro K. (2019b). Temporal distribution, seed damage and notes on the natural history of *Acanthoscelides quadridentatus* and *Acanthoscelides winderi* (Coleoptera: Chrysomelidae: Bruchinae) on their host plant, *Mimosa setosa* var. *paludosa* (Fabaceae), in the Brazilian Cerrado. *J Nat Hist*, 53(9-10):611–623.
- Sousa-Lopes B, Bächtold A, Del-Claro K. (2016). Biology, natural history and temporal fluctuation of the geometrid *Oospila pallidaria* associated with host plant phenology. *Stud Neotrop Fauna Environ.* 51:135–143.
- Sousa-Lopes B, Whitfield JB, Salgado-Neto G, Del-Claro K. (2019c). *Cotesia itororensis* sp. nov. from Brazilian savanna: a new reared microgastrinae wasp described using an integrative taxonomic approach. *Zootaxa*, 4544 (3): 437–445.
- Stefani V, Pires TL, Torezan-Silingardi HM, Del-Claro K. (2015). Beneficial effects of ants and spiders on the reproductive value of *Eriotheca gracilipes* (Malvaceae) in a tropical savanna. *PLoS One* 10:e0131843. doi:10.1371/journal.pone.0131843.
- Strassburg BBN, Brooks T, Feltran-Barbieri R, Iribarrem A, Crouzeilles R, Loyola R, Latawiec AE, Filho FJBO, Scaramuzza CAM, Scarano FR, Soares-Filho B, Balmford A. (2017). Moment of truth for the Cerrado hotspot. *Nat. Ecol. Evol.* 99, <http://dx.doi.org/10.1038/s41559-017-0099>.
- Strong DR, Lawton JH, Southwood R. (1984). *Insects on Plants: Community Patterns and Mechanisms*. Blackwell, Oxford.
- Tylianakis JM, Laliberté E, Nielsen A, Bascompte J. (2010). Conservation of species interaction networks. *Biological Conservation* 143: 2270–2279.
- Vilela AA, Torezan-Silingardi HM, Del-Claro K. (2014). Conditional outcomes in ant–plant–herbivore interactions influenced by sequential flowering. *Flora* 209:359–366. doi:10.1016/j.flora. 2014.04.004.
- Zhang S, Zhang Y, Keming MA. (2015). The equal effectiveness of different defensive strategies. *Sci Repo* 5. Article number:13049. doi:10.1038/srep13049.

Table 1. Richness (Rich.), Shannon diversity index (H), robustness (R), and Strength of Interactions (SI) of the Fabaceae-insect herbivore network in Brazilian Cerrado.

Metrics	Host plants				
	<i>A. Humilis</i>	<i>B. rufa</i>	<i>C. cathartica</i>	<i>M. setosa</i> var. <i>paludosa</i>	<i>S. polyphyllum</i>
Rich.	22	21	12	17	22
H	2.74	2.39	1.28	1.74	2.16
R	0.12	0.13	0.22	0.16	0.12
SI	20.64	20.20	11.20	16.04	18.90

Table 2. Host plant, prey, and absolute frequency of natural enemies of insect herbivores associated with Fabaceae species in Brazilian Cerrado.

Natural enemy	Host plant	Prey/Host	Absolute frequency
<i>Apiomerus</i> sp. (Hemiptera: Reduviidae)	<i>Chamaecrista cathartica</i>	Generalist	09
<i>Camponotus blandus</i> (Smith, 1858) (Hymenoptera: Formicidae)	<i>Bauhinia rufa</i>	Generalist	45
<i>Camponotus crassus</i> May, 1862 (Hymenoptera: Formicidae)	<i>Andira humilis</i>	Generalist	147
	<i>Bauhinia rufa</i>		377
	<i>Chamaecrista cathartica</i>		27
<i>Camponotus renggeri</i> Emery, 1894 (Hymenoptera: Formicidae)	<i>Stryphnodendron polyphyllum</i>	Generalist	453
	<i>Andira humilis</i>		02
<i>Camponotus</i> sp.1 (Hymenoptera: Formicidae)	<i>Bauhinia rufa</i>	Generalist	01
	<i>Stryphnodendron polyphyllum</i>		01
<i>Camponotus</i> sp2 (Hymenoptera: Formicidae)	<i>Andira humilis</i>	Generalist	01
<i>Cephalotes</i> aff. <i>angustus</i> (May, 1862) (Hymenoptera: Formicidae)	<i>Bauhinia rufa</i>	Generalist	01
<i>Cephalotes pusillus</i> (Klug, 1824)	<i>Bauhinia rufa</i>	Generalist	06
<i>Cotesia itororensis</i> Sousa-Lopes & Whitfield 2019 (Hymenoptera: Braconidae)	<i>Mimosa setosa</i> var. <i>paludosa</i>	<i>Oospila pallidaria</i>	06
<i>Ectatoma tuberculatum</i> (Olivier, 1792) (Hymenoptera: Formicidae)	<i>Andira humilis</i>	Generalist	05
	<i>Bauhinia rufa</i>		34
	<i>Chamaecrista cathartica</i>		33
	<i>Stryphnodendron polyphyllum</i>		17
Eupelmidae sp. (Hymenoptera)	<i>Mimosa setosa</i> var. <i>paludosa</i>	<i>Acanthoscelides winderi</i>	110
Myrmicinae sp. (Hymenoptera: Formicidae)	<i>Stryphnodendron polyphyllum</i>	Generalist	02
<i>Peucetia flava</i> Keyserling, 1877	<i>Mimosa setosa</i> var. <i>paludosa</i>	Generalist	101

(Araneae: Oxyopidae)	<i>Chamaecrista catártica</i>		108
<i>Pheidole</i> sp. (Hymenoptera: Formicidae)	<i>Stryphnodendron polyphyllum</i>	Generalist	09
<i>Pseudomyrmex flavidus</i> (Smith, 1858) (Hymenoptera: Formicidae)	<i>Andira humilis</i>	Generalist	02
<i>Pseudomyrmex gracilis</i> (Fabricius, 1804) (Hymenoptera: Formicidae)	<i>Stryphnodendron polyphyllum</i>		01
	<i>Andira humilis</i>	Generalist	04
	<i>Bauhinia rufa</i>		31
	<i>Stryphnodendron polyphyllum</i>		02
<i>Stenocorse bruchivora</i> (Crawford, 1909) (Braconidae, Doryctinae)	<i>Mimosa setosa</i> var. <i>paludosa</i>	<i>Acanthoscelides</i> <i>quadridentatus</i>	48

Table S1. Host plant, guild, strength of interactions, and absolute frequency of insect herbivores sampled on Fabaceae species in Brazilian Cerrado. Herbivorous guilds were adapted from Novotny et al. (2010). Ex. = external (exophytic) and In. = internal (endophytic).

Host plant	Taxonomic affiliation	Guild	Strength of interactions	Absolute frequency
<i>Andira humilis</i>	<i>Thasus</i> sp. (Hemiptera: Coreidae)	Ex. stem sucking	0.0408	02
<i>Andira humilis</i>	<i>Chinavia</i> sp. (Hemiptera: Coreidae)	Ex. leaf sucking	0.0204	01
<i>Andira humilis</i>	Pentatomidae sp.1 (Hemiptera)	Ex. leaf sucking	0.0408	02
<i>Andira humilis</i>	Coreidae sp.3 (Hemiptera)	Ex. leaf sucking	0.0892	04
<i>Stryphnodendron polyphyllum</i>				01
<i>Andira humilis</i>	Coreidae sp.1 (Hemiptera)	Ex. leaf sucking	0.0204	01
<i>Andira humilis</i>	<i>Hyalymenus</i> sp. (Hemiptera: Alydidae)	Ex. leaf sucking	0.1504	07
<i>Stryphnodendron polyphyllum</i>				01
<i>Andira humilis</i>	Lygaeidae sp.1 (Hemiptera)	Ex. leaf sucking	0.0204	01
<i>Andira humilis</i>	Tettigoniidae sp.6 (Orthoptera)	Ex. leaf chewer	0.1836	09
<i>Andira humilis</i>	Pleminiini sp. (Tettigoniidae: Orthoptera)	Ex. leaf chewer	0.0204	01
<i>Andira humilis</i>	Proscopiidae sp.1 (Orthoptera)	Ex. leaf chewer	0.0359	01
<i>Chamaecrista cathartica</i>				02
<i>Mimosa setosa</i> var. <i>paludosa</i>				03
<i>Andira humilis</i>	Acrididae sp.1 (Orthoptera)	Ex. leaf chewer	0.0204	01
<i>Andira humilis</i>	Acrididae sp.2 (Orthoptera)	Ex. leaf chewer	0.0204	01
<i>Andira humilis</i>	<i>Orophus</i> sp. (Orthoptera: Tettigoniidae)	Ex. leaf chewer	0.0204	01
<i>Andira humilis</i>	Entiminae sp.2 (Coleoptera: Curculionidae)	Ex. leaf chewer	0.0408	02
<i>Andira humilis</i>	<i>Naupactus</i> sp. (Coleoptera: Curculionidae)	Ex. leaf chewer	0.0408	01
<i>Andira humilis</i>	<i>Naupactus tarsalis</i> Boheman, 1840 (Coleoptera: Curculionidae)	Ex. leaf chewer	0.0612	03
<i>Andira humilis</i>	Entiminae sp.1 (Coleoptera: Curculionidae)	Ex. leaf chewer	0.0408	02
<i>Andira humilis</i>	<i>Anthonomus</i> sp. (Coleoptera: Curculionidae)	Ex. leaf chewer	0.1012	04

<i>Bauhinia rufa</i>	Curculionidae)			01
<i>Andira humilis</i>	Galerucinae sp.4 (Coleoptera: Chrysomelidae)	Ex. leaf chewer	0.0204	01
<i>Andira humilis</i>	Galerucinae sp.2 (Coleoptera: Chrysomelidae)	Ex. leaf chewer	0.0204	01
<i>Andira humilis</i>	Lycaenidae sp.1 (Lepidoptera)	Ex. leaf chewer	0.0204	01
<i>Andira humilis</i>	Notodontidae sp.1 (Lepidoptera)	Ex. leaf chewer	0.0204	01
<i>Bauhinia rufa</i>	<i>Empoasca</i> sp. (Hemiptera: Cicadellidae)	Ex. leaf sucking	0.0196	01
<i>Bauhinia rufa</i>	<i>Enchenopa</i> sp. (Hemiptera: Membracidae)	Ex. stem sucking	0.0196	01
<i>Bauhinia rufa</i>	<i>Crinocerus sanctus</i> (Fabricius, 1775) (Hemiptera: Coreidae)	Ex. leaf sucking	0.0196	01
<i>Bauhinia rufa</i>	Eumastacidae sp.1 (Orthoptera)	Ex. leaf chewer	0.0196	01
<i>Bauhinia rufa</i>	Tettigoniidae sp.3 (Orthoptera)	Ex. leaf chewer	0.0196	01
<i>Bauhinia rufa</i>	Molytinae sp.2 (Coleoptera: Curculionidae)	Ex. leaf chewer	0.0196	01
<i>Bauhinia rufa</i>	<i>Sternechus</i> sp. (Coleoptera: Curculionidae)	Ex. leaf chewer	0.0196	01
<i>Bauhinia rufa</i>	Molytinae sp.1 (Coleoptera: Curculionidae)	Ex. leaf chewer	0.0196	01
<i>Bauhinia rufa</i>	<i>Aphrastus</i> sp. (Coleoptera: Curculionidae)	Ex. leaf chewer	0.0196	01
<i>Bauhinia rufa</i>	<i>Eubulus</i> sp. (Coleoptera: Curculionidae)	Ex. leaf chewer	0.0196	01
<i>Bauhinia rufa</i>	<i>Eumolpus</i> sp. (Coleoptera: Chrysomelidae)	Ex. leaf chewer	0.0392	10
<i>Bauhinia rufa</i>	<i>Xystus sanguinicollis</i> (Germar, 1824) (Coleoptera: Curculionidae)	Ex. leaf chewer	0.0196	02
<i>Bauhinia rufa</i>	Cryptorhynchinae sp.1 (Coleoptera: Curculionidae)	Ex. leaf chewer	0.0196	01

<i>Bauhinia rufa</i>	<i>Anthonomus</i> sp.2 (Coleoptera: Curculionidae)	Ex. leaf chewer	0.0196	01
<i>Bauhinia rufa</i>	<i>Gibbobruchus</i> cf. <i>bergamini</i> Manfio & Ribeiro Costa, 2014 (Coleoptera: Chrysomelidae)	Int. seed chewer	0.3333	17
<i>Bauhinia rufa</i>	<i>Gibbobruchus</i> cf. <i>scurra</i> Boheman, 1833 (Coleoptera: Chrysomelidae)	Int. seed chewer	0.1960	10
<i>Bauhinia rufa</i>	Chrytocephalinae sp.2 (Coleoptera: Chrysomelidae)	Ex. leaf chewer	0.0196	01
<i>Bauhinia rufa</i>	Cryptocephalinae sp.1 (Coleoptera: Chrysomelidae)	Ex. leaf chewer	0.0588	03
<i>Bauhinia rufa</i>	<i>Chlamisus</i> sp.2 (Coleoptera: Chrysomelidae)	Ex. leaf chewer	0.0392	02
<i>Bauhinia rufa</i>	Oecophoridae sp.2 (Lepidoptera)	Ex. leaf chewer	0.0392	02
<i>Chamaecrista cathartica</i>	<i>Dichelops</i> sp. (Hemiptera: Pentatomidae)	Ex. leaf sucking	0.0065	01
<i>Chamaecrista cathartica</i>	<i>Hypselonotus interruptus</i> Hahn, 1833 (Hemiptera: Coreidae)	Ex. leaf sucking	0.0261	04
<i>Chamaecrista cathartica</i>	Tettigoniidae sp.2 (Orthoptera)	Ex. leaf chewer	0.0588	09
<i>Chamaecrista cathartica</i>	Acrididae sp.3 (Orthoptera)	Ex. leaf chewer	0.0588	09
<i>Chamaecrista cathartica</i>	<i>Teratopactus nodicollis</i> (Boheman, 1833) (Coleoptera: Curculionidae)	Ex. leaf chewer	0.0065	01
<i>Chamaecrista cathartica</i>	<i>Naupactus</i> sp.2 (Coleoptera: Curculionidae)	Ex. leaf chewer	0.0261	04
<i>Chamaecrista cathartica</i>	Baridinae sp.2 (Coleoptera: Curculionidae)	Ex. stem chewer	0.0065	01
<i>Chamaecrista cathartica</i>	Galerucinae sp.3 (Coleoptera: Chrysomelidae)	Ex. leaf chewer	0.0065	01
<i>Chamaecrista cathartica</i>	<i>Pachybrachis</i> sp. (Coleoptera: Chrysomelidae)	Ex. leaf chewer	0.6862	105
<i>Chamaecrista cathartica</i>	Hesperiidae sp.1 (Lepidoptera)	Ex. leaf chewer	0.0588	09

<i>Chamaecrista cathartica</i>	<i>Atta</i> sp. (Hymenoptera: Formicidae)	Ex. leaf chewer	0.0533	07
<i>Stryphnodendron polyphyllum</i>				01
<i>Mimosa setosa</i> var. <i>paludosa</i>	<i>Cebrenis cauta</i> Brailovsky, 1995 (Hemiptera: Coreidae)	Ex. leaf sucking	0.0008	01
<i>Mimosa setosa</i> var. <i>paludosa</i>	Coreidae sp.4 (Hemiptera)	Ex. leaf sucking	0.0008	01
<i>Mimosa setosa</i> var. <i>paludosa</i>	Alydidae sp.1 (Hemiptera)	Ex. leaf sucking	0.0008	01
<i>Mimosa setosa</i> var. <i>paludosa</i>	Membracidae sp.2 (Hemiptera)	Ex. stem sucking	0.0008	01
<i>Mimosa setosa</i> var. <i>paludosa</i>	Tettigonidae sp.4 (Orthoptera)	Ex. leaf chewer	0.0008	01
<i>Mimosa setosa</i> var. <i>paludosa</i>	Baridinae sp.1 (Coleoptera: Curculionidae)	Ex. leaf/stem chewer	0.0016	02
<i>Mimosa setosa</i> var. <i>paludosa</i>	<i>Aracanthus</i> sp. (Coleoptera: Curculionidae)	Ex. leaf chewer	0.0435	54
<i>Mimosa setosa</i> var. <i>paludosa</i>	Curculioninae sp.1 (Coleoptera: Curculionidae)	Ex. leaf chewer	0.0072	09
<i>Mimosa setosa</i> var. <i>paludosa</i>	Galerucinae sp.1 (Coleoptera: Chrysomelidae)	Ex. leaf chewer	0.0016	02
<i>Mimosa setosa</i> var. <i>paludosa</i>	Galerucinae sp.5 (Coleoptera: Chrysomelidae)	Ex. leaf chewer	0.3263	45
<i>Stryphnodendron polyphyllum</i>				38
<i>Mimosa setosa</i> var. <i>paludosa</i>	<i>Acanthoscelides winderi</i> Kingsolver, 1984 (Coleoptera: Chrysomelidae)	Int. seed chewer	0.3389	420
<i>Mimosa setosa</i> var. <i>paludosa</i>	<i>Acanthoscelides quadridentatus</i> (Schaeffer, 1907) (Coleoptera: Chrysomelidae)	Int. seed chewer	0.2937	364
<i>Mimosa setosa</i> var. <i>paludosa</i>	<i>Chlamisus</i> sp.1 (Coleoptera: Chrysomelidae)	Ex. leaf chewer	0.0169	21
<i>Mimosa setosa</i> var. <i>paludosa</i>	Gelechiidae sp.1 (Lepidoptera)	Int. seed chewer	0.1452	180
<i>Mimosa setosa</i> var. <i>paludosa</i>	<i>Oospila pallidaria</i> (Schaus, 1897) (Lepidoptera: Geometridae)	Ex. leaf/flower chewer	0.0718	89
<i>Mimosa setosa</i> var. <i>paludosa</i>	<i>Macaria</i> sp. (Lepidoptera: Geometridae)	Ex. leaf/flower chewer	0.0363	45
<i>Stryphnodendron polyphyllum</i>	Miridae sp.1 (Hemiptera)	Ex. leaf sucking	0.0076	01

<i>Stryphnodendron polyphyllum</i>	<i>Leptoglossus</i> sp.1 (Hemiptera: Coreidae)	Ex. leaf sucking	0.0152	02
<i>Stryphnodendron polyphyllum</i>	Coreidae sp.2 (Hemiptera)	Ex. leaf sucking	0.0152	02
<i>Stryphnodendron polyphyllum</i>	Pentatomidae sp.2 (Hemiptera)	Ex. leaf sucking	0.0204	03
<i>Stryphnodendron polyphyllum</i>	Membracidae sp.1 (Hemiptera)	Ex. stem sucking	0.0076	01
<i>Stryphnodendron polyphyllum</i>	Entiminae sp.3 (Coleoptera: Curculionidae)	Ex. leaf chewer	0.0152	02
<i>Stryphnodendron polyphyllum</i>	Chrysomelidae sp.1 (Coleoptera)	Ex. leaf chewer	0.0534	07
<i>Stryphnodendron polyphyllum</i>	Chrysomelidae sp.2 (Coleoptera)	Ex. leaf chewer	0.0076	01
<i>Stryphnodendron polyphyllum</i>	Chrysomelinae sp.1 (Coleoptera)	Ex. leaf chewer	0.0076	01
<i>Stryphnodendron polyphyllum</i>	Eumolpinae sp.1 (Coleoptera: Chrysomelidae)	Ex. leaf chewer	0.0076	01
<i>Stryphnodendron polyphyllum</i>	<i>Acanthoscelides</i> cf. <i>gregorioi</i> (Pic, 1931) (Coleoptera: Chrysomelidae)	Int. seed chewer	0.0992	13
<i>Stryphnodendron polyphyllum</i>	Cryptocephalinae sp.2 (Coleoptera: Chrysomelidae)	Int. seed chewer	0.0196	01
<i>Stryphnodendron polyphyllum</i>	Pachybrachina sp.1 (Coleoptera: Chrysomelidae)	Ex. leaf chewer	0.0076	01
<i>Stryphnodendron polyphyllum</i>	<i>Macroductylus pumilio</i> Burm, 1855 (Coleoptera: Scarabidae)	Ex. flower chewer	0.0152	02
<i>Stryphnodendron polyphyllum</i>	Crambidae sp.1 (Lepidoptera)	Ex. leaf chewer	0.2824	37
<i>Stryphnodendron polyphyllum</i>	Crambidae sp.2 (Lepidoptera)	Ex. leaf chewer	0.0305	04
<i>Stryphnodendron polyphyllum</i>	Oecophoridae sp.1 (Lepidoptera)	Ex. leaf chewer	0.0763	10
<i>Stryphnodendron polyphyllum</i>	Lycaenidae sp.1 (Lepidoptera)	Ex. flower chewer	0.0076	01

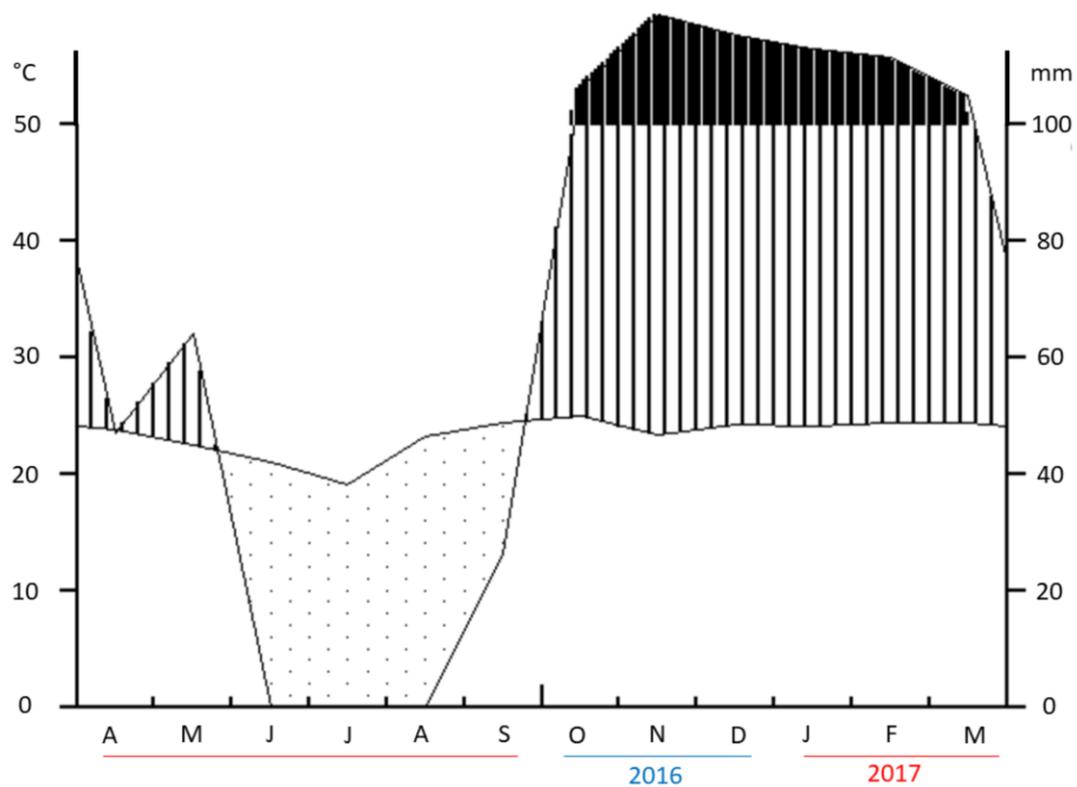


Fig. 1. Climatogram, according to Walter & Lieth 1960, for Uberlândia, Minas Gerais, Brazil, from October 2016 to September 2017. Black areas higher than the striped ones above the temperature line represent rainfall over 100 mm per month in the wet season. The dotted areas under the temperature line show the dry season. Annual precipitation: 1426 mm. Annual mean temperature: 23.2°C.

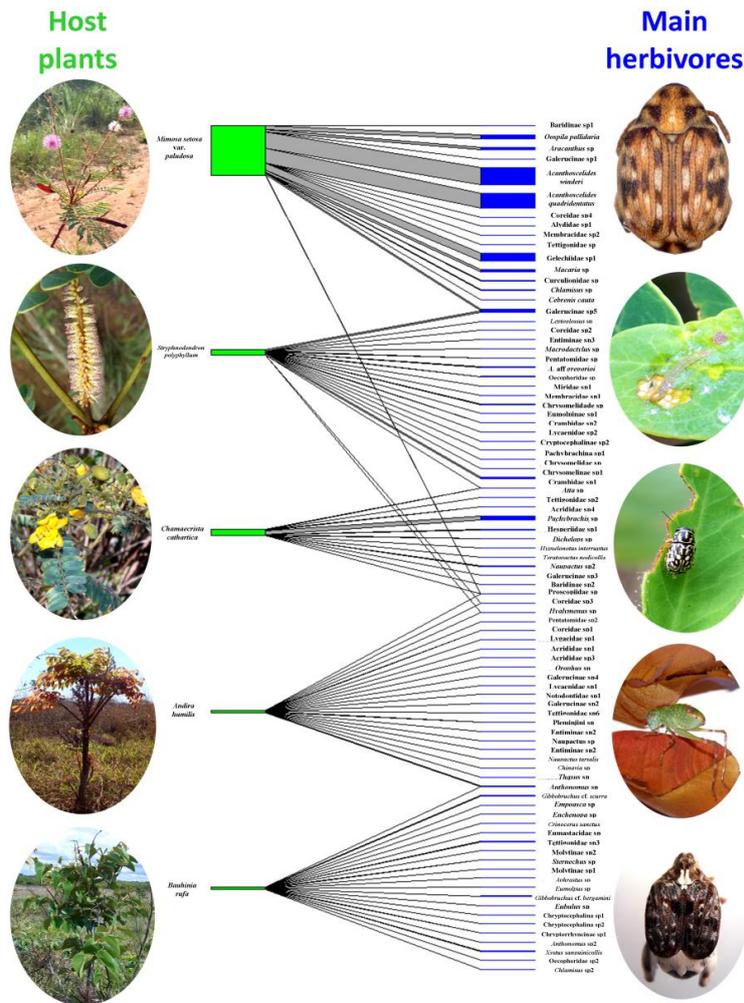


Fig. 2. Fabaceae-insect herbivore network in Brazilian Cerrado. Host plants are in the left side and insect herbivores are in the right side. Host plants from top-down are: *Mimosa setosa* var. *paludosa*, *Stryphnodendron polyphyllum*, *Chamaecrista cathartica*, *Andira humilis* and *Bauhinia rufa*. Main insect herbivores from top-down are: *Acanthoscelides winderi*, Crambidae sp. 1, *Pachybrachis* sp., Tettigoniidae sp. 6 and *Gibbobruchus* cf. *scurra*. The thickness of the branches represents the number of interactions.

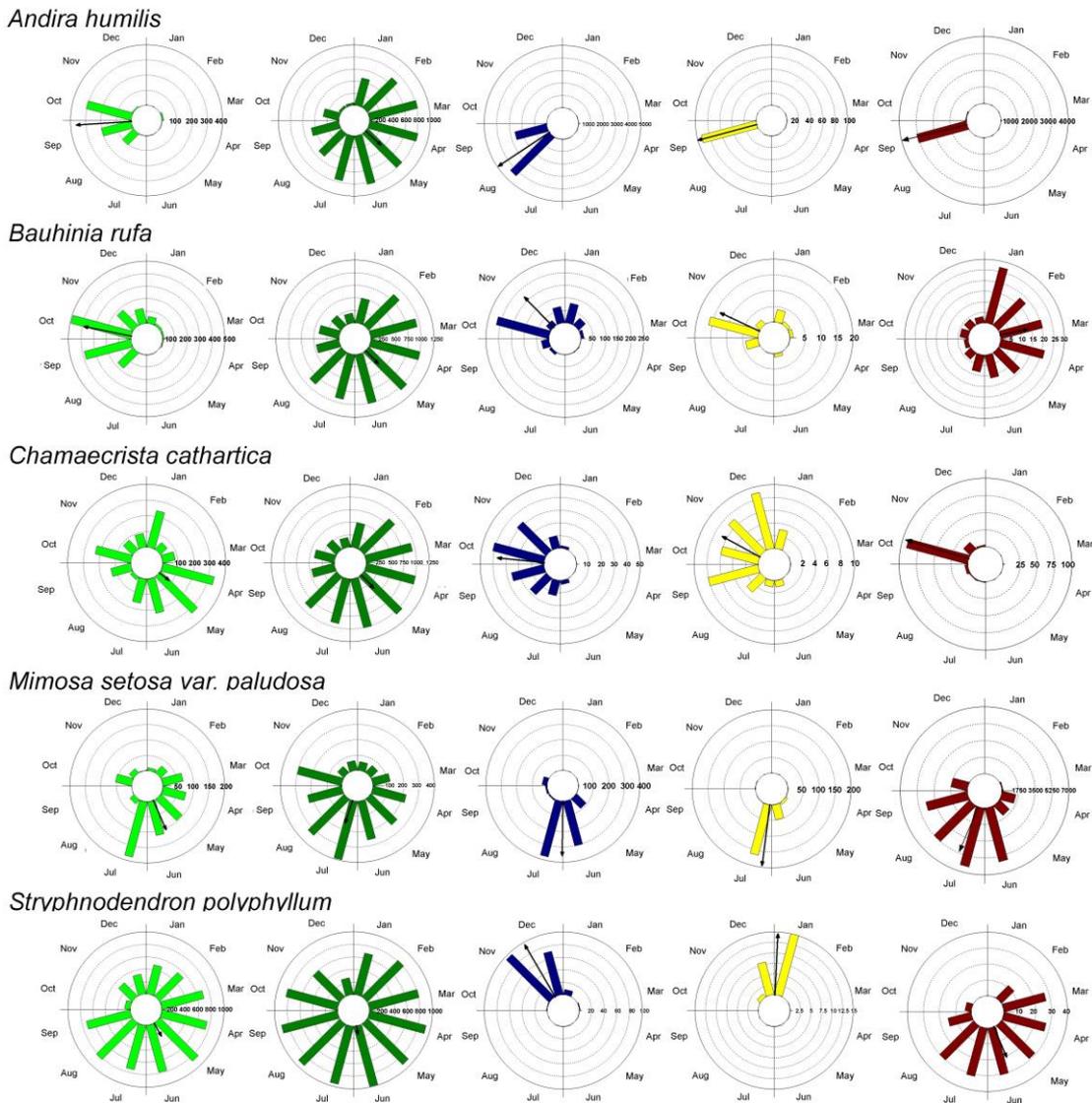


Fig. 3. Annual phenology of *Andira humilis*, *Bauhinia rufa*, *Chamaecrista cathartica*, *Mimosa setosa var. paludosa* and *Stryphnodendron polyphyllum*. Colors light green, dark green, blue, yellow and dark red represent new leaves, mature leaves, flower buds, flowers and fruits, respectively. Numbers within circles are the monthly means for each plant phenophase, and arrows are the mean months of occurrence.

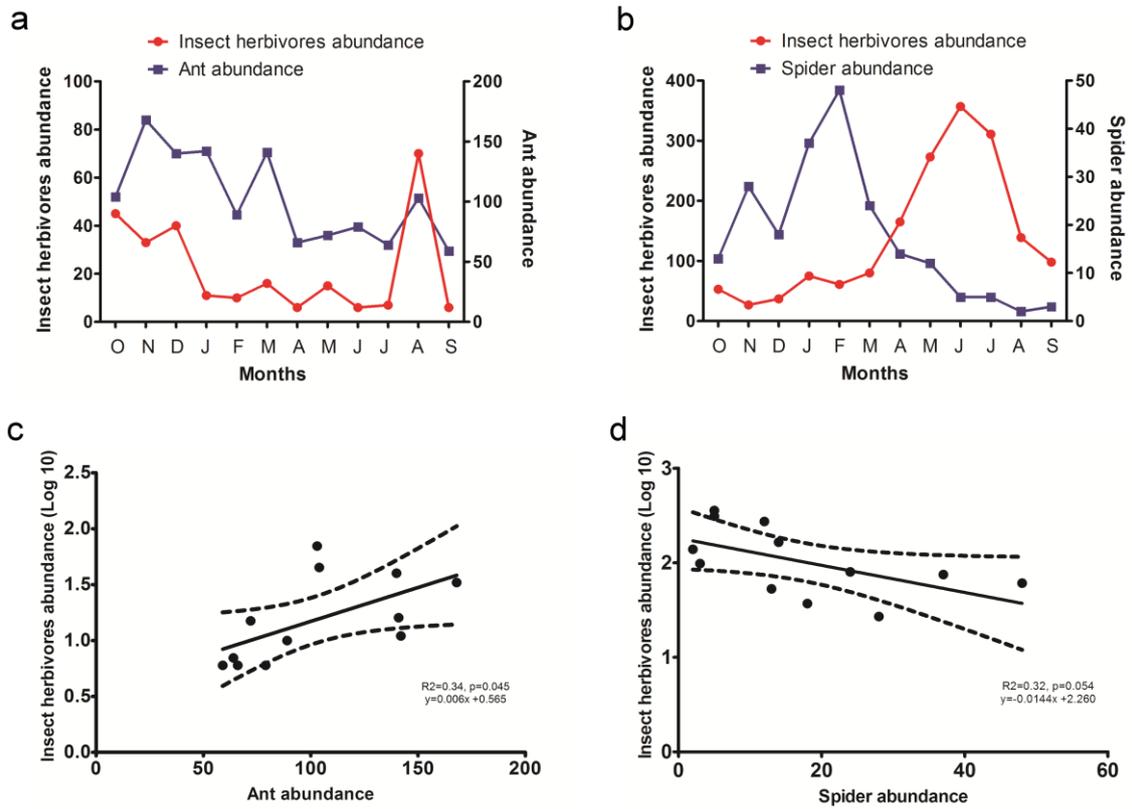


Fig. 4. Relationships between the abundance of insect herbivores and their main natural enemies: (a) insect herbivores and ant abundance on extrafloral-nectaried plants, (b) insect herbivores and spider abundance on glandular plants, (c) simple linear regression between insect herbivores and ant abundance, and (d) simple linear regression between insect herbivores and spider abundance. Dashed lines in figures c and d represent intervals of confidence at 95%.

Capítulo 2

Temporal distribution, seed damage and notes on the natural history of *Acanthoscelides quadridentatus* and *Acanthoscelides winderi* (Coleoptera: Chrysomelidae: Bruchinae) on their host plant, *Mimosa setosa* var. *paludosa*, in the Brazilian Cerrado



Este capítulo foi publicado no Journal of Natural History, 53: 611–623.

Temporal distribution, seed damage and notes on the natural history of *Acanthoscelides quadridentatus* and *Acanthoscelides winderi* (Coleoptera: Chrysomelidae: Bruchinae) on their host plant, *Mimosa setosa* var. *paludosa*, in the Brazilian Cerrado

Bruno de Sousa-Lopes, Nayane Alves-da-Silva, Cibele Stramare Ribeiro-Costa and
Kleber Del-Claro

Abstract

The seed beetles, *Acanthoscelides quadridentatus* and *Acanthoscelides winderi* are here recorded for the first time feeding on seeds of *Mimosa setosa* var. *paludosa* in the Brazilian Cerrado. Our main aims were to describe the temporal distribution, seed damage, and notes on the natural history of these two species on their host plant. We hypothesised that: (a) healthy seeds from infested fruits would have worse germination rate than healthy seeds from noninfested fruits, and (b) females of seed beetles would lay more eggs on large fruits. We made field observations and an experimental field study with the presence of seed beetles versus their exclusion on plants. Results revealed that seed beetles are synchronised with fruiting, with a temporal partitioning in occurrence. Attacked seeds did not germinate, whereas healthy seeds from infested fruits had worse germination rate than healthy seeds from noninfested fruits. Females of seed beetles laid more eggs on large fruits. These results suggest that seed beetles avoid competition through a temporal partitioning crucial for their coexistence, and select large fruits to oviposit as these fruits probably provide more food resource for their offspring. Furthermore, plants might perceive seed beetles' damage and then reduce resource allocation on infested fruits.

Keywords: Insect-plant interactions; oviposition guild; resource allocation; seed beetles.

Introduction

Pre-dispersal seed predation occurs when seeds are predated while they remain on the mother plant (Stachurska-Sawakoń et al. 2018), which may directly affect reproductive success, survival and population dynamics of plants (Janzen 1970; Harper 1977; Kolb et al. 2007). The damage varies among plant species (e.g. El Atta 1993; Klips et al. 2005; Fenner & Thompson 2005); however, it must reach $\geq 80\%$ to regulate plant populations (van Klinken 2005; van Klinken & Flack 2008; Rodrigues et al. 2012). This happens because seed predation may only remove individuals that were doomed to die later through competition and/or other density-dependent processes (Lewis & Gripenberg 2008). Therefore, high levels of seed predation may not represent significant effects on plant population (Crawley 1992; Kolb et al. 2007).

Bruchinae (Coleoptera: Chrysomelidae) is a group of seed-feeding insects in which approximately two-thirds of the 1700 known species feed in a single seed during all larval stages (Center & Johnson 1974; Alvarez et al. 2006; Ribeiro-Costa & Almeida 2012). Some are synchronized with the fruiting, mainly in the early phase when fruits are soft and it is apparently easy for the first instar larvae to perforate the exocarp and seeds (Rossi et al. 2011). In this short time, species of bruchines may avoid competition through a temporal partitioning, with one species arriving and exploring the resources first, then another (Maia et al. 2017). This partition in foraging time is paramount for species with highly discrete internal feeding niches, such as many bruchine species, as they have little ability to switch among resources (Denno et al. 1995; Maia et al. 2017). Thus, temporal partitioning may be crucial to promoting the coexistence of species that

rely strongly on common resources and would otherwise experience potentially exclusive interspecific competition (Richards 2002).

Besides synchrony with fruiting and temporal partitioning, bruchines may lay more eggs on large fruits since these fruits provide more food resources, allowing increases in offspring survival and fitness (Mitchell 1975; Cope & Foz 2003; Ostergård et al. 2007; Morales-Silva et al. 2018). Moreover, to ensure offspring survival females may also lay eggs with filaments, also called anchoring strands, which possibly help the eggs to fix on the fruit wall, avoiding detachment when fruits are growing and dehydrating (Johnson & Kingsolver 1975; Ribeiro-Costa & Costa 2002). Johnson and Romero (2004) reviewed the oviposition behavior of 114 bruchine species and found that these beetles can oviposit in different ways. According to these authors, bruchine species can be classified as that which only oviposit on fruits while on the plant (Guild A, 77%), that which only oviposit on seeds exposed in fruits while still on the plant (Guild B, 10%), and that which only oviposit on seeds once they are exposed on the substrate (Guild C, 13%).

Larvae of bruchines bore into the seed, consume the endosperm, and when the seed embryo is widely damaged, kill the seeds (Ribeiro-Costa & Almeida 2012). Due to the high abundance of bruchines, plants lose a lot of seeds, commonly which ranges from 4% to 42% (Klips et al. 2005; Rodrigues et al. 2012) but can reach up to 90% (e.g., *Acacia nilotica*; El Atta 1993). In addition to the loss of predated seeds, healthy seeds may also suffer with fruit damage (Tomaz et al. 2007). This happens because plants may perceive seed beetles' elicitors or cues during their oviposition and/or feeding and, as a response, plants may decrease resource allocation in damaged but also in undamaged parts (e.g. Doss et al. 2000; Wu & Baldwin 2010; Menezes et al. 2010). Furthermore, it is also possible that the physical damage inflicted by seed beetles may

simply change environmental conditions sufficiently (e.g., through humidity changes, the entrance of fungi, etc.) that negatively impacts the development of seeds. Thus, it is possible that healthy seeds from infested fruits have smaller resource allocation and, consequently, worse germination rate than healthy seeds from noninfested fruits.

Acanthoscelides Schilsky, 1905 is the largest American genus of Bruchinae with approximately 340 species (Nápoles & Kingsolver 2009), some of them important pests of cultivated plants (e.g., *Acanthoscelides obtectus* Say, 1831 in beans, *Phaseolus vulgaris* L.; Baier & Webster 1992). However, little is known about the natural history of some species as *Acanthoscelides winderi* Kingsolver, 1984 and *Acanthoscelides quadridentatus* (Schaeffer, 1907). For *A. winderi* there is apparently only one study with the species description and record of an unidentified species of host plant of the genus *Mimosa* L. (Fabaceae: Mimosoideae) in Brazil (Kingsolver 1984). *Acanthoscelides quadridentatus* was recorded feeding on *Mimosa pigra* L., *Mimosa invisa* Colla, and *Mimosa strigillosa* Torrey and Gray, and considered a potential agent for biological control of the weed, *M. pigra*, in Australia (Bottimer 1969; Johnson 1979; Kassulke et al. 1990).

Here we provide the first record of *A. winderi* and *A. quadridentatus* feeding in pre-dispersal seeds of *Mimosa setosa* var. *paludosa* (Benth.) Barneby. The main goals of this study were: (1) to describe the temporal distribution of *Acanthoscelides* beetles, (2) describe the percentage of seed damage imposed by *Acanthoscelides* beetles on *M. setosa* var. *paludosa*, (3) evaluate whether *Acanthoscelides* beetles negatively affect the germination rate of healthy seeds from infested fruits, and (4) describe notes on the natural history of *Acanthoscelides* beetles. We tested the following hypotheses: (i) the germination rate is worse in healthy seeds from infested fruits possibly due to a decreasing in resource allocation by plants, and (ii) large fruits have a greater abundance

of eggs of *Acanthoscelides* beetles possibly because females select fruits that promise more food resources for their offspring.

Material and Methods

Study area

Fieldwork was carried out by BSL and NAS in the Cerrado sensu stricto vegetation of the Ecological Reserve of Clube Caça e Pesca Itororó de Uberlândia (CCPIU), Minas Gerais, Brazil. The reserve is located at 18°59'00''S, 48°17'44''W, with an elevation of 863 m and a natural area of approximately 640 ha (Del-Claro & Marquis 2015). The climate in the region is markedly seasonal, characterized by a rainy summer (October to March) which may account for up to 75% of the annual rainfall, and a dry winter (April to September) (Laboratory of Climatology, Federal University of Uberlândia; Sousa-Lopes et al. 2016).

Plant species and phenology

Mimosa setosa var. *paludosa* (Figure 1) is a swampy shrub that is endemic to Brazil and Paraguay. It reaches 1.5–5 m tall (Barneby 1991; Dutra & Garcia 2014); however, in this study area, it rarely exceeded 3 m. This species has thorns in its stems and aculeate and glandular trichomes all over the rachis, stems, inflorescences, and fruits (Barneby 1991; Dutra & Garcia 2014). The fruits are red-brown colored and partially dehiscent (craspedium fruits). Plants were found growing on the edge of a trail (3 m wide and c.1.5 km long) inside the Cerrado reserve, near a vereda, a swampy area located in the headwater of a stream (Sousa-Lopes et al. 2016). This species is important for apiarian activity and the restoration of wetlands (Pott et al. 2006); however, it is also an important weed in pasture areas as its large thorny branches can injure cattle (Lorenzi 2000; Pott et al. 2006). Despite its importance, little attention has

been given to the ecological interactions of this plant species, although there is a record of the herbivore *Oospila pallidaria* (Schaus, 1897) feeding on its leaves (Sousa-Lopes et al. 2016). The plant species used in this study was identified by the specialist in leguminous plants from Brazil, Dr. Rubens Teixeira de Queiroz.

A total of 20 individuals of *M. setosa* var. *paludosa* were tagged and inspected once a month from January to December 2016. During inspections, the number of nearly- or completely-mature fruits was counted in each plant to describe the phenology and associate it with the temporal distribution of seed beetles.

Temporal distribution of Acanthoscelides beetles and seed damage

To assess the temporal distribution of *Acanthoscelides* beetles from March to October 2016, when fruits were available, 20 nearly- or completely-mature fruits were collected from each of the 20 previously-tagged *M. setosa* var. *paludosa* plants (n = 400 fruits per month). Fruits were taken to the Behavioral Ecology and Interactions Laboratory (LECI) at the Federal University of Uberlândia and maintained for 60 days in transparent plastic pots (500 mL) covered with voile under laboratory conditions (12-h light and 20–30°C). After that, the abundance of *Acanthoscelides* beetles and damaged seeds were counted. The two species of bruchine, *A. quadridentatus* and *A. winderi*, were identified by the specialist in seed beetles, Dr. Cibele Stramare Ribeiro-Costa, and deposited at the Coleção de Entomologia Pe. J.S. Moure, Departamento de Zoologia, Universidade Federal do Paraná.

Germination test

To assess the germination rate of seeds, we randomly selected two flowering branches in each 10 of the previously-tagged plants in February 2017. Plants were at least 5 m apart and their branches had the same phenology and height. One branch was

carefully bagged with voile to prevent infestation with *Acanthoscelides* beetles and to obtain only healthy seeds. Conversely, another branch was not bagged to allow infestation with *Acanthoscelides* beetles and to evaluate whether the larval activity of these beetles may affect resource allocation in fruits and, thus, the germination rate of healthy seeds in these same fruits. When fruits from the two branches of each plant matured, after approximately 40 days, they were collected, placed in plastic pots covered with voile, and labeled (bagged or nonbagged). Fruits were maintained for 60 days under laboratory conditions, then they were dissected and seeds were separated into three groups: (1) healthy seeds from infested fruits, (2) healthy seeds from noninfested fruits, and (3) attacked seeds. Before germination tests, the healthy seeds were scarified with sandpaper to overcome the physical dormancy (seed coat) and to allow free access to water. Attacked seeds were not scarified as *Acanthoscelides* beetles left exit holes, which already allowed water to enter (Rodrigues et al. 2012). Finally, seeds were placed in filter-paper lined plastic pots (500 mL) with 10 mL of water. Each seed group had 10 replicates with 10 seeds, totaling 300 seeds. Germination was defined as the appearance of a 2 mm primary root, and the number of germinated seeds was counted daily for each group until it to reach the plateau on the fifth day of trials.

Effects of fruit size on Acanthoscelides beetles

To assess fruit size and correlate it with the abundance of eggs of *Acanthoscelides* beetles, in May 2017 we randomly collected another 76 fruits of the same-tagged *M. setosa* var. *paludosa* plants. These fruits were taken to the laboratory and the length of each fruit was obtained using a digital caliper (mm). After that, we counted the number of eggs by using a stereomicroscope (according to Morales-Silva et al. 2018).

Data analysis

Circular statistical analysis of directional data was used to evaluate whether there was seasonality in fruit availability (mean monthly) and *Acanthoscelides* spp. abundance (monthly). In this analysis, months were converted into angles (30° intervals) and these angles were combined with the respective value of plant phenology and *Acanthoscelides* spp. abundance to describe the temporal distribution (Table 1, Figure 2). Circular statistics provide: (1) the mean angle (μ), which is the period when a given variable (e.g., fruits or *Acanthoscelides* spp.) occurred most often; (2) the vector (r), which is a direct measure of seasonality (closer to “1” being more seasonal); and (3) the Rayleigh test (z), which indicates whether seasonality is significant (Sousa-Lopes et al. 2016). The cumulative germination rate of seeds from infested fruits, noninfested fruits, and attacked seeds was compared using repeated measures ANOVA followed by Bonferroni’s post-hoc test (Figure 4). We used also a simple linear regression to evaluate the relationship between fruit size (independent variable) and abundance of eggs of *A. quadridentatus* (Figure 5). Oriana 4, R Studio (R Development Core Team 2016) and Graph Pad Prism 5 were used to perform the analyses and graphics.

Results

We counted a total of 26,478 fruits on the 20 tagged plants throughout the year, with July being the month with the highest amount (24%) and March the month with the lowest amount (0.4%; Figure 2a). Statistical analyses suggest that the occurrence of fruit was seasonal (concentrated in the dry season), with the mean month being July (Figure 2a; Table 1). We also found 420 *A. winderi* (Figure 3a) and 365 *A. quadridentatus* (Figure 3b) individuals feeding in the seeds of *M. setosa* var. *paludosa* throughout the dry season (Figure 2b and c). The peak infestations for *A. winderi* and *A.*

quadridentatus were in May (n = 206) and July (n = 122), respectively. Circular statistical analyses showed that the occurrence of *Acanthoscelides* beetles was overlapped with the availability of fruits, with mean angle μ being very similar among them, especially between *A. quadridentatus* and fruits (Table 1). During this overlap, we found a temporal partitioning between *Acanthoscelides* beetles, with *A. winderi* being more abundant during early fruiting (April-June, 91%) and *A. quadridentatus* more abundant after that (June-August, 74%). Although we collected 785 individuals of *Acanthoscelides* beetles, we found 2000 seeds predated by these beetles in our sampling, which represented approximately 15.20% of the total counted seeds. It happened because collects were done once a month and thus many seed beetles probably completed their development among collects and left only the exit holes (Figure 3d), which justify a higher predation rate than compared to seed beetle abundance.

The germination rate among attacked seeds, healthy seeds from infested and noninfested fruits differed statistically ($F_{2,4} = 87.27, p < 0.0001$). Attacked seeds did not germinate; whereas healthy seeds from noninfested fruits contained 94% germinated seeds, and healthy seeds from infested fruits contained 70% germinated seeds (Figure 4).

Females of both *Acanthoscelides* species laid eggs on the external surface and trichomes of fruits (Figure 3c). Eggs are elongated, with filaments (anchoring strands) at the anterior and posterior ends that possibly help to fix on the fruit wall and directly on trichomes (Figure 3c). Simple linear regression showed that the abundance of *Acanthoscelides* eggs was predicted by fruit size ($F_{1,74} = 10.40, p < 0.001, R^2 = 0.123$; Figure 4). In this analysis, we found 323 eggs of *Acanthoscelides* beetles, with the highest abundance of eggs (n = 16) in a fruit with 56 mm length and the lowest (n = 1)

in a fruit with 42 mm length. The mean number of eggs was 4.25 (± 3.24) and the mean size of fruits was 47.55 mm (± 6.68). Larvae of the two species studied here developed feeding on only one seed (Figure 3d).

Discussion

Our two initial hypotheses were corroborated: first, the germination rate was worse in healthy seeds from infested fruits; and second, large fruits had a greater abundance of *Acanthoscelides* eggs. Some studies have shown that the presence of insects can facilitate seed germination (Takakura 2002; Vega et al. 2011). For instance, Takakura (2002) showed that the seed beetle, *Bruchidus dorsalis* Fahraeus, plays a crucial role for *Gleditsia japonica* Miquel (Fabaceae) since it opens the hard coat of seeds and facilitates the entrance of water and, consequently, the germination. In contrast, our study showed that *Acanthoscelides* beetles negatively affected the germination rate of *M. setosa* var. *paludosa* seeds, as none of the attacked seeds germinated. This was similar to what was found in *Leucaena leucocephala* (Lam.) de Wit, in which seeds were predated by *Acanthoscelides macrophtalmus* (Schaeffer 1907) (Rodrigues et al. 2012). Furthermore, our study also showed that *Acanthoscelides* beetles could affect unattacked seeds, as healthy seeds from infested fruits had a worse germination rate than healthy seeds from noninfested fruits, like in *Mimosa bimucronata* (DC.) Kuntze (Menezes et al. 2010). These results suggest that larval activity of *Acanthoscelides* beetles during fruit development in *M. setosa* var. *paludosa* may result in a change in resource allocation by plants. It has been shown that plants can perceive insects' elicitors or cues during their oviposition or feeding and, through electrical and/or chemical cascade events, plants may induce responses not only in damaged but also in undamaged parts (Doss et al. 2000; Wu & Baldwin 2010). For

instance, Doss et al. (2000) showed that the plant, *Pisum sativum* L. (Fabaceae), stimulates cell division and the formation of neoplasms when the seed beetle, *Bruchus pisorum* L., oviposits on its fruits. These neoplasms develop beneath the egg and impede larval entry into the fruits. Menezes et al. (2010) showed that the healthy seeds from infested fruits of *M. bimucronata* had lower nitrogen concentration and worse germination rate than healthy seeds from noninfested fruits. These authors suggested that the damage inflicted by the beetle *Acanthoscelides schrankiae* Horn may affect the resource allocation on *M. bimucronata*. Therefore, it is also possible that *M. setosa* var. *paludosa* perceives seed beetles' attack and reduces energetic investment in infested fruits and, consequently, affects the germination rate of healthy seeds in these fruits.

Females of *Acanthoscelides* beetles laid more eggs on large fruits. Several other studies also have shown that the oviposition of seed beetles is correlated with fruit size as large fruits have a higher availability of food resources which may positively affect offspring development and fitness (Mitchell 1975; Cope & Foz 2003; Ostergård et al. 2007; Morales-Silva et al. 2018). However, this is not a rule since the oviposition in some species of bruchines is not positively correlated with fruit size (see Ribeiro-Costa et al. 2011; Modena et al. 2012). Here, we highlight that females of *A. quadridentatus* and *A. winderi* appear to assess the fruit size before oviposition, suggesting that large fruits may be better for offspring survival and fitness.

An overlap was found between the availability of fruits of *M. setosa* var. *paludosa* and *Acanthoscelides* beetles, especially for *A. quadridentatus* and fruits whose mean month was July. Similar to this study, Rossi et al. (2011) also found synchrony between *A. schrankiae* and fruits of *M. bimucronata*. This reinforces that for insects with restricted diets, like *Acanthoscelides* beetles, the availability of their host plants is

a crucial factor accounting for their maintenance in natural environments, especially because fruiting occurs in a short time (Bernays & Chapman 1994).

We also showed that *A. winderi* occurred mainly in the early phase of the fruiting period (April-June), whereas *A. quadridentatus* occurred in higher abundance after that (June-August). This temporal partitioning may be an adaptation to avoid direct competition when seeds are available. Maia et al. (2017) also showed that the seed beetles, *Merobruchus terani* (Kingsolver 1980) and *Stator maculatopygus* (Pic 1930), can avoid competition through a temporal partitioning in *Senegalia tenuifolia* (L.) Britton & Rose (Fabaceae: Mimosoideae). In this case, the authors explained that the less abundant species, *S. maculatopygus*, arrived later probably to avoid competition with the dominant species, *M. terani*. Here, we also showed that the less abundant species, *A. quadridentatus*, arrived later than the most abundant species, *A. winderi*. Besides avoiding competition, it is also possible that *A. quadridentatus* arrives later to avail the holes made by first instar larvae of *A. winderi*. This could happen if the eggs of *A. winderi* detach from fruits, especially when fruits are growing and dehydrating (see Ribeiro-Costa & Costa 2002), thus, allowing *A. quadridentatus* to oviposit on them and first instar larvae use the same holes left by *A. winderi*. The use of pre-existing holes made on fruits is a behavior already recorded in other species of bruchines, as for *Zabrotes interstitialis* (Chevrolat) that avails the holes made by *Pygiopachymerus lineola* (Chevrolat) on *Cassia grandis* L. (Janzen 1971). Herein, we reinforce that temporal partitioning may be crucial to avoid competition and promote the coexistence of species that rely strongly on common resources (see also Richards 2002).

Our data on seed damage (15.20%) corroborate the infestation range of other studies that focused on seed beetles (i.e., varying from 4% to 42%) (Klips et al. 2005; Rodrigues et al. 2012). This damage rate decreases seed set and abundance of *M. setosa*

var. *paludosa*; however, according to van Klieben (2005), van Klieben and Flack (2008), and Rodrigues et al. (2012), the damage rate must reach $\geq 80\%$ to regulate plant populations. Thus, the damage imposed by *Acanthoscelides* beetles on *M. setosa* var. *paludosa* seems unable to regulate this plant population.

On the natural history, we showed that *A. quadridentatus* and *A. winderi* laid eggs on the external surface of *M. setosa* var. *paludosa* fruits. This behavior was observed in approximately 77% of all bruchine species studied (Johnson & Romero 2004), including *Acanthoscelides* species (Silva et al. 2007). According to Johnson and Romero (2004), species of seed beetles that glue their eggs to the outside of the fruit while it remains on the plants belong to the oviposition Guild A. Species belonging to this guild probably face the challenge of avoiding the detachment of eggs from the fruit wall before the hatching. The strategy that seed beetles apparently developed to overcome that pressure was the use of anchoring strands, which help the eggs to fix on fruit wall when fruits are growing and dehydrating, as showed here for the two studied species (see also Ribeiro-Costa & Costa 2002). After the hatching, larvae of seed beetles from Guild A, such as *A. quadridentatus* and *A. winderi*, burrow through the fruit wall and then to seed coat, different from other guilds (B and C) that face only one barrier (seed coat). Thus, descriptions on the oviposition behavior, including our report for *A. quadridentatus* and *A. winderi*, are paramount to understand ecological questions on seed beetle-host plant interactions.

Overall, we showed that *A. quadridentatus* and *A. winderi* decreased the seed set as well as the performance of healthy seeds from infested fruits of *M. setosa* var. *paludosa*, although they did not reach the degree of damage necessary to regulate plant population. Females of these two *Acanthoscelides* species selected large fruits to lay their eggs, and the temporal partitioning in the occurrence of *Acanthoscelides* beetles

suggests a survival strategy to avoid competition and promote the coexistence between species.

References

- Alvarez N, Romero-Napoles J, Anton KW, Benrey B, Hossaert-McKey M. 2006. Phylogenetic relationships in the Neotropical bruchid genus *Acanthoscelides* (Bruchinae, Bruchidae, Coleoptera). *J Zool Syst Evol Res.* 44:63–74.
- Baier AH, Webster BD. 1992. Control of *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae) in *Phaseolus vulgaris* L. seed stored on small farms I. Evaluation of damage. *J. Stored Prod. Res.* 28:289–293.
- Barneby CR. 1991. Sensitivae censitae. A description of the genus *Mimosa* L. (Mimosaceae) in the New World. *Mem N Y Bot Gard.* 65:1–835.
- Bernays EA, Chapman RF. 1994. Host-plant selection by phytophagous insects. New York (NY): Chapman & Hall Press.
- Bottimer LJ. 1969. Bruchidae associated with *Mimosa* with the description of a new species. *Can Entomol.* 101:1186–1198.
- Center TD, Johnson CD. 1974. Coevolution of some seed beetles (Coleoptera: Bruchidae) and their hosts. *Ecology.* 55:1096–1103.
- Cope JM, Fox CW. 2003. Oviposition decisions in the seed beetle, *Callosobruchus maculatus* (Coleoptera: Bruchidae): effects of seed size on superparasitism. *J Stored Prod Res.* 39:355–365.
- Crawley MJ. 1992. Seed predators and plant population dynamics. In Fenner M (ed.). *Seeds, The Ecology of Regeneration in Plant Communities*, pp. 157– 191. Wallingford, UK: CABI International.
- Del-Claro K, Marquis RJ. 2015. Ant species identity has a greater effect than fire on the outcome of an ant protection system in Brazilian cerrado. *Biotropica.* 47: 459–467.
- Denno RF, McClure MS, Ott JR. 1995. Interspecific interactions in phytophagous insects: competition reexamined and resurrected. *Annu Rev Entomol* 40:297– 331.
- Dutra VF, Garcia FCP. 2014. *Mimosa* L.(Leguminosae-Mimosoideae) dos campos rupestres de Minas Gerais, Brasil. *Iheringia Série Botânica.* 69:49–88.
- Doss RP, Oliver JE, Proebsting WM, Potter SW, Kuy S, Clement SL, Williamson RT, Carney JR, DeVilbiss ED. 2000. Bruchins: insect-derived plant regulators that stimulate neoplasm formation. *PNAS.* 97:6218–6223.
- El Atta HA. 1993. The effect of *Caryedon serratus* Olivier (Col., Bruchidae) on viability and germination of seeds of *Acacia nilotica* (L. Willd. ex Del.) in the Sudan. *Forest Ecol Manag.* 57:169–177.
- Fenner M, Thompson K. 2005. *Ecology of Seeds*. Cambridge: Cambridge University Press.
- Harper JL. 1977. *Population Biology of Plants*. New York: Academic Press.
- Janzen DH. 1970. Herbivores and number of tree species in tropical forests. *Am Nat.* 104:501–528.
- Janzen DH. 1971. Escape of *Cassia grandis* L. beans from predators in time and space. *Ecology.* 52:964–979.
- Johnson CD. 1979. New host records for *Acanthoscelides* (Coleoptera: Bruchidae). *Pan-Pac Entomol.* 55(1): 61–71.
- Johnson CD, Kingsolver JM. 1975. Ecology and redescription of the Arizona grape bruchid, *Amblycerus vitis* (Coleoptera). *Coleopt. Bull.* 29(4):321–331.

- Johnson CD, Romero J. 2004. A review of evolution of oviposition guilds in the Bruchidae (Coleoptera). *Rev Bras Entomol.* 48:401–408.
- Kassulke RC, Harley KLS, Maynard GV. 1990. Host specificity of *Acanthoscelides quadridentatus* and *A. puniceus* (Coleoptera: Bruchidae) for biological control of *Mimosa pigra* (with preliminary data on their biology). *Entomophaga.* 35(1):85–96.
- Kingsolver JM. 1984. *Acanthoscelides winderi*, new species, (Coleoptera, Bruchidae) associated with *Mimosa* spp. (Leguminosae, Mimosoideae) from Brazil. *Entomol News.* 95:87–90.
- Klips RA, Sweeney PM, Bauman EKK, Snow AA. 2005. Temporal and geographic variation in predispersal seed predation on *Hibiscus moscheutos* L. (Malvaceae) in Ohio and Maryland, USA. *Am. Midl. Nat.* 154:286–95.
- Kolb A, Ehrlen J, Eriksson O. 2007. Ecological and evolutionary consequences of spatial and temporal variation in pre-dispersal seed predation. *Perspect Plant Ecol Syst.* 9:79–100.
- Lewis OT, Gripenberg S. 2008. Insect seed predators and environmental change. *J Appl Ecol.* 45:1593–1599.
- Lorenzi H. 2000. Plantas daninhas do Brasil: terrestres, aquáticas, parasitas e tóxicas. Nova Odessa: Instituto Plantarum, SP.
- Maia LF, Tuller J, Faria LDB. 2017. Morphological traits of two seed-feeding beetle species and the relationship to resource traits. *Neotrop Entomol.* 46:36–44.
- Menezes LCCR, Klein J, Kestring J, Rossin MN. 2010. Bottom-up and top-down effects in a pre-dispersal seed predation system: are non-predated seeds damaged? *Basic and Applied Ecology.* 11:126–134.
- Mitchell R. 1975. The evolution of oviposition tactics in the vean weevil, *Calosobruchus maculatus* (F). *Ecology.* 56:696–702.
- Modena EdeS, Pires ACV, Barônio GJ, Inforzato I, Demczuk SDB. 2012. Do fruit traits of the *Senna occidentalis* weed influence seed predation by Bruchinae? *Rev Bras Bioc.* 10:293–297.
- Morales-Silva T, Monteiro A, Faria LDB. 2018. Multitrophic web of insects associated with *Piptadenia gonoacantha* (Mart.) Macbr. (Fabaceae) and their relationship with resource traits. *Arthropod Plant Interac.* 12:553–565.
- Nápoles JR, Kingsolver JM. 2009. A new species of *Acanthoscelides* Schilsky (Coleoptera: Bruchidae) from Mexico with some biological notes. *Neotrop. Entomol.* 38(4):497–500.
- Ostergård H, Hambäck PA, Ehrlén J. 2007. Pre-dispersal seed predation: the role of fruit abortion and selective oviposition. *Ecology.* 88:2959–2965.
- Pott A, Pott VJ, Souza TW. 2006. Plantas daninhas de pastagem na região dos Cerrados. Campo Grande, MS: EMBRAPA Gado de Corte.
- R Development Core Team. 2016. R: A language and environment for statistical computing. <http://www.R-project.org>. Accessed 02 March 2016.
- Ribeiro-Costa CS, Almeida LM. 2012. Seed-Chewing Beetles (Coleoptera: Chrysomelidae, Bruchinae). In: Panizzi AR, Parra JRP (orgs.). *Insect Bioecology and Nutrition for Integrated Pest Management*. 1ed. Boca Raton: CRC Press, 2012, v. 1, p. 325–352.
- Ribeiro-Costa CS, Costa, AS. 2002. Oviposition behavior of Bruchidae (Coleoptera) seed predators of *Cassia leptophylla* Vogel (Caesalpinacea), eggs morphology and description of a new species. *Rev Bras Zool.* 19:305–316.
- Ribeiro-Costa CS, Sari LT, Viana JH, Manfio D. 2011. *Ecologia de bruquíneos*

- (Coleoptera: Chrysomelidae) em frutos de *Bauhinia holophylla* Steud (Caesalpinioideae) no Parque Estadual do Cerrado, Jaguariaíva, Paraná. Coletânea de Pesquisa do Parque Estadual de Vila Velha, Cerrado e Guartelá. 1:273–281.
- Richards SA. 2002. Temporal partitioning and aggression among foragers: modeling the effects of stochasticity and individual state. *Beh Ecol.* 13:427–438.
- Rodrigues LMS, Viana JH, Ribeiro-Costa CS, Rossi MN. 2012. The extent of seed predation by bruchine beetles (Coleoptera: Chrysomelidae: Bruchinae) in a heterogeneous landscape in southeastern Brazil. *Coleopt Bull*, 66 (3):271–279.
- Rossi MN, Rodrigues LMS, Ishino MN, Kestring D. 2011. Oviposition pattern and within-season spatial and temporal variation of pre-dispersal seed predation in a population of *Mimosa bimucronata* trees. *Arthropod Plant Interact.* 5:209–217.
- Silva LA, Maimoni-Rodella CS, Rossi MN. 2007. A preliminary investigation of pre-dispersal seed predation by *Acanthoscelides schrankiae* Horn (Coleoptera: Bruchidae) in *Mimosa bimucronata* (DC.) Kuntze trees. *Neotrop Entomol.* 36 (2):197–202.
- Sousa-Lopes B, Bächtold A, Del-Claro K. 2016. Biology, natural history and temporal fluctuation of the geometrid *Oospila pallidaria* associated with host plant phenology. *Stud Neotrop Fauna Environ.* 51:135–143.
- Stachurska-Sawakoń A, Barabasz-Krasny B, Klasa A, Palaczyk A. 2018. Reduced plant fitness by pre-dispersal seed predation in the threatened plant species *Cirsium decussatum*. *Seed Sci Res.* 28:123–130.
- Takakura K. 2002. The specialist seed predator *Bruchidius dorsalis* (Coleoptera: Bruchidae) plays a crucial role in the seed germination of its host plant, *Gleditsia japonica* (Leguminosae). *Funct Ecol.* 16:252–257.
- Tomaz CA, Kestring D, Rossi MN. 2007. Effects of the seed predator *Acanthoscelides schrankiae* on viability of its host plant *Mimosa bimucronata*. *Biol Res.* 40:281–290.
- Van Klinken RD. 2005. Total annual seed loss on a perennial legume through predation by insects: the importance of within-season seed and seed feeder dynamics. *Austral Ecol.* 30:414–425.
- Van Klinken RD, Flack LK. 2008. What limits predation rates by the specialist seed-feeder *Penthobruchus germaini* on an invasive shrub? *J Appl Ecol.* 45:1600–1611.
- Vega C, Arista M, Ortiz PL, Herrera CM, Talavera S. 2011. Endozoochory by beetles: a novel seed dispersal mechanism. *Ann Bot.* 107:629–637.
- Wu J, Baldwin IT. 2010. New insights into plant responses to the attack from insect herbivores. *Annu Rev Genet.* 44:1–24.

Table caption

Table 1 Circular statistical analysis of the seasonality of *Acanthoscelides* beetles abundance together with fruits of their host plant, *M. setosa* var. *paludosa*.

	Mean angle (μ)	Mean month	Vector (r)	Rayleigh Z test
Fruits	195.83°	July	0.643	623.34***
<i>A. winderi</i>	148.48°	May	0.925	359.67***
<i>A. quadridentatus</i>	184.80°	July	0.790	227.76***

*** $p < 0.0001$.



Fig. 1. The host plant of the seed beetles, *Mimosa setosa* var. *paludosa*, in the fruiting phase in the Brazilian Cerrado. The arrow indicates some fruits.

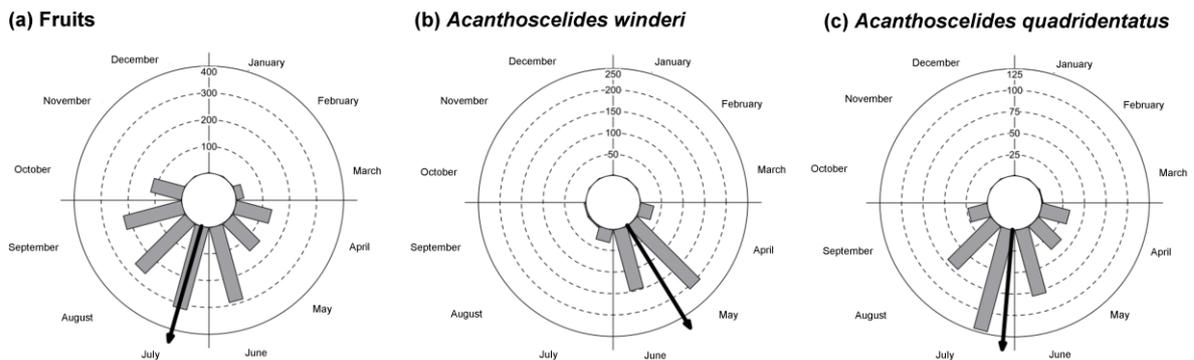


Fig. 2. (a) Annual distribution of fruit availability of *M. setosa* var. *paludosa*, (b) abundance of *A. winderi*, and (c) abundance of *A. quadridentatus*. Numbers within circles are the monthly means of fruits (a) and the absolute frequency of seed beetles (b, c). Arrows are the mean month of fruits or the occurrence of *Acanthoscelides* beetles.

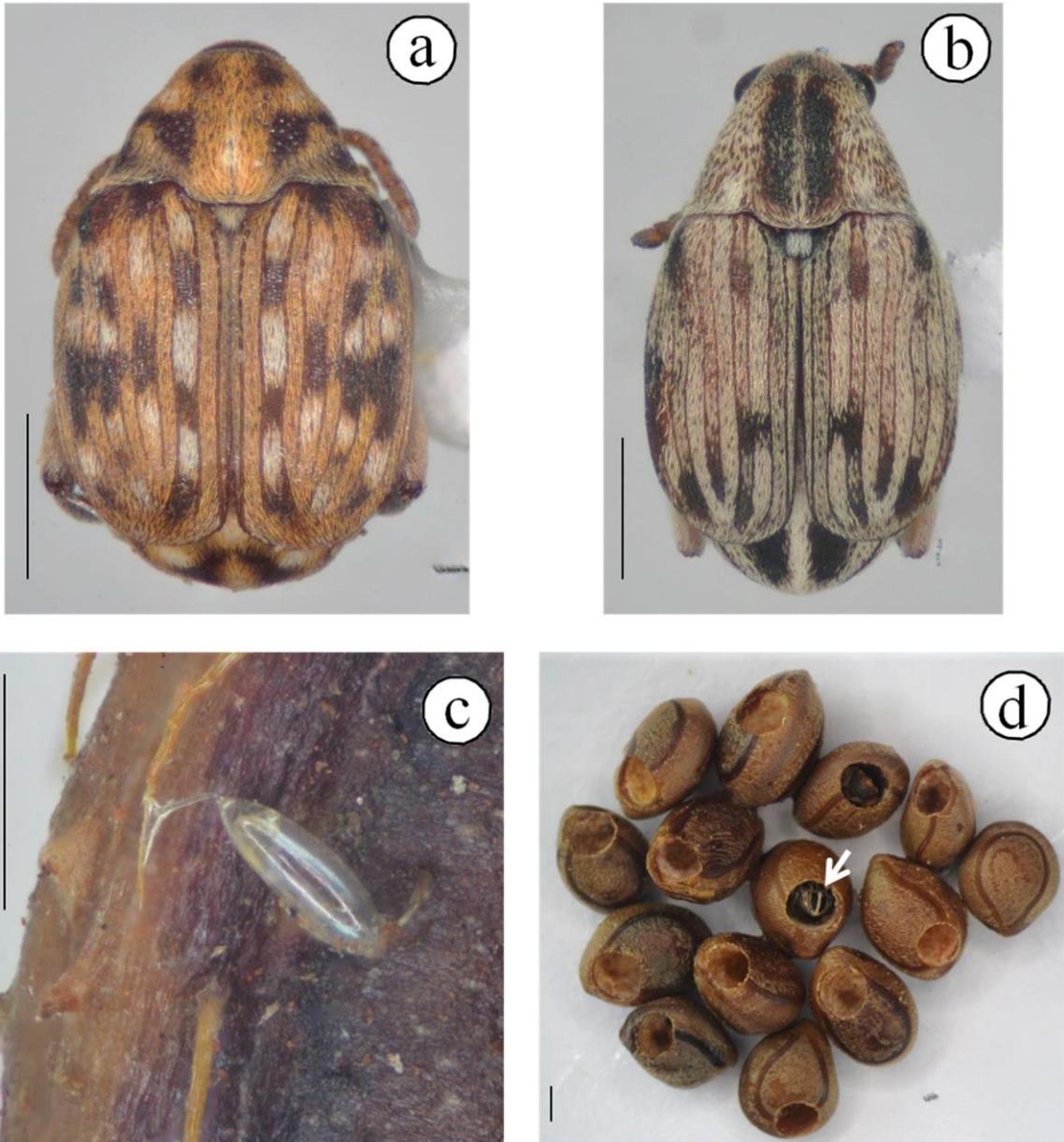


Fig. 3. Seed beetles (Bruchinae) associated with *M. setosa* var. *paludosa*: (a) *A. winderi*, (b) *A. quadridentatus*, (c) seed beetle egg on the fruit surface, and (d) attacked seeds. The arrow indicates a single *Acanthoscelides* beetle inside a seed. Scale: 1 mm.

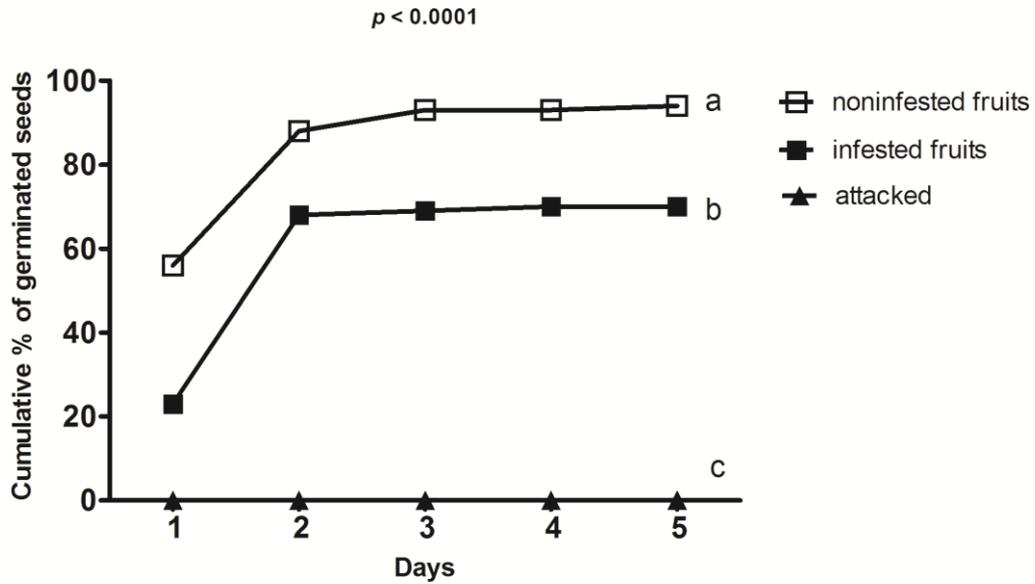


Fig. 4. Cumulative percentage of germination of healthy seeds from noninfested and infested fruits, and attacked seeds of *M. setosa* var. *paludosa*. Letters indicate significant statistical difference among treatments by Bonferroni's post hoc test at $p < 0.0001$.

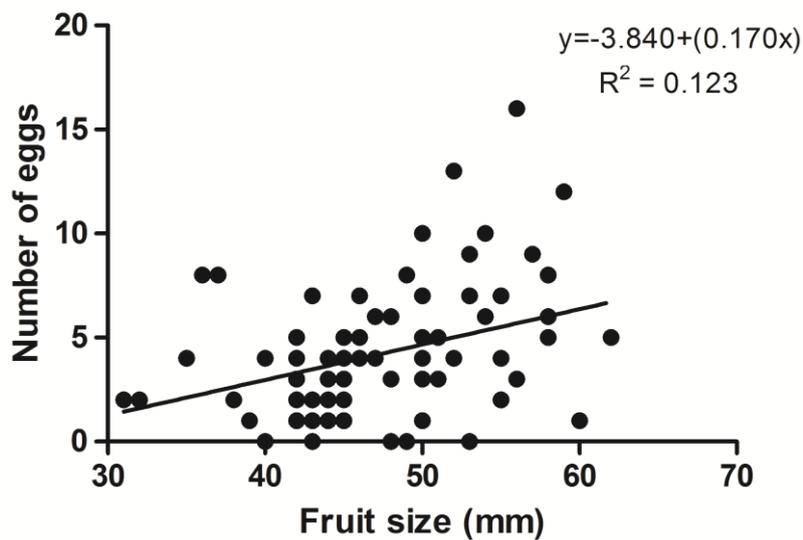
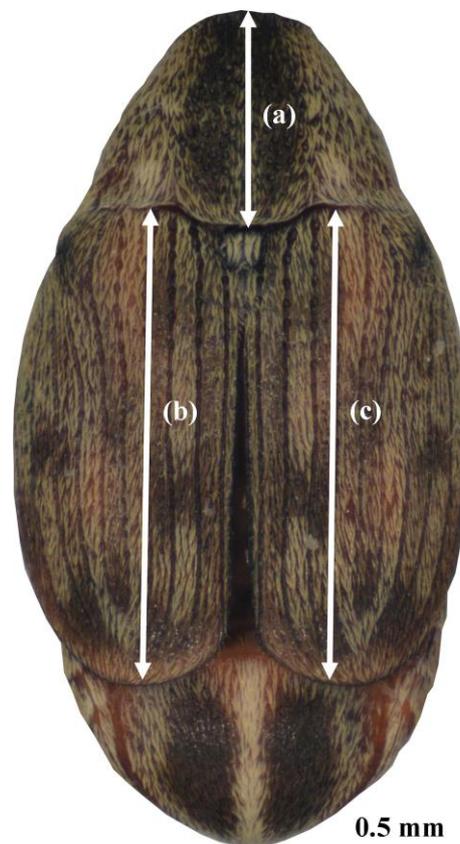


Fig. 5. Simple linear regression of fruit size (independent variable) and number of *Acanthoscelides* beetles' eggs ($F_{1,74} = 10.40$, $p < 0.001$).

Capítulo 3

**Spatio-temporal variation in seed traits affects the occurrence
and body-size pattern of a seed-feeding beetle
(Chrysomelidae: Bruchinae) in Brazilian Cerrado**



Este capítulo foi publicado em Acta Oecologica, 105(2020): 103579.

**Spatio-temporal variation in seed traits affects the occurrence and
body-size pattern of a seed-feeding beetle (Chrysomelidae: Bruchinae)
in Brazilian Cerrado**

Bruno de Sousa-Lopes, Aluska Tavares dos Santos, Cibele Stramare Ribeiro-Costa &
Kleber Del-Claro

Abstract

Seed quality is an important bottom-up factor driving life-history traits of seed beetles (Chrysomelidae: Bruchinae). However, information on how seed traits change spatiotemporally and affect herbivores' survival is incipient in Neotropics. We evaluated how the spatio-temporal variation in seed weight, hardness, size, and water content of *Mimosa setosa* var. *paludosa* (Fabaceae) affects the occurrence and body-size pattern of the seed beetle, *Acanthoscelides quadridentatus*, in Brazilian Cerrado. We collected 400 fruits from four populations of the plant in roadsides from Goiás and Minas Gerais states and then we assessed in laboratory seed traits along with the weight and body size of *A. quadridentatus*. Additionally, we collected a total of 76 fruits (immature plus mature) in beginning and end of the fruiting phase and counted the frequencies of unhatched and hatched eggs of the seed beetle to evaluate the effect of seed hardening on beetle posture. We found greater seed damage in the population with softer, smaller and lighter seeds. Larger and heavier seeds were harder than smaller and lighter seeds; and male and female reared from larger and heavier seeds were respectively 15 and 25% larger than those reared from smaller and lighter seeds. We also found that weight and body size of *A. quadridentatus* were positively correlated with seed length, weight and water content. Unhatched eggs were found only in the beginning of fruiting phase when

seeds were immature and softer, suggesting the occurrence of a window of time in which females lay their eggs before seed hardening to facilitate the entry of first instar larvae. These results highlight that larger and heavier seeds are most protected against herbivory, but individuals of *A. quadridentatus* that overcome plant hardness can grow larger in size. We suggest that seed traits change across a spatio-temporal scale and affect life-history traits of *A. quadridentatus*.

Keywords: *Acanthoscelides*, food resource quality, life-history traits, seed weight, seed hardness, *Mimosa setosa* var. *paludosa*

Introduction

Host plant quality is an important bottom-up factor driving the performance and life-history traits of herbivore insects such as body-size pattern, lifespan, fecundity, and reproductive success (Janzen 1969, Fox et al. 2007, González-Teuber et al. 2008, Price et al. 2011). In the case of seed beetles (Chrysomelidae: Bruchinae), the quality of food resource is important because many species feed in a single seed during all larval development, then any change in seed quality can affect seed-beetles' body size and other traits (Amarillo-Suárez et al. 2011, Ribeiro-Costa and Almeida 2012). The wide phenotypic plasticity of these beetles has been occasionally associated with seasonality (variance in humidity, precipitation, and temperature; Stillwell et al. 2007), but in most cases their plasticity is related to the variation in seed quality among host plant populations (*i.e.*, variation in weight, hardness, size, and water content) (Takakura 2004, Haga and Rossi 2016, Canto et al. 2019).

Seeds are mature ovules containing an embryo and stored nutrients such as carbohydrates, lipids, and proteins inside a protective coat (Wall et al. 2005, Fenner and Thompson 2005). Larger and heavier seeds may be crucial for seed beetles as they

possibly contain high nutrient concentration, providing better opportunities to beetles' development (Moegenburg 1996, González-Teuber et al. 2008, Canto et al. 2019). For example, the seed beetles *Merobruchus terani* Kingsolver, 1980 and *Stator maculatopygus* (Pic, 1930) grow to larger sizes when feeding on heavier and larger seeds of *Senegalia tenuifolia* (L.) Britton & Rose (Maia et al. 2017). In addition, the water content in seeds is crucial for seed beetles because it may facilitate nutrient assimilation, besides to represent approximately 50% of beetle's weight (Ribeiro-Costa and Almeida 2012). Indeed, it has been suggested that variations in water content in seeds of different populations of *Leucaena leucocephala* (Lam.) de Wit (Fabaceae) explain changes in body-size pattern of the seed beetle *Acanthoscelides macrophthalmus* (Schaeffer, 1907) (Haga and Rossi 2016).

In comparison to other plant parts, seeds are most protected from herbivores since they are responsible for plant reproduction and therefore have a higher supply of nutrients (Bridwell 1918, Wall et al. 2005). According to the Optimal Defense Theory (ODT), plants should allocate more defenses to structures in relation to their value and probability of attack (McKey 1974, 1979). In this sense, we expect that seeds (especially the large ones) may be most protected against herbivory with a very thick seed coat which may affect the entry and survival of the first instar larvae (Howe and Currie 1964, Janzen 1969, Seifelnasr 1991). Therefore, seed beetles can face a trade-off between the quality (*e.g.*, weight and size of seeds) and defenses of host plants (*e.g.*, hardness; Fox 2000, Fox and Czesak 2006).

In order to find food sources of superior quality and avoid plant defenses (*e.g.*, seed hardening), many seed beetle species synchronize their occurrence with the host-plant phenology (temporal pattern), especially in beginning of the fruiting phase, when the seeds are soft thus favoring the entry by first instar larvae that perforate the exocarp

(Szentesi 2006, Rossi et al. 2011). For instance, *Acanthoscelides schrankiae* (Horn, 1873) present higher abundance in the field before the fruit maturation of *Mimosa bimucronata* (DC) Kuntze probably because larvae fail to penetrate the tough inner lining of the mature fruit wall (Rossi et al. 2011). However, species of seed beetles may also develop other behavioral strategies to overcome the natural hardening of seeds that apparently become a plant defense. Female seed beetles of the multivoltine species *Bruchidius dorsalis* (Fahraeus, 1839) can regulate egg size according to seasonal changes in hardness of host-plant seeds (Takakura 2004). In this case, females lay larger eggs in harder seeds in the overwintering generation, which allow larvae to drill farther into these seeds reducing larval mortality and providing large adult beetles (Takakura 2004).

As female seed beetles face substantial fecundity selection for egg size shaped by seed traits (*e.g.*, seed hardness), they generally are larger and respond faster to host plant changes in comparison to males (Fox et al. 2007). This is observed in *A. macrophthalmus* on *L. leucocephala*, in which females are commonly larger than males when collected in a wide spatial scale from Northern to South of Brazil (Minas Gerais to Rio Grande do Sul states; Haga and Rossi 2016). However, exceptions include males of a few species of *Stator* and *M. terani* that can be larger in size than females (Fox et al. 2007, Maia et al. 2017). In addition, it has been observed that females varied more in size than males among plant populations due to their greater sensitivity to seed traits such as water content (Haga and Rossi 2016). Therefore, food resource quality plays a major role on the life-history traits of herbivore insects such as seed beetles, with fundamental implications in the ecology and evolution of these animals (Blanckenhorn and Demont 2004, Kolb et al. 2007), especially on females.

Since the last century, the attention on how the variation in seed traits among plant populations affects life-history traits of seed beetles has increased exponentially (Szentesi and Jermy 1995, Messina 2004, Yang et al. 2006, Stotz et al. 2013, Maia et al. 2017). However, the large Neotropical genus *Acanthoscelides* Schilsky, 1905 has been relatively poorly explored (but see Kestring et al. 2009, Haga and Rossi 2016, Sousa-Lopes et al. 2019a), although this genus has approximately 300 species (Johnson 1981, Nápoles and Kingsolver 2009, Morse 2014) and contains important pest species of stored legumes (Southgate 1979, Baier and Webster 1992). This lack of ecological information is mainly related to species that are not associated with stored seeds such as beans (*Phaseolus vulgaris* L.).

In order to provide more ecological information about *Acanthoscelides*, our main goals are: (1) to describe the damage rate inflicted by *Acanthoscelides quadridentatus* (Schaeffer, 1907) among populations of the host plant, *Mimosa setosa* var. *paludosa* (Benth.) Barneby (Fabaceae: Mimosoideae), in a spatial scale encompassing Goiás and Minas Gerais states (Brazil); (2) to analyze how the variation in seed traits (*i.e.*, weight, size, and water content) among populations of *M. setosa* var. *paludosa* affects the weight and body-size pattern of *A. quadridentatus* (spatial scale); (3) to analyze whether possible high quality seeds (*e.g.*, larger and heavier seeds) are more protected in hardness than possible low quality seeds (*i.e.* smaller and lighter seeds); and (4) to evaluate if postures of *A. quadridentatus* are synchronized with the beginning of the fruiting phase, possibly avoiding mature fruits and harder seeds (temporal scale). We hypothesise that: (i) the variation in seed traits among plant populations, that probably changes seed quality in a spatial scale (Stillwell et al. 2007, Haga and Rossi 2016), explains the variation in the body-size pattern of *A. quadridentatus*, where plant population with larger and heavier seeds will provide larger seed beetles; (ii) larger and

heavier seeds have also a harder seed coat since they are most expensive than smaller and lighter seeds, and according to ODT they must be most protected (McKey 1974, 1979); (iii) *A. quadridentatus* egg laying is synchronized with the beginning of the fruiting phase possibly to avoid seed hardening (temporal scale) (Rossi et al. 2011); and (iv) females are larger than males as shown for the major part of seed beetle species, with few exceptions (Fox et al. 2007), since they must be able to lay larger eggs for the first instar larvae overcome seed coat barrier.

Materials and Methods

Study system

Mimosa setosa var. *paludosa* is an endemic shrub to Brazil and Paraguay, and it has been recorded in several Brazilian states including Acre, Bahia, Ceará, Goiás, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Rio de Janeiro, São Paulo and Tocantins (Pott et al. 2006, Dutra and Garcia 2014). This species occurs predominantly clumped in floodplains, margin of rivers, lakes, and roadsides, in the Brazilian Cerrado (Lorenzi 2000, Dutra and Garcia 2014). The fruits are red-brown colored, dehiscent (craspedium fruits), and found mainly in the dry season (April–September). Immature fruits are bright red-brown with soft and green seeds, whereas mature fruits are dark red-brown with hard and brown seeds (Sousa-Lopes pers. obs.). This plant is important for the restoration of wetlands, but it is also an important weed in pasture areas as its large thorny branches can cause injures to cattle (Lorenzi 2000, Pott et al. 2006). Recently, this plant species has been extensively studied as to its ecological interactions in Brazilian Cerrado with relevant implications to the knowledge of trophic interactions (Sousa-Lopes et al. 2016, 2019a, 2019b, 2019c).

Acanthoscelides quadridentatus and *Acanthoscelides winderi* Kingsolver, 1984 are American species of seed beetles recorded only for the genus *Mimosa* L.

(Kingsolver 1984, Bottimer 1969, Kassulke et al. 1990, Escobar-Domínguez et al. 2018). Individuals develop feeding in a single seed which they kill the embryo and leave exit holes to adult emergence (Sousa-Lopes et al. 2019a). In *M. setosa* var. *paludosa*, these beetles inflicted a seed damage of about 15%, representing a decrease in seed set and therefore on plant fitness (Sousa-Lopes et al. 2019a). However, further studies on seed beetles are needed to better understand how ecological attributes such as the spatio-temporal scale influence the trophic interactions in which these beetles are involved.

In this study, we analyze the effect of host plant quality in a spatio-temporal scale on *A. quadridentatus* since we found this species in all sampled plant populations and always with the highest abundance. This may be related to time of collection, since most fruits were collected at the end of the fruiting phase (September), and also to the temporal partitioning already recorded between *A. quadridentatus* and *A. winderi* (Sousa-Lopes et al. 2019a). Individuals were deposited at Coleção Entomológica Padre Santiago Jesus Moure (DZUP), Departamento de Zoologia, Universidade Federal do Paraná.

Assessment of fruits and beetles

In September 2017, we randomly collected 100 mature fruits from each of the four populations of *M. setosa* var. *paludosa* distributed in roadsides from the Brazilian Cerrado in the states of Goiás (GO) and Minas Gerais (MG), near the cities of Morrinhos-GO (P1 = 17° 38' 01.17" S, 49° 10' 32.26" W), Goiatuba-GO (P2 = 18° 08' 28.13"S, 49° 17' 17.59"W), Tupaciguara-MG (P3 = 18° 39' 38.39" S, 48° 39' 42.73" W) and Uberlândia-MG (P4 = 18° 58' 10.38" S, 48° 15' 55.91" W) (Fig. 1). The minimum distance among populations was 60 km (P1-P2) and the maximum distance was 240 km (P1-P4). We selected the minimum of three different plants at least 1 m distant from

each other to randomly collect the fruits of each population. After collecting, we set the fruits individually into transparent plastic containers (500 ml) covered with voile fabric. Containers were taken to the Behavioral Ecology and Interactions Laboratory at the Federal University of Uberlândia and maintained for 150 days under laboratory conditions (12-h light and 20-30°C) until all adults emerged and posteriorly were analyzed. Damaged and undamaged seeds and *Acanthoscelides* individuals were counted.

Assessment of seed traits

To assess seed size we randomly selected 50 undamaged (with no exit holes and no wither) seeds from each population of *M. setosa* var. *paludosa* and photographed them by using a stereomicroscope with scale nearest 0.1 mm (adapted from Kestring et al. 2009). Then, we used the program Image J (Schneider et al. 2012) to obtain individual seed measurements (length and width, mm). To assess seed weight (mg) we randomly chose another 50 undamaged seeds from each population of *M. setosa* var. *paludosa* and weighted them individually with a precision analytical balance (M 163 II Meter Bel Mark Balance; Monza, Milan, Italy). To assess water content, we used the same data from seed weight (above) as fresh weight, and then we dried seeds at 60°C for 48h. After drying all seeds, we reweighed individual seeds and water content was determined as the following: $\% = (\text{fresh weight} - \text{dry weight}) \times 100 / \text{fresh weight}$ (according to Haga and Rossi 2016). Finally, to assess seed hardness we randomly selected another 50 undamaged seeds from each population and used the fruit hardness tester (Instrutherm PTR-300; São Paulo, Brazil) to measure the necessary strength (Newtons) to perforate individually each seed surface (adapted from Constant et al. 1996).

Assessment of weight and body size of Acanthoscelides quadridentatus

To assess the weight (mg) and body size (mm) of *A. quadridentatus* we randomly selected 50 individuals from each population of *M. setosa* var. *paludosa* and carefully dried those at 60°C for 48h, then we weighted them using a precision analytical balance (according to Maia et al. 2017). After, we measured the length (mm) of pronotum, left and right elytra through photos made with a digital camera coupled to a stereomicroscope at a standard magnification (1.5X) and with scale nearest 0.1 mm (Fig. 2). Measurements were obtained individually by analyzing photos in the software Image J. The lengths of traits (pronotum, left and right elytra) of each *A. quadridentatus* were summed to obtain the body size and evaluate its relationship with seed traits (adapted from Szentesi and Jermy 1995, Kestring et al. 2009). Male and female beetles were separated by examining the shape of the last one abdominal ventrite, which is slightly emarginated in males and straight in females. Differences between sexes can also be seen in the pygidium, which is laterally more convex in males in comparison to females (Ribeiro-Costa and Almeida 2012). Individuals of *A. quadridentatus* in which the sex determination was difficult and/or doubtful by analysis of the last abdominal ventrite were excluded from the assessment of weight and body size.

Assessment of seed beetles' eggs on fruits

To assess the number of seed beetles' eggs on fruits we used 76 fruits of *M. setosa* var. *paludosa* collected in September 2017 ($n = 20$) and April 2018 ($n = 56$) in Uberlândia-MG that have not been used in other analyses. We used fruits only from Uberlândia since that was the single locality in which we were able to collect in both dry and wet season. When these fruits were taken to the laboratory we computed their stage (immature or mature; see characteristic of these fruits in the section Study system), and counted the number of hatched (*i.e.*, opaque after larval emergence; Ribeiro-Costa and Almeida 2012) and unhatched eggs (*i.e.*, with visible chorion) with a

stereomicroscope. Since it was not possible to differentiate the eggs of *A. quadridentatus* and *A. winderi*, we counted all eggs of seed beetles independently of species.

Data analysis

We used the Chi-square test (χ^2) to evaluate whether frequencies of damaged and undamaged seeds differed from the expected frequencies among populations of *M. setosa* var. *paludosa* and to evaluate whether frequencies of unhatched and hatched eggs differed between immature and mature fruits. To test whether there was difference among the means of seeds and *A. quadridentatus* traits among populations we used Analyses of Variance (one-way ANOVA) followed by Tukey's post-hoc test. The unpaired *t*-test was used to evaluate whether there was a difference in the means of body size between male and female, and also between the means of damaged seeds of Minas Gerais in comparison to Goiás. Since the conjunct of data from the spatial scale (all populations together) did not meet the assumptions of normality and homoscedasticity, we used Spearman's correlations to test which seed traits influenced weight and body size of *A. quadridentatus*. Analyses were performed in R 3.1.2 with the package "PerformanceAnalytics" (R Development Core, 2016) at the 5% probability level. We also used GraphPad Prism 5 (GraphPad Company, San Diego, CA, USA) to generate graphics.

Results

We found 314 individuals of *Acanthoscelides* associated with the four populations of *M. setosa* var. *paludosa*, being 82% ($n = 258$) of them belonging to *A. quadridentatus*. Regarding seed predation, we found a significant difference in frequencies of damaged and undamaged seeds among populations of *M. setosa* var. *paludosa* ($\chi^2 = 120.1$, $P < 0.0001$). The highest percentage of damage occurred in P4

(Minas Gerais), whereas the smallest was in P1 (Goiás; Table 1), although there was no significant difference between the means of damaged seeds of Minas Gerais in comparison to Goiás ($t = -1.14, P = 0.29$).

We found significant differences in seed traits among plant populations, with highest mean values in P1 followed by P2, P3 and P4, respectively (hardness: $F = 52.36, P < 0.0001$; Length: $F = 54.49, P < 0.0001$; weight: $F = 92.99, P < 0.001$; Width: $F = 84.80, P < 0.0001$). The exception occurred for water content in which P3 had higher amount compared with other populations ($F = 26.06, P < 0.0001$; Table 2). There was a positive and significant relationship between seed length, weight, hardness, and water content (Fig. 3), indicating that larger and heavier seeds possess a higher amount of water, and they are also most protected in hardness.

Beetles were larger in P1 followed by P2, P3 and P4, although a significant difference was only observed between males of P1 and P4 ($F = 36.86, P < 0.001$, Tukey post-hoc test = $P < 0.01$), and females of P1, P2 and P3 in comparison to P4 ($F = 26.49, P < 0.0001$, Tukey post-hoc test = $P < 0.01$). Males and females from P1 were approximately 15 and 25% larger than P4, respectively. In general, females were significantly larger than males ($t = 2.16, P = 0.03$), although in P4 the mean value of female's body size was lower than the male's (Table 3). On the other hand, we did not find difference in weight of male ($F = 2.51, P > 0.05$) and female ($F = 4.48, P > 0.05$) among populations (Table 3). We also found a positive and significant correlation between seed length, weight, hardness, and water content with body size of male and female, and weight of female *A. quadridentatus* (Fig. 3).

Unhatched eggs were found only in immature fruits collected in April 2018, and it was found a significant difference in frequencies of unhatched and hatched eggs between immature and mature fruits ($\chi^2 = 32.35, P < 0.0001$; Fig. 4).

Discussion

Our initial hypotheses were corroborated: first, changes in seed traits explained the variation in the body-size pattern of *A. quadridentatus* among populations; second, larger and heavier seeds were harder than smaller and lighter seeds; third, *A. quadridentatus* egg laying was synchronized with the beginning of fruiting phase; and fourth, female *A. quadridentatus* were larger than males among populations (with the exception of P4). We also found a higher percentage of seed damage in *M. setosa* var. *paludosa* population (P4) which seeds were softer. Although we showed some evidence of synchronicity between beetle egg laying and immature fruit availability, we suggest that this phenomenon must be carefully evaluated. It is because we were able to assess egg laying only in one population and long-term studies are still necessary to confirm this pattern.

Seed weight, size, and water content proved to be important factors influencing weight and body size of seed beetles (Moegenburg 1996, Maia et al. 2017). For example, the weight of *Caryobruchus gleditsiae* (Linnaeus, 1763) was positively correlated with the weight of seed consumed, which was also correlated with total seed biomass of *Sabal palmetto* (Walter) Lodd. ex Schult. & Schult. f. (Moegenburg 1996). It was also shown that larger individuals of *M. terani* and *S. maculatopygus* were found feeding on larger and heavier seeds of *S. tenuifolia* (Maia et al. 2017). In addition, water content was the factor that most explained variation in body size of *A. macrophthalmus* in a spatial scale (Haga and Rossi 2016). We also found that seed length, weight, and water content were positively correlated with the weight and body size of *A. quadridentatus*, being seed weight one of the most important factors affecting the body-size pattern of this beetle species. Indeed, in P1, where we found heavier seeds, we found larger beetles, whereas, in P4, where we found lighter seeds, we found smaller

beetles. In this sense, it is possible that heavier seeds might be more nutritious because they contain a higher proportion of carbohydrates, lipids, and proteins as well as water content which provides more available resources to seed beetles, and thus affecting their body-size pattern, survival, and reproductive success (Mitchell 1975, Messina 2004, Fox et al. 2007, Amarillo-Suárez et al. 2011).

We found greater seed hardness in P1 and lower seed hardness in P4 and, consequently, P1 had lower seed damage than P4. This result fits in the ODT (McKey 1974, 1979, Rhoades 1979), suggesting that expensive resources (*i.e.*, larger and heavier seeds) are also most protected and therefore less damaged by seed beetles. However, individuals of *A. quadridentatus* that had overcome seed hardness in P1 could enter in seeds, intake high amounts of nutrients and then grow to larger sizes. Seed hardness was the reason that Seifelnasr (1991) provided for the nondevelopment of some individuals of *Callosobruchus maculatus* (Fabricius, 1775) in seeds of *P. vulgaris*. Nevertheless, as shown here, there is no indication that large seeds with a hard seed coat are physically inimical to seed beetles' development since some individuals can overcome this plant defense (Janzen 1969).

Other studies on the tropical region suggested that seed beetles seem to have developed a temporal strategy to avoid seed hardness by synchronizing their life cycle with the beginning of the fruiting phase (*e.g.*, Brazil; Sari et al. 2005). In this way, when females lay their eggs on immature seeds and fruits that therefore have softer fruit/seed surface, they probably facilitate the penetration of first instar larvae in fruits and seeds. For instance, it was shown that *A. schrankiae* and *Sennius crudelis* Ribeiro-Costa & Reynaud, 1998 occur in higher abundance in the field before fruit maturation of *M. bimucronata* and *Senna multijuga* (Rich.) H. S. Irwin & Barneby, respectively. That apparently occurs because it is easier for the first instar larvae to penetrate the fruits'

wall (Rossi et al. 2011, Sari et al. 2005). In this study we found unhatched eggs only in the beginning of the fruiting phase, which suggests that females can make postures before fruit ripping in order to avoid seed hardening for first instar larvae. Thus, seed hardness is an important bottom-up factor which plays a crucial role on the occurrence and survival of *A. quadridentatus*, although further long-term studies are still necessary to confirm that. Other plant traits such as age, size and defensive chemical compounds may also change among populations of *M. setosa* var. *paludosa* and play an important role (spatial scale), although unexplored here (see Janzen 1969, Sari et al. 2005, Kergoat et al. 2005, Ribeiro-Costa and Almeida 2012). Our data suggest that earliest arriving females may found a window of time with better opportunities (*i.e.*, softer seeds) to lay their eggs.

In general, females of seed beetles are larger than males, with few exceptions such as in *M. terani* and *Stator* spp. (Fox et al. 2007, Maia et al. 2017). Here we also found that females of *A. quadridentatus* are larger than males, although in P4 males were larger than females. It is possible that the selective pressure for fecundity is higher in females from populations with harder seeds (P1, P2, and P3) since they must lay larger eggs to enable big larvae to overcome seed hardness. Indeed, it has been showed that female of the seed beetle, *B. dorsalis*, can regulate egg size according to the hardness of host-plant seeds (Takakura 2004). On the other hand, as P4 seeds are softer, the selective pressure to females oviposit larger eggs may be weak compared to other populations, which explains smaller females. In this case, it is possible that stronger selective pressure occurs in males. For example, in many species of seed beetles males transfer nuptial gifts to females in the form of a large volume of seminal fluid, in many cases contributing with 6–10% of their weight to females during mating (Savalli and Fox 1998, Fox et al. 2007). Thus, in poor seeds as that from P4, it is possible that

females need male nuptial gifts to survive and produce eggs, which would explain a male-biased size in this population. Nevertheless, we neither address fecundity questions or measure egg size in this study then further studies on these subjects are still necessary to better understand the ecology of *A. quadridentatus*.

Overall, we showed that the variation in seed traits in the spatial scale (~240 km), especially weight, affected the body-size pattern of *A. quadridentatus*, highlighting a phenotypic plasticity in this beetle among host plant populations. Additionally, we showed that the temporal variation in seed hardness during the fruiting phase is another important bottom-up factor influencing postures and possibly the survival of *A. quadridentatus*. This study reports new information about the ecology of *A. quadridentatus*, and the results found here suggest that the spatio-temporal scale is an important ecological component driving variations in seed traits and consequently life-history traits of the associated seed beetle. However, large scale studies including questions about synchrony, fecundity and egg size of *A. quadridentatus*, and other host plant traits such as age, size and amount of chemical compound will contribute to a deeper understanding of the ecology and evolution of this interaction.

References

- Amarillo-Suárez, A.R., Stillwell, R.C., Fox, C.W. 2011. Natural selection on body size is mediated by multiple interacting factors: a comparison of beetle populations varying naturally and experimentally in body size. *Ecol Evol*.1:1–14. <https://doi.org/10.1002/ece3.1>.
- Baier, A.H., Webster, B.D. 1992. Control of *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae) in *Phaseolus vulgaris* L. seed stored on small farms I. Evaluation of damage. *J. Stored Prod. Res.* 28:289–293. [https://doi.org/10.1016/0022-474X\(92\)90011-E](https://doi.org/10.1016/0022-474X(92)90011-E).
- Blanckenhorn, W.U., Demont, M. 2004. Bergmann and converse Bergmann latitudinal clines in arthropods: two ends of a continuum? *Integr Comp Biol.* 44:413–424. <https://doi.org/10.1093/icb/44.6.413>
- Bottimer, L.J. 1969. Bruchidae associated with *Mimosa* with the description of a new species. *Can Entomol.* 101:1186–1198. <https://doi.org/10.4039/Ent1011186-11>.
- Bridwell, J.C. 1918. Notes on the Bruchidae and their parasites in the Hawaiian Islands. *Proc Hawaiian Entomol Soc.* 3:465–505.

- Canto, A., Rodríguez, R., Reyes-Novelo, E. 2019. Relationship between the weights of seed beetles of the genus *Megacerus* Fåhraeus, 1839 (Coleoptera: Chrysomelidae: Bruchinae) and their host seeds of the family Convolvulaceae. *Sci Rep.* 9:8438. <https://doi.org/10.1038/s41598-019-44761-8>.
- Constant, B. Grenier, S., Febvay, G., Bonnot, G. 1996. Host plant hardness in oviposition of *Macrolophus caliginosus* (Hemiptera: Miridae). *J Econ Entomol.* 89:1446–1452. <https://doi.org/10.1093/jee/89.6.1446>.
- Dutra, V.F., Garcia, F.C.P. 2014. *Mimosa* L.(Leguminosae-Mimosoideae) dos campos rupestres de Minas Gerais, Brasil. *Iheringia Serie Bot.* 69:49–88.
- Escobar-Domínguez, A.A., Romero-Nápoles, J., Equihua-Martínez, A., Carrilo-Sánchez, J.L., Ramínez-Alarcón, S. 2018. Bruchids (Coleoptera: Bruchidae) associated with seeds os species of *Mimosa* L. genus (Leguminosae: Mimosoideae). *Acta Zool Mex.* 34: 1–17. <https://doi.org/10.21829/azm.2018.3412149>.
- Fenner, M. and Thompson, K. 2005. *The ecology of seeds.* Cambridge University Press, Cambridge.
- Fox, CW. 2000. Natural selection on seed-beetle eggs size in nature and the laboratory: variation among environments. *Ecology,* 81(11):3029–3035. [https://doi.org/10.1890/0012-9658\(2000\)081\[3029:NSOSBE\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2000)081[3029:NSOSBE]2.0.CO;2).
- Fox, C.W., Czesak, M.E. 2006. Selection on body size and sexual size dimorphism differs between host species in a seed-feeding beetle. *J Evolution Biol.* 19(4):1167–1174. <https://doi.org/10.1111/j.1420-9101.2006.01092.x>.
- Fox, C.W., Stillwell, R.C., Moya-Laraño, J. 2007. Variation in selection, phenotypic plasticity, and ecology of sexual size dimorphism in two seed-feeding beetles, in Fairbairn, D.J., Blanckenhorn, W.U., Szekely, T. (Eds.), *Sex, size and gender roles: evolutionary studies of sexual size dimorphism.* Oxford University Press, Oxford, NY, pp. 88–96.
- González-Teuber, M., Segovia, R., Gianoli, E. 2008. Effects of maternal diet and host quality on oviposition patterns and offspring performance in a seed beetle (Coleoptera: Bruchidae). *Naturwissenschaften.* 95:609–615. <https://doi.org/10.1007/s00114-008-0361-4>.
- Haga, E.B., Rossi, M.N. 2016. The effect of seed traits on geographic variation in body size and sexual size dimorphism of the seed-feeding beetle *Acanthoscelides macrophthalmus*. *Ecol Evol.* 6(19):6892–6905. <https://doi.org/10.1002/ece3.2364>.
- Howe, R.W., Currie, J.E. 1964. Some laboratory observations on the rates of development, mortality and oviposition of several species of Bruchidae breeding in stored pulses. *Bull. Entomol. Res.* 55:437–477. <https://doi.org/10.1017/S0007485300049580>.
- Janzen, D.H. 1969. Seed-eaters versus seed size, number, toxicity and dispersal. *Evolution.* 23(1):1–27. DOI: 10.2307/2406478.
- Johnson, C.D. 1981. Interactions between bruchid (Coleoptera) feeding guilds and behavioral patterns of fruits of the Leguminosae. *Environ Entomol.* 10:249–253. DOI: 10.1093/ee/10.2.249.
- Kassulke, R.C., Harley, K.L.S., Maynard, G.V.1990. Host specificity of *Acanthoscelides quadridentatus* and *A. puniceus* (Col.: Bruchidae) for biological control of *Mimosa pigra* (with preliminary data on their biology). *Entomophaga.* 35(1):85–96. <https://doi.org/10.1007/BF02374305>.
- Kergoat, G.J., Delobel, A., Fédière, G., Rü, B.L., Silvain, J-F. 2005. Both host-plant phylogeny and chemistry have shaped the African seed-beetle radiation. *Mol Phylogenetics Evol.* 35(3):602–611. <https://doi.org/10.1016/j.ympev.2004.12.024>.

- Kestring, D., Menezes, L.C.R., Tomaz, C.A., Lima, G.P.P., Rossi, M.N. 2009. Relationship among phenolic contents, seed predation, and physical seed traits in *Mimosa bimucronata* plants. *J. Plant Biol.* 52:569–576. <https://doi.org/10.1007/s12374-009-9073-3>.
- Kingsolver, J.M. 1984. *Acanthoscelides winderi*, new species, (Coleoptera, Bruchidae) associated with *Mimosa* spp. (Leguminosae, Mimosoideae) from Brazil. *Entomol News.* 95:87–90.
- Kolb, A., Ehrlen, J., Eriksson, O. 2007. Ecological and evolutionary consequences of spatial and temporal variation in pre-dispersal seed predation. *Perspect Plant Ecol Syst.* 9:79–100. <https://doi.org/10.1016/j.ppees.2007.09.001>.
- Lorenzi, H. 2000. Plantas daninhas do Brasil: terrestres, aquáticas, parasitas e tóxicas. Instituto Plantarum, Nova Odessa, SP.
- Maia, L.F., Tuller, J., Faria, L.D.B. 2017. Morphological traits of two seed-feeding beetle species and the relationship to resource traits. *Neotrop Entomol.* 46:36–44. DOI: 10.1016/j.rbe.2017.04.003.
- McKey, D. 1974. Adaptative patterns in alkaloid physiology. *Amer Nat.* 108(961): 305–320.
- McKey, D. 1979. The distribution of plant secondary compounds within plants, in Rosenthal, G.A., Janzen, D.H. (Eds.). *Herbivores: their interactions with secondary plant metabolites.* Academic press, New York, pp. 55–133.
- Mitchell, R. 1975. The evolution of oviposition tactics in the vean weevil, *Calosobruchus maculatus* (F). *Ecology.* 56:696–702.
- Messina, F.J. 2004. Predictable modification of body size and competitive ability following a host shift by a seed beetle. *Evolution.* 58:2788–2797. <https://doi.org/10.1554/04-372>.
- Moegenburg, S.M. 1996. *Sabal palmetto* seed size: causes of variation, choices of predators, and consequences for seedlings. *Oecologia.* 106(4):539–543. <https://doi.org/10.1007/BF00329713>.
- Morse, G.E. 2014. Bruchinae Latreille, 1802, in Leschen, R.A.B., Beutel, R.G. *Handbook of Zoology, Coleoptera volume 3: morphology and systematics (Phytophaga).* Walter de Gruyter, Berlin, pp. 189–198.
- Nápoles, J.R., Kingsolver, J.M. 2009. A New Species of *Acanthoscelides* Schilsky (Coleoptera: Bruchidae) from Mexico with Some Biological Notes. *Neotrop Entomol.* 38(4):497–500. <https://doi.org/10.1590/S1519-566X2009000400009>.
- Pott, A., Pott, V.J., Souza, T.W. 2006. Plantas daninhas de pastagem na região dos Cerrados. Campo Grande, MS: EMBRAPA Gado de Corte.
- Price, P.W., Denno, R.F., Eubanks, M.D., Finke, D.L., Kaplan, I. 2011. *Insect Ecology: behavior, populations and communities.* Cambridge University Press, New York, 764p.
- R Development Core Team. 2016. R: A language and environment for statistical computing. Available form: <http://www.R-project.org>
- Rhoades, D.F. 1979. Evolution of plant defense against herbivores, in Rosenthal, G.A. and Janzen, D.H. (Ed.). *Herbivores: their interactions with secondary plant metabolites.* Academic press, New York, pp. 1–55.
- Ribeiro-Costa, C.S., Almeida, L.M. 2012. Seed-chewing beetles (Coleoptera: Chrysomelidae, Bruchinae), in Panizzi, A.R., Parra, J.R.P. (Org.), *Insect Bioecology and Nutrition for Integrated Pest Management.* 1ed. CRC Press, Boca Raton, pp. 325–352.

- Rossi, M.N., Rodrigues, L.M.S., Ishino, M.N., Kestring, M. 2011. Oviposition pattern and within-season spatial and temporal variation of pre-dispersal seed predation in a population of *Mimosa bimucronata* trees. *Arthropod Plant Interact.* 5:209–217. <https://doi.org/10.1007/s11829-011-9130-y>.
- Sari, L.T., Ribeiro-Costa, C.S., Roper, J.J. 2005. Dinâmica populacional de bruquíneos (Coleoptera, Chrysomelidae) em *Senna multijuga* (Rich.) H. S. Irwin & Barneby (Caesalpinaceae). *Rev. Bras. Zool.* 22(1):169–174. <https://doi.org/10.1590/S0101-81752005000100019>.
- Savalli, U.M., Fox, C.W. 1998. Genetic variation in paternal investment in a seed beetle. *Anim Behav.* 56(4):953–961. <https://doi.org/10.1006/anbe.1998.0853>.
- Schneider, C.A., Rasband, W.S., Eliceiri, K.W. 2012. [NIH Image to ImageJ: 25 years of image analysis](https://doi.org/10.1038/nmeth.2089), *Nature methods* 9(7): 671–675. <https://doi.org/10.1038/nmeth.2089>.
- Seifelnasr, Y.E. 1991. The role of asparagine and seed coat thickness in resistance of *Phaseolus vulgaris* (L.) to *Callosobruchus maculatus* (F.) (Col., Bruchidae). *J. Appl. Entomol.* 111:412–417. <https://doi.org/10.1111/j.1439-0418.1991.tb00342.x>.
- Stillwell, R.C., Morse, G.E., Fox, C.W. 2007. Geographic variation in body size and sexual size dimorphism of a seed-feeding beetle. *Am. Nat.* 170:358–369.
- Stotz, G.C., Suarez, L.H., Gonzales, W.L., Gianoli E. 2013. Local host adaptation and use of a novel host in the seed beetle *Megacerus eulophus*. *PLoS One* 8:e53892. <https://doi.org/10.1371/journal.pone.0053892>.
- Szentesi, A. 2006. Pre-dispersal seed predation by *Bruchidius villosus* (Coleoptera, Bruchidae) in *Laburnum anagyroides* (Fabaceae, Genisteae). *Community Ecol.* 7:13–22. <https://doi.org/10.1556/ComEc.7.2006.1.2>.
- Szentesi, A., Jermy, T. 1995. Predispersal seed predation in leguminous species: seed morphology and bruchid distribution. *Oikos.* 73:23–32.
- Sousa-Lopes, B., Bächtold, A., Del-Claro, K. 2016. Biology, natural history and temporal fluctuation of the geometrid *Oospila pallidaria* associated with host plant phenology. *Stud Neotrop Fauna Environ.* 51:135–143. <https://doi.org/10.1080/01650521.2016.1199140>.
- Sousa-Lopes, B., Alves-da-Silva, N., Ribeiro-Costa, C.S., Del-Claro, K. 2019a. Temporal distribution, seed damage and notes on the natural history of *Acanthoscelides quadridentatus* and *Acanthoscelides winderi* (Coleoptera: Chrysomelidae: Bruchinae) on their host plant, *Mimosa setosa* var. *paludosa* (Fabaceae), in the Brazilian Cerrado. *J Nat Hist*, 53(9-10):611–623. <https://doi.org/10.1080/00222933.2019.1606358>.
- Sousa-Lopes, B., Alves-da-Silva, N., Alves-Martins, F., Del-Claro, K. 2019b. Antiherbivore protection and plant selection by the lynx spider *Peucetia flava* (Araneae: Oxyopidae) in the Brazilian Cerrado. *J. Zool*, 308(2): 121–127. <https://doi.org/10.1111/jzo.12662>.
- Sousa-Lopes, B., Whitfield, J.B., Salgado-Neto, G., Del-Claro, K. 2019c. *Cotesia itororensis* sp. nov. from Brazilian savanna: a new reared microgastrinae wasp described using an integrative taxonomic approach. *Zootaxa*, 4544 (3): 437–445. <http://dx.doi.org/10.11646/zootaxa.4544.3.9>.
- Southgate, B.J. 1979. Biology of the Bruchidae. *Annu. Rev. Entomol.* 24:449–73. <https://doi.org/10.1146/annurev.en.24.010179.002313>.
- Takakura, K. 2004. Variation in egg size within and among generations of the bean weevil, *Bruchidius dorsalis* (Coleoptera: Bruchidae): effects of host plant quality and paternal nutritional investment. *Ann. Entomol. Soc. Am.* 97:346–352. [https://doi.org/10.1603/0013-8746\(2004\)097\[0346:VIESWA\]2.0.CO;2](https://doi.org/10.1603/0013-8746(2004)097[0346:VIESWA]2.0.CO;2).

- Wall, S.B.V., Forget, P.M., Lambert, J.E., Hulme, P.E. 2005. Seed fate pathways: filling the gap between parent and offspring, in Forget, P.M., Lambert, J.E., Hulme, P.E., Wall, S.B.V (Eds.), Seed fate: predation, dispersal and seedling establishment. CABI Publishing, Wallingford, pp. 1–8.
- Yang, R.L., Fushing, H., Horng, S.B. 2006. Effects of search experience in a resource-heterogeneous environment on the oviposition decisions of the seed beetle, *Callosobruchus maculatus* (F.). *Ecol Entomol.* 31(4):285–293. <https://doi.org/10.1111/j.1365-2311.2006.00696.x>.

Table Caption

Table 1. Damaged and undamaged seeds (total number and percentage) for each population of *Mimosa setosa* var. *paludosa* (Fabaceae: Mimosoideae) in roadsides from Brazilian Cerrado. P1= Morrinhos-GO, P2 = Goiatuba-GO, P3 = Tupaciguara-MG and P4 = Uberlândia-MG. Observed frequencies differed statistically from the expected frequencies by the Chi-square test at $P < 0.0001$.

Population	Damaged seeds		Undamaged seeds	
	N°	%	N°	%
P1	246	32	518	68
P2	306	36	547	64
P3	260	52	241	48
P4	316	58	229	42

Table 2. Seed traits (mean \pm SD) from each population of *Mimosa setosa* var. *paludosa* (Fabaceae: Mimosoideae) in roadsides from Brazilian Cerrado. P1= Morrinhos-GO, P2 = Goiatuba-GO, P3 = Tupaciguara-MG and P4 = Uberlândia-MG. Different letters represent that means differed statistically by Tukey's post-hoc test at $P < 0.05$.

Population	Hardness (N)	Length (mm)	Water content (%)	Weight (mg)	Width (mm)
P1	154.13(\pm 27.84)a	3.27(\pm 0.32)a	37(\pm 20)a	0.028(\pm 0.030)ab	2.37(\pm 0.23)a
P2	151.91(\pm 37.46)a	3.20(\pm 0.47)a	37(\pm 25)a	0.024(\pm 0.005)a	2.38(\pm 0.31)a
P3	144.37(\pm 39.31)a	3.18(\pm 0.50)a	68(\pm 19)b	0.021(\pm 0.005)a	2.34(\pm 0.38)a
P4	72.41(\pm 45.95)b	2.34(\pm 0.36)b	32(\pm 27)a	0.016(\pm 0.017)ac	1.55(\pm 0.30)b

Table 3. Weight (mean \pm SD) and body size (mean \pm SD) of *Acanthoscelides quadridentatus* (Chrysomelidae: Bruchinae) according to the population of *Mimosa setosa* var. *paludosa* (Fabaceae: Mimosoideae) where they were collected. P1= Morrinhos-GO, P2 = Goiatuba-GO, P3 = Tupaciguara-MG and P4 = Uberlândia-MG; M = male and F = female. Body size is the sum of left elytra, pronotum, and right elytra. Different letters represent that means differed statistically by Tukey's post-hoc test at $P < 0.05$.

Population	Weight (mg)		Body size (mm)	
	M	F	M	F
P1	0.0011(\pm 0.0003)a	0.0013(\pm 0.0003)a	3.92(\pm 0.30)ab	4.28(\pm 0.28)a
P2	0.0009(\pm 0.0003)a	0.0012(\pm 0.0002)a	3.84(\pm 0.23)a	3.96(\pm 0.14)a
P3	0.0009(\pm 0.0002)a	0.0010(\pm 0.0004)a	3.77(\pm 0.44)a	3.96(\pm 0.31)a
P4	0.0009(\pm 0.0003)a	0.0009(\pm 0.0004)a	3.44(\pm 0.42)ac	3.39(\pm 0.47)b

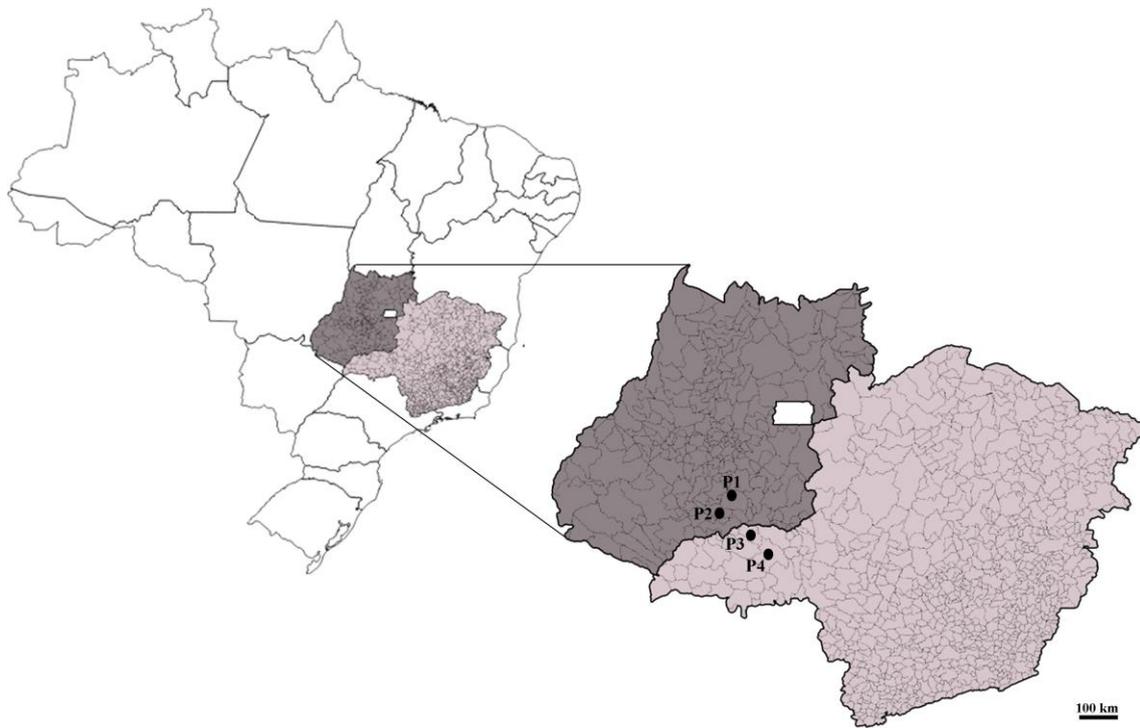


Figure 1. Spatial distribution of the populations (P1 = Morrinhos-GO, P2 = Goiatuba-GO, P3 = Tupaciguara-MG and P4 = Uberlândia-MG) of *Mimosa setosa* var. *paludosa* (Fabaceae: Mimosoideae) from which *Acanthoscelides quadridentatus* (Chrysomelidae: Bruchinae) individuals were collected. Dark and light grey colors indicate the Brazilian states Goiás and Minas Gerais, respectively.

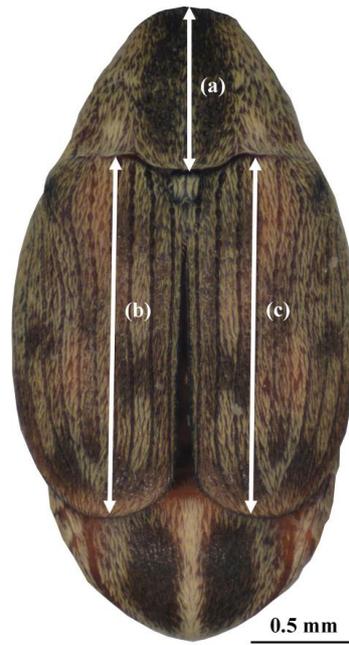


Figure 2. Body traits of *Acanthoscelides quadridentatus* (Chrysomelidae: Bruchinae) taken to estimate its body-size pattern among populations (P1 = Morrinhos-GO, P2 = Goiatuba-GO, P3 = Tupaciguara-MG and P4 = Uberlândia-MG). The arrows indicate: (a) pronotum, (b) left elytra, and (c) right elytra.

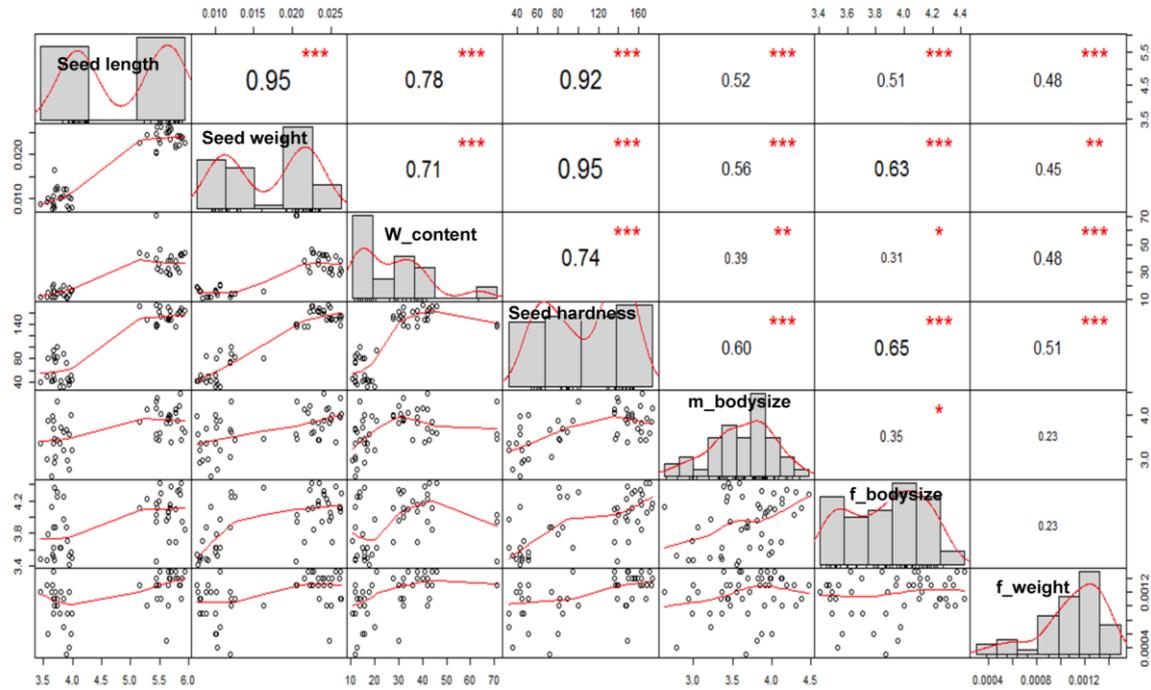


Figure 3. Correlation matrix between traits of the host plant *Mimosa setosa* var. *paludosa* (seed length, weight, water content, and hardness) and *Acanthoscelides quadridentatus* (male body size, and female body size and weight). Data are presented in bars, scatter plots and with Spearman correlation values for each relationship. Analyses must be performed taking into account lines versus columns. * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$.

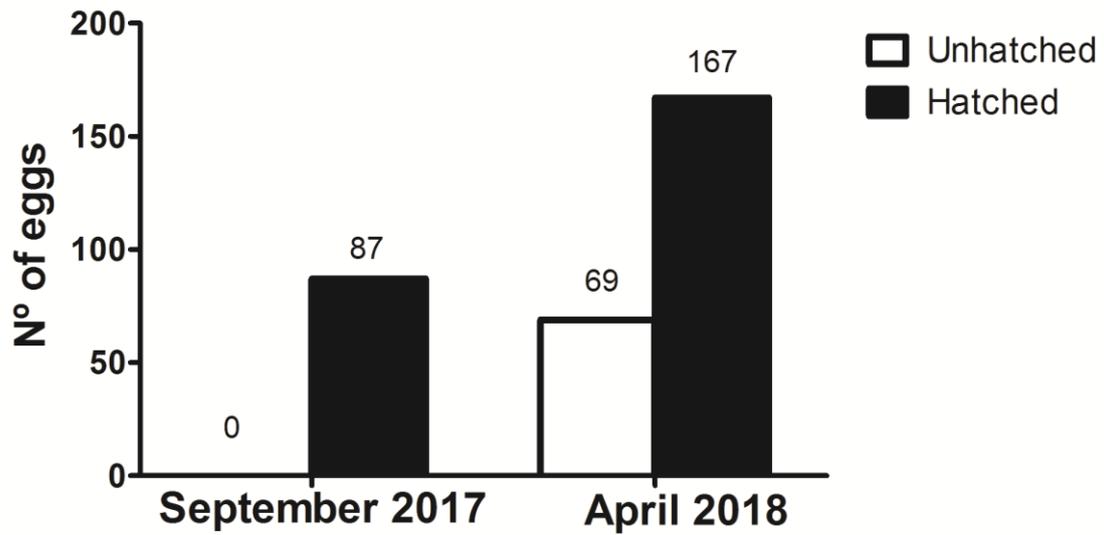
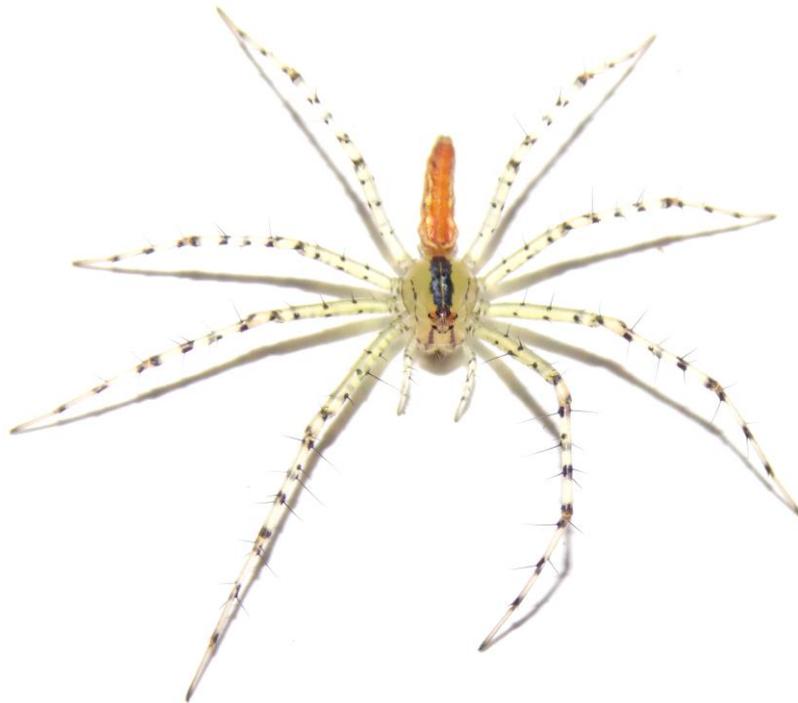


Figure 4. Number of unhatched and hatched eggs of *Acanthoscelides* beetles (Chrysomelidae: Bruchinae) in the end (September 2017) and beginning of fruiting phase (April 2018) on fruits of *Mimosa setosa* var. *paludosa* (Fabaceae: Mimosoideae).

Capítulo 4

Antiherbivore protection and plant selection by the lynx spider *Peucetia flava* in the Brazilian Cerrado



Este capítulo foi publicado no Journal of Zoology, 308(2): 121-127.

Antiherbivore protection and plant selection by the lynx spider *Peucetia flava* in the Brazilian Cerrado

Bruno de Sousa-Lopes, Nayane Alves-da-Silva, Fernanda Alves-Martins & Kleber Del-Claro

Abstract

Spiders are ubiquitous in most vegetation, however very little empirical data are available on specific spider–plant interactions and their reciprocal outcomes. In the Brazilian Cerrado, the plant *Mimosa setosa* var. *paludosa*, (Fabaceae) has glandular trichomes in its leaves and stems, commonly entrapping insects (i.e. carrion) as well as hosting the lynx spider, *Peucetia flava*. We hypothesized that: (1) the damage inflicted by exophytic (leaves) and endophytic (seeds) herbivore insects that overcome the glandular trichomes is lower in plants where the lynx spider is present; and (2) the presence of this predator is positively related to food availability (live insects and/or carrion) and plant size. We performed field observations and an experimental field study in terms of the spider’s presence versus absence on the Fabaceae plants. Our results showed that the proportion of damaged foliolules on the Fabaceae plants differed between the spiders-present and spiders-absent treatments, and that the absence of spiders led to a 3.3-fold increase in the number of damaged foliolules. However, there was no significant difference in the proportion of seeds taken by endophytic herbivores from branches with and without spiders. We also found that the presence of *Peucetia flava* was positively related to the presence of entrapped carrion on plants, and that there was a positive and marginally significant effect of increasing abundance of the spiders on taller plants. The results of this study suggest that it is more common to find lynx

spiders interacting with *M. setosa* var. *paludosa* in larger plants with higher carrion abundance (food source), and that *P. flava* and *M. setosa* var. *paludosa* interact in a facultative mutualism, in which plants provide entrapped carrion for spiders to feed on and possibly facilitate prey manipulation. In return, lynx spiders decrease the damage inflicted by exophytic, but not by endophytic herbivores. These results also contribute to a better understanding of which ecological factors may affect plant selection by lynx spiders and what the influence of this predator is on the structure of food webs in glandular plants.

Keywords: Entrapped carrion, Fabaceae, facultative mutualisms, herbivore damage, *Mimosa*, spider-plant interactions, tri-trophic interactions.

Introduction

Plants represent a complex of microhabitats hosting several animals and giving place to different ecological interactions, which vary from antagonisms to mutualisms (Janzen, 1967; Heil, 2008; Price *et al.*, 2011). Spiders are among the most ubiquitous arthropods found on plants (Foelix, 1996), and in some cases, they play an important role as plant guards into facultative mutualisms (Whitney, 2004; Vasconcellos-Neto *et al.*, 2017). In these relationships, plants provide suitable microhabitats for spiders forage, breed, and shelter (Romero & Vasconcellos-Neto, 2005); spiders, in return, can benefit the host plant by preying on herbivores and decreasing the damage in plant tissues (Morais-Filho & Romero, 2010). However, very few empiric data are available in the literature regarding specific spider–plant interactions and which plant traits affect plant selection by spiders (Vasconcellos-Neto *et al.*, 2017), despite the fact that their impact on the reproductive success of plants have received increased attention in recent years (Del-Claro *et al.*, 2016).

The plant selection by spiders may be strongly influenced by the availability of live arthropods on plants, which represent a food source for spiders (Morais-Filho & Romero, 2008). However, increasing the abundance of several predators including spiders may be also related to the abundance of dead insects adhered to plant surfaces (hereafter: carrion) (Krimmel & Pearse, 2014; LoPresti *et al.*, 2018). The carrion would represent an accessible food source and without costs associated with attacking and manipulating live preys, making that predators remain on plants with reliable carrion sources (LoPresti *et al.*, 2015). On the other hand, plant size is also another important factor influencing plant selection by spiders (Lawton, 1983; Halaj *et al.*, 2000; Langelloto & Denno, 2004). Large plants are more conspicuous and attractive for insects from different herbivore guilds (e.g. endophytic and exophytic herbivores) due to their wide range of microhabitats and resources (Schlinkert *et al.*, 2015), thus providing more abundant and variable food sources for spiders (Romero & Vasconcellos-Neto 2005). Indeed, spiders can select the host plant according to the plant height (Morais-Filho & Romero, 2008).

The lynx spider *Peucetia flava* Keyserling 1877 (Araneae: Oxyopidae; Fig. 1a) is commonly found on glandular trichome-bearing plants worldwide (Vasconcellos-Neto *et al.*, 2007; Morais-Filho & Romero, 2009). These plants have sticky hairs that, in addition to the defensive functions against herbivores, facilitate prey capture and provide carrion as a food source for lynx spiders, and thereby increasing spider fidelity to the host plant (Romero *et al.*, 2008). Indeed, lynx spiders have been recorded feeding on carrion (scavenging behavior; Romero *et al.*, 2008), although the effect of the carrion abundance on plant selection by lynx spiders is poorly understood. Lynx spiders, in return to the benefits given by the host plant, may reduce the damage inflicted by herbivores that overcome the glandular trichomes on leaves and floral buds

(Vasconcellos-Neto *et al.*, 2017). For instance, the damage on leaves and floral buds in *Rhynchanthera dichotoma* (Ness) C.B. Clarke (Melastomataceae) was reported to decrease in 74 and 85%, respectively, in the presence of the lynx spiders (Morais-Filho & Romero, 2010). However, the effect of lynx spiders as plant guards may be taxon and guild specific (Romero *et al.*, 2008). If on the one hand, these spiders may be effective in protecting the *R. dichotoma* plants against exophytic herbivores (e.g., Miridae sp. and Cicadellidae sp.) (Morais-Filho & Romero, 2010). On the other hand, lynx spiders may not be effective against endophytic herbivores, as it was reported for the endophytic dipterans (e.g., *Melanogromyza* spp.; Agromyzidae) on *Trichogoniopsis adenantha* (DC.) King & Rob (Asteraceae) (Romero *et al.*, 2008). Indeed, endophytic herbivores can be protected against some predators as they feed inside plant tissues (Alves-Silva *et al.*, 2014).

Spiders and ants together exert a positive and complementary effect on the reproductive success of extrafloral nectaried plants by reducing or deterring herbivores (Nahas *et al.*, 2012, 2017; Stefani *et al.*, 2015; Del-Claro *et al.*, 2013, 2017). Glandular trichome-bearing plants also have received more attention currently, especially Asteraceae, Melastomataceae, and Solanaceae (Romero & Vasconcellos-Neto, 2012). However, little attention has been paid to the relationship between spiders and plant species of the large family Fabaceae and its role in structuring food webs. In order to fill these gaps, we record here the impact of the presence of the lynx spider, *P. flava*, on *Mimosa setosa* var. *paludosa* (Benth.) Barneby (Fabaceae: Mimosoideae; Fig. 1b), a glandular trichome-bearing plant commonly found in the Brazilian Cerrado (Dutra & Garcia, 2014).

Our main aims were: (1) to evaluate the effectiveness of lynx spiders as a plant guard against exophytic and endophytic herbivores, and (2) to evaluate the effects of

insect abundance (live and carrion) and *M. setosa* var. *paludosa* size on the presence and abundance of lynx spiders. We hypothesized that: (a) the damage inflicted by exophytic (leaves) and endophytic (seeds) herbivores will be lower in plants where the lynx spider is present, and (b) the presence of this predator will be positively related to the food availability (live insects and/or entrapped carrion) and plant size.

Material and Methods

Study site and plant species

The experiments were performed between February and July 2017 and December 2017 and February 2018, in the Ecological Reserve of Clube Caça e Pesca Itororó de Uberlândia (CCPIU), Minas Gerais state, southeastern Brazil (18°59'00''S, 48°17'44''W). The climate in the region is markedly seasonal, characterized by a rainy summer (October to April) which may account for up to 75% of the annual rainfall, and a dry winter (May to September) (Laboratory of Climatology, Federal University of Uberlândia; Sousa-Lopes *et al.*, 2016).

Mimosa setosa var. *paludosa* is a shrub that rarely exceeds a height of 3 m, has compound leaves, craspedium fruits, glandular trichomes over all its parts (Dutra & Garcia, 2014), and occurs on the edge of CCPIU near a vereda, a swampy area located in the headwater of a stream (Sousa-Lopes *et al.*, 2016). The glandular trichomes present a sticky substance that entraps insects (Fig. 1c, d) but not lynx spiders, which are adapted to glandular plants and use them to feed on, breed and shelter (Vasconcellos-Neto *et al.*, 2007; Fig. 1e, f). The plant produces leaves all year round, (Sousa-Lopes *et al.*, 2016). Flowering and fruiting is from February to May and March to October, respectively (B. Sousa-Lopes, pers. obs.).

Experimental manipulations

To test whether lynx spiders would have a negative impact on the damage inflicted by exophytic herbivores, we tagged 20 plants with similar height and phenology and separated them into two treatments, 10 plants in spiders-present treatment and 10 plants in spiders-absent treatment. On the spiders-present treatment, we kept one individual of *P. flava*, similarly to what we used to find on plants (Sousa-Lopes, pers. obs.), whereas spiders were removed on plants belonging to spiders-absent treatment. Every 3 days, we inspected all plants and spiders, and after 15 days of trials (5 visits), we randomly selected 10 leaves of the 20 plants, and we counted the number of foliolules damaged (chewed) and undamaged (not chewed). Then, we calculated the proportion of foliolules damaged relative to the total number of foliolules found in the 10 selected leaves per plant for both spiders-present and spiders-absent treatments (sensu Romero *et al.*, 2008).

To test whether lynx spiders would have a negative impact on the damage inflicted by endophytic herbivores, we tagged another 13 plants with similar height and phenology. At the beginning of the flowering (February 2017), we carefully bagged two branches of each plant with a polyester net ($n = 26$ branches) to allow free access for the most abundant endophytic seed feeder, the seed beetle *Acanthoscelides winderi* Kingsolver, 1984 (Chrysomelidae: Bruchinae; Fig. 1g), and to prevent damage by exophytic herbivores such as lepidopterans (see Sousa-Lopes *et al.*, 2016). We used a polyester net with small holes (3 x 3 mm) so that seed beetles could come in and lynx spiders could not come out. In each plant, we randomly chose one of the two bagged branches and put one spider inside the net ($n = 13$), while the other branch had no spider ($n = 13$). We inspected the branches every 3 days to check for spider survival, and after 60 days of trials (20 visits) we removed the nets, collected fruits, and took them to the laboratory in plastic containers (500 ml), where we counted the number of damaged and

healthy seeds. Finally, we calculated the proportion of seeds damaged relative to the total number of seeds produced per plant per treatment (sensu Romero *et al.*, 2008).

Assessing the abundance of insects, carrion, lynx spiders and plant size

In the field, we performed visual inspections lasting 20 minutes in another 53 randomly selected *M. setosa* var. *paludosa* plants, in which we counted the abundance of carrion, live insects and lynx spiders by examining all plant structures. Then, we measured the maximum height of branches with leaves, the maximum canopy diameter, and the perpendicular length to this diameter in each of the 53 plants. Measures were transformed in cubic meters to estimate a plant's real size (sense Jacobucci *et al.*, 2009).

Spider–herbivore interactions

In order to record interactions between *P. flava* and the herbivores of *M. setosa* var. *paludosa* we conducted 30 hours of *ad libitum* *sense* observation (Altmann, 1974) in sessions lasting 20 minutes in each plant ($n = 90$ plants) in field. In addition, to evaluate the effectiveness of lynx spiders as predators of the main endophytic seed feeder, *A. winderi*, we staged 10 encounters between a lynx spider and an endophytic beetle (totaling 10 spiders and 10 seed beetles). We performed encounters under laboratory conditions (12-h light and 20-30 °C), and we used transparent plastic containers (500 ml) to facilitate the observations. For acclimation, all lynx spiders were maintained in the containers omit 24 hours before the trials. The observation period started after the first physical encounter, when spiders touched seed beetles, and lasted 10 minutes. We recorded the duration of encounters in seconds.

Statistical analyses

We used a hurdle model approach with a zero-truncated Poisson regression to assess whether the presence and abundance of *P. flava* is increased by plant size, carrion abundance, and live insect's abundance. In short, the hurdle model approach separates

zero and non-zero data and allows modeling of both presence/absence and abundance in an integrated framework (Potts & Elith, 2006). The predictors were log-transformed to improve model fit. We used the paired *t*-test to compare the proportion of damaged foliolules and the proportion of predated seeds between spiders-present and spiders-absent treatments. Statistical analyses were performed in R Studio and package lme4 (R Development Core Team, 2016). We used Graph Pad Prism 5 to generate the graphics.

Results

The lynx spiders provided protection for plants against exophytic, but not against endophytic herbivory. On the one hand, the proportion of foliolules damaged differed between the spiders-present (0.10 ± 0.08) and spider-absent treatments (0.43 ± 0.10) ($t = 7.96$, $df = 18$, $P < 0.001$) and the absence of spiders led to a 3.3-fold increase in the number of damaged foliolules (Fig. 2). On the other hand, there was no significant statistical difference between the proportion of seeds damaged in the spiders-present (0.16 ± 0.10) and spiders-absent treatments (0.19 ± 0.16) ($t = 0.98$, $df = 12$, $P = 0.34$; Fig. 3).

During our field observations, we recorded 23 *P. flava* spiders on *M. setosa* var. *paludosa* plants, in individuals larger than 0.0840 m^3 (plant size varied from 0.0019 to 4.1231 m^3 , $X = 0.4911$, $SD = 0.7942$). Overall, we found only one spider per plant, except in four cases when we found two ($n = 3$) and four ($n = 1$) spiders on plants with 0.5152 , 0.8114 , 1.1609 , and 3.8250 m^3 , respectively. The hurdle regression model showed that the presence of *P. flava* increases with increasing carrion abundance ($P < 0.050$; Table 1), and spiders showed a trend (positive, marginally significant effect) in choosing taller plants ($P = 0.057$, in the Zero hurdle model). The results also indicated a trend (positive, marginally significant effect) in increasing the abundance of lynx spiders in taller plants ($P = 0.059$; Table 1).

In our field observations, we could also notice that *P. flava* lay egg sacs on the fruit surface (from September to October) and stay near them offering parental care (Fig. 1e) until the plant produces new leaves, and then juvenile spiders disperse towards the leaves (October; Fig. 1f). We also observed *P. flava* feeding two times on the carrion of an unidentified species of isopteran and preying on the herbivore species *Oospila pallidaria* (Schaus, 1897) (Lepidoptera: Geometridae) ($n = 1$) and *Naupactus* sp. (Coleoptera: Curculionidae) ($n = 1$; Fig. 1h), and two unidentified species of hymenopterans ($n = 1$ for each species; Fig. 1i).

In our laboratory observations, we found that lynx spiders spent on average 28s (± 14) to encounter and attack *A. winderi*, and interactions lasted 115s (± 22 ; $n = 10$). Spiders could not prey on any of *A. winderi* individuals since these beetles protected their head and legs under the pronotum and elytra and stayed immobile until spiders stopped the attack.

Discussion

Our results corroborate the hypotheses: (a) the damage inflicted by exophytic herbivores was lower in lynx spider presence since there was a decrease in foliolules damaged on plants with spiders; and (b) the presence of lynx spiders on plants is positively related to the food availability (i.e., carrion). We also showed a trend in choosing and increasing the abundance of spiders in taller plants, possibly because large plants provide more suitable sites for spiders to find carrion and preys, and also sheltering and breeding.

Other studies focused on lynx spiders showed that their presence decreased the leaf herbivory on plants, but the influence of these spiders was guild-dependent (Romero *et al.*, 2008; Morais-Filho & Romero 2010). The spiders decreased the abundance and damage inflicted by several exophytic herbivores, but had no effect on

some herbivores, particularly those that are endophytic. For example, Romero *et al.* (2008) showed that lynx spiders decreased by 16-fold the proportion of damage inflicted by exophytic Geometridae larvae (Lepidoptera). However, spiders did not affect the endophytic dipterans *Melanogromyza* since their larvae feed inside plant tissues and the adults have little movements on plants, making them less vulnerable to spider attack. Similarly, our study showed that the endophytic seed beetles *A. winderi* are less vulnerable to *P. flava* attack as these beetles feed inside plant tissue (seeds) during immature stages (B. Sousa-Lopes, pers. obs.) and adults avoid spider attack by the physical protection provided by their elytra (see also Linz *et al.*, 2016). Therefore, we suggest that *P. flava* is an important plant guard for *M. setosa* var. *paludosa*, but the outcomes of this interaction may be dependent on the taxon and herbivore guilds. This is true since the defensive behavior in some taxonomic groups (e.g. seed beetles) and the habit of feeding inside plant tissues (i.e. endophytic guild; immature stages of seed beetles) can make these herbivores less vulnerable to spider attack.

We found that the presence of *P. flava* is positively related to the carrion abundance as well as showed by other studies focused on several predators on glandular trichome-bearing plants (LoPresti *et al.*, 2015, 2018). Since the scavenging behavior has been recorded for lynx spiders (Romero *et al.*, 2008; and herein), it is possible they take into account the carrion abundance before choosing their host plants. Higher carrion abundance would be advantageous for spiders because they represent an accessible food source free of costs associated with attacking and manipulating live preys. Thus, the presence of *P. flava* may be not only related to the abundance of live arthropods (Morais-Filho & Romero 2008) but also carrion, which reinforce that these spiders choose sites of high food availability, independently if the food sources are alive or dead.

Morais-Filho and Romero (2008) and Jacobucci *et al.* (2009) showed that lynx spiders might select their host plants based on size. For instance, the abundance of *P. flava* was positively related to the size of *Solanum thomasiifolium* Sendtner (Solanaceae), and the authors suggested that larger plants are more suitable for spiders forage (Jacobucci *et al.* 2009), and possibly for sheltering and breeding (herein). Indeed, large plants are more conspicuous and attractive for arthropods due to the wide range of microhabitats and resources (Lawton, 1983; Schlinkert *et al.*, 2015), including preys for predators such as lynx spiders and also sites for these spiders hid of natural enemies (Foelix, 1996). Although we found a marginally significant effect in choosing and increasing the abundance of *P. flava* on taller plants, we suggest that these spiders can take into account the plant size prior to select their host plant.

The results of this study suggest that it is more common to find lynx spiders interacting with *M. setosa* var. *paludosa* in larger plants with higher carrion abundance (food source). *Peucetia flava* and *M. setosa* var. *paludosa* interact into a facultative mutualism, in which plants provide entrapped carrion for spiders to feed on and possibly facilitate prey manipulation. In return, lynx spiders decrease the damage inflicted by exophytic, but not by endophytic herbivores. Our findings contribute to a better understanding of which ecological factors may affect plant selection by lynx spiders and what is the influence of this predator on the structure of the food webs in glandular plants.

References

- Altmann, J. (1974). Observational study of behavior: sampling methods. *Behaviour* **49**, 227–266.
- Alves-Silva, E., Bächtold, A., Barônio, G.J., Torezan-Silingardi, H.M., & Del-Claro, K. (2014). Ant-herbivore interactions in an extrafloral nectaried plant: are ants good plant guards against curculionids beetles? *J. Nat. Hist.* **49**, 841–851.
- Del-Claro, K., Stefani, V., Lange, D., Vilela, A.A., Nahas, L., Velasques, M. & Torezan-Silingardi, H.M. (2013). The importance of natural history studies for a better comprehension of animal-plant interactions networks. *Biosci J.* **29**, 439–448.

- Del-Claro, K., Rico-Gray, V., Torezan-Silingardi, H.M., Alves-Silva, E., Fagundes, R., Lange, D., Dáttilo, W., Vilela, A.A., Aguirre, A. & Rodriguez-Morales, D. (2016). Loss and gains in ant–plant interactions mediated by extrafloral nectar: Fidelity, cheats, and lies. *Insectes Soc.* **63**, 207–221.
- Del-Claro, K., Stefani, V., Nahas, L. & Torezan-Silingardi, H.M. (2017). Spiders as plant partners: complementing ant services to plants with extrafloral nectaries. In *Behaviour and Ecology of spiders: contribution from the Neotropical region*: 215–226. Viera, C. & Gonzaga, M.O. (Ed.). Gewerbestrasse: Springer.
- Dutra, V.F. & Garcia, F.C.P. (2014) *Mimosa* L.(Leguminosae-Mimosoideae) dos campos rupestres de Minas Gerais, Brasil. *Iheringia* **69**, 49–88.
- Foelix, R.F. (1996). *Biology of Spiders*. Second Edition. Oxford, UK: Oxford University Press, 330 pp.
- Halaj, J., Ross, D.W. & Moldenke, A.R. (2000). Importance of habitat structure to arthropod food-webs in Douglas-fir canopies. *Oikos* **90**, 139–152.
- Heil, M. 2008. Indirect defence via tritrophic interactions. *New Phytologist* **178**:41–61.
- Jacobucci, G.B., Medeiros, L., Vasconcellos-Neto, J. & Romero, G.Q. (2009). Habitat selection and potential antiherbivore effects of *Peucetia flava* (Oxyopidae) on *Solanum thomasiifolium* (Solanaceae). *J. Arachnol.* **37**, 365–367.
- Janzen, D. 1967. Interaction of the bull’s-horn acacia (*Acacia cornigera* L.) with an ant inhabitant (*Psuedomyrmex ferruginea* F. Smith) in eastern Mexico. *Univ. Kans. Sci. bull.* **47**, 315–558.
- Krimmel, B. A., and I. S. Pearse. (2014). Generalist and sticky plant specialist predators suppress herbivores on a sticky plant. *Arthropod Plant Interact.* **8**, 403–410.
- Langellotto, G.A. & Denno, R.F. (2004). Responses of invertebrate natural enemies to complex-structured habitats: a meta-analytical synthesis. *Oecologia* **139**, 1–10.
- Lawton, J.H. (1983). Plant Architecture and the Diversity of Phytophagous Insects. *Annu. Rev. Entomol.* **28**, 23–39.
- Linz, D.M., Hu, A.W., Sitvarin, M.I., & Tomoyasu, Y. (2016) Functional value of elytra under various stresses in the red flour beetle, *Tribolium castaneum*. *Scientific Reports* **6**, 34813.
- LoPresti, E.F., Pearse, I.S. & Charles, G.K. (2015). The siren of a sticky plant: columbines provision mutualist arthropods by attracting and killing passerby insects. *Ecology* **96**, 2862–2869.
- LoPresti, E.F., Krimel, B. & Pearse, I.S. (2018). Entrapped carrion increases indirect plant residence and intra-guild predation on a sticky tarweed. *Oikos* **127**, 1033–1044.
- Morais-Filho, J.C. & Romero, G.Q. (2008). Microhabitat use by *Peucetia flava* (Oxyopidae) on the glandular plant *Rhynchanthera dichotoma* (Melastomataceae). *J. Arachnol.* **36**, 374–378.
- Morais-Filho, J.C. & Romero, G.Q. (2009). Natural history of *Peucetia flava* (Araneae: Oxyopidae): seasonal density fluctuation, phenology and sex ratio on the glandular plant *Rhynchanthera dichotoma* (Melastomataceae). *J. Nat. Hist.* **43**, 701–711.
- Morais-Filho, J.C. & Romero, G.Q. (2010). Plant glandular trichomes mediate protective mutualism in a spider–plant system. *Ecol. Entomol.* **35**, 485–494.
- Nahas, L., Gonzaga, M.O. & Del-Claro, K. (2012). Emergent impacts of ant and spider interactions: herbivory reduction in a tropical savanna tree. *Biotropica* **44**, 498–505.
- Nahas, L., Gonzaga, M.O. & Del-Claro, K. (2017). Wandering and web spiders feeding on the nectar from extrafloral nectaries in neotropical savanna. *J. Zool.* **301**, 125–132.

- Potts, J. & Elith, J. (2006). Comparing species abundance models. *Ecol. Model.* **199**, 153–163.
- Price, P.W., Denno, R.F., Eubanks, M.D., Finke, D.I. & Kaplan, I. (2011). *Insect Ecology, Behavior, Populations and Communities*. New York: Cambridge University Press.
- R Development Core Team. (2016). *R: A language and environment for statistical computing*. <http://www.R-project.org>. Accessed 02 March 2016.
- Romero, G.Q. & Vasconcellos-Neto, J. (2005) The effects of plant structure on the spatial and microspatial distribution of a bromeliad-living jumping spider (Salticidae). *J Anim Ecol* **74**, 12–21.
- Romero, G.Q., Souza, J.C. & Vasconcellos-Neto, J. (2008). Anti-herbivore protection by mutualistic spiders and the role of plant glandular trichomes. *Ecology* **89**, 3105–3115.
- Romero, G.Q. & Vasconcellos-Neto, J. (2012). Interações entre aranhas e plantas: associações específicas e mutualismos. In *Ecologia das Interações Plantas-Animais: uma abordagem ecológico-avaliativa*: pp. 241–256. Del-Claro, K. & Torezan-Silingardi, H.M. (Ed.). Rio de Janeiro: Technical Books.
- Schlinkert, H., Westphal, C., Clough, Y., László, Z., Ludwig, M. & Tscharrntke, T. (2015). Plant Size as Determinant of Species Richness of Herbivores, Natural Enemies and Pollinators across 21 Brassicaceae Species. *Plos One*, <https://doi.org/10.1371/journal.pone.0135928>
- Sousa-Lopes, B., Bächtold, A. & Del-Claro, K. (2016). Biology, natural history and temporal fluctuation of the geometrid *Oospila pallidaria* associated with host plant phenology. *Stud. Neotrop. Fauna Environ.* **51**, 135–143.
- Stefani, V., Pires, T.L., Torezan-Silingardi, H.M. & Del-Claro, K. (2015). Beneficial effects of ants and spiders on the reproductive value of *Eriotheca gracilipes* (Malvaceae) in a tropical savanna. *Plos One*, <https://doi.org/10.1371/journal.pone.0131843>.
- Vasconcellos-Neto, J., Romero, G.Q., Santos, A.J. & Dippenaar-Schoeman, A.S. (2007). Associations of spiders of the genus *Peucezia* (Oxyopidae) with plants bearing glandular hairs. *Biotropica* **39**, 221–226
- Vasconcellos-Neto, J., Messas, Y.F., Souza, H.S., Villanueva-Bonilla, G.A. & Romero, G.Q. (2017). Spider–plant interactions: An Ecological Approach. In *Behaviour and Ecology of spiders: contribution from the Neotropical region*. pp.165–214. Viera, C. & Gonzaga, M.O. (Ed.). Gewerbestrasse: Springer.
- Whitney, K.D. (2004). Experimental evidence that both parties benefit in a facultative plant-spider mutualism. *Ecology* **6**, 1642–1650.

Table caption

Table 1 Hurdle regression model between the presence and abundance of *P. flava* and plant size, carrion abundance and abundance of live insects on *Mimosa setosa* var. *paludosa*. The zero hurdle model (above) accounts for the effect of the predictors (first column) on the presence of *P. flava*. The count model (below) accounts for the effect of the predictors (first column) on the abundance of *P. flava*. In bold, significant result. Marginally significant results are underlined.

Zero hurdle model coefficients (binomial with logit links)				
	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	-6.9540	4.6349	-1.500	0.1335
plant_size	1.9112	1.0040	1.904	<u>0.0570</u>
Carrion	1.5714	0.7182	2.188	0.0287
insect_count	0.9837	0.5569	1.766	0.0773

Count model coefficients (truncated poisson with log link)				
	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	0.7397	6.2375	0.119	0.9060
plant_size	2.3373	1.2379	1.888	<u>0.0590</u>
Carrion	-0.4889	0.5904	-0.828	0.4080
insect_count	0.3111	0.6781	0.459	0.6460

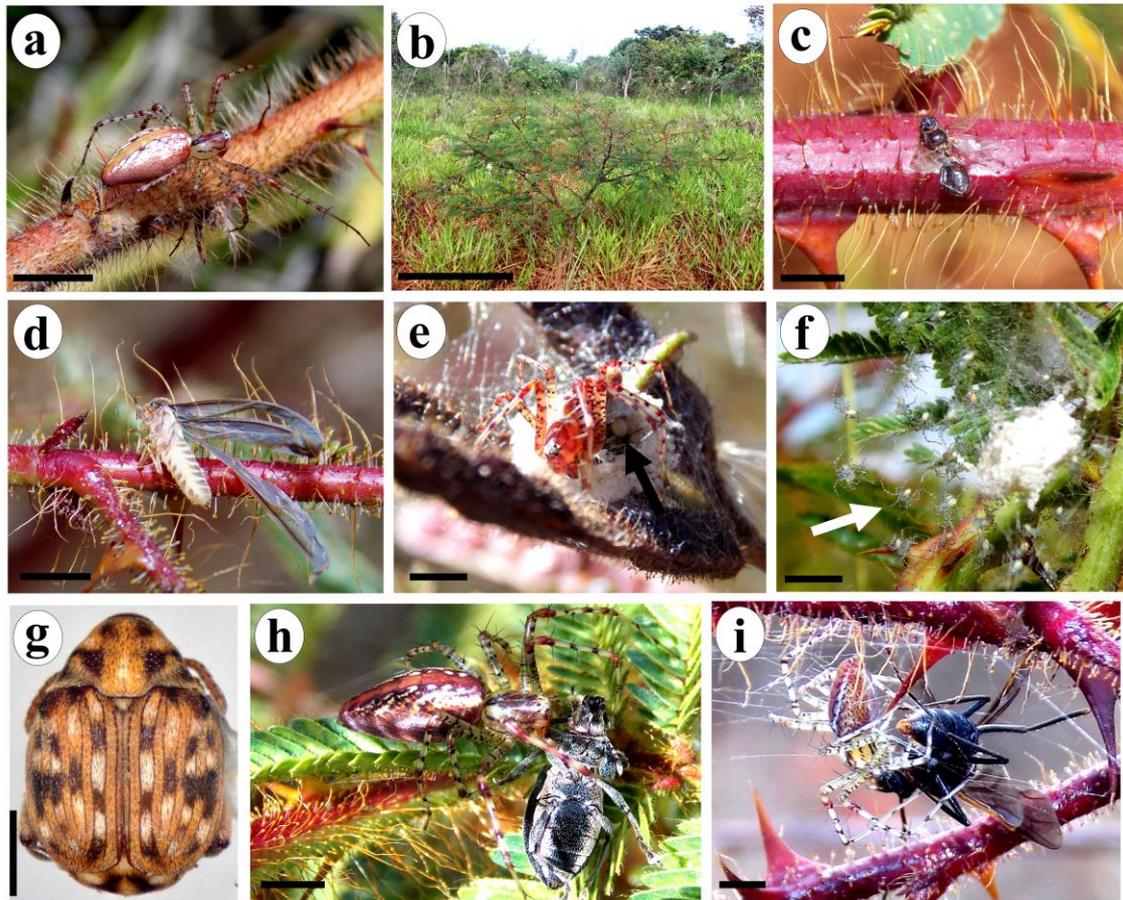


Figure 1 Lynx spider–herbivore interactions on *Mimosa setosa* var. *paludosa* in the Brazilian Cerrado. (a) The lynx spider, *Peucetia flava* Keyserling 1877; (b) *Mimosa setosa* var. *paludosa*; (c) glandular trichomes with a hymenopteran entrapped; (d) glandular trichomes with an isopteran entrapped; (e) *P. flava* with egg sac (black arrow) on fruit surface, offering parental care; (f) juveniles of *P. flava* dispersing (white arrow); (g) the endophytic seed beetle, *Acanthoscelides winderi* Kingsolver, 1984; (h) *P. flava* preying on the exophytic herbivore, *Naupactus* sp; (i) *P. flava* preying on an unidentified hymenopteran entrapped on the host plant. Scale bar: 1 mm, except in (b) with 1 m.

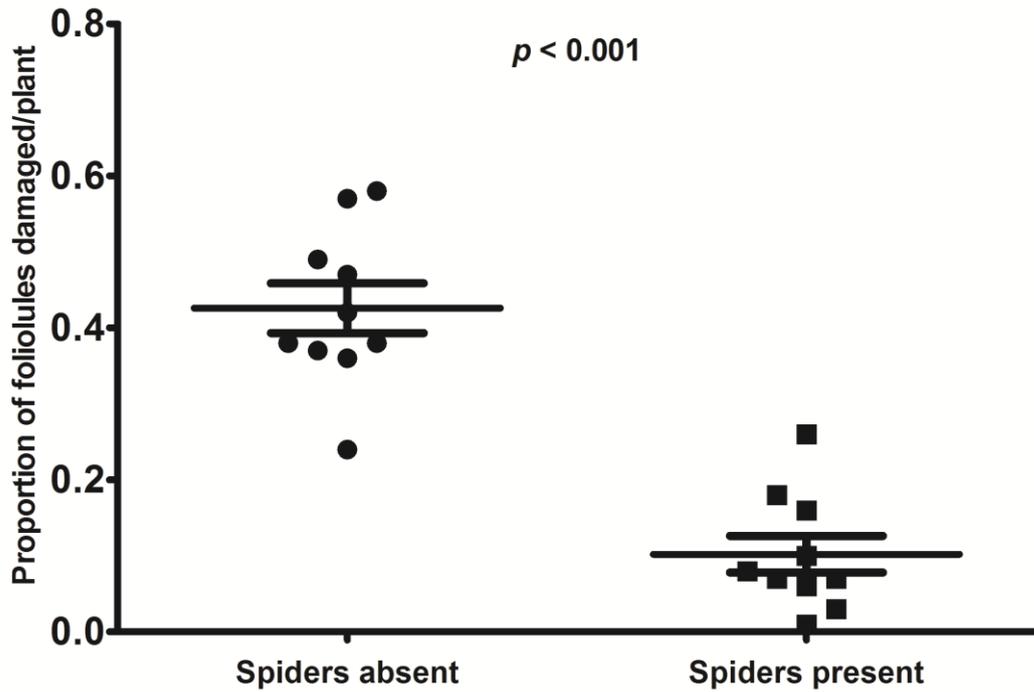


Figure 2 Mean proportion (\pm SE) of foliolules damaged in spiders-absent and spiders-present treatments on *Mimosa setosa* var. *paludosa*. Note a significant statistical difference at unpaired *t*-test ($P < 0.0001$, $n = 10$ plants). The total number of foliolules damaged on spiders absent and spiders present were 1012 (30%) and 305 (10%), respectively.

Capítulo 5

***Cotesia itororensis* sp. nov. from Brazilian savanna: a new
reared microgastrine wasp (Hymenoptera: Braconidae)
described using an integrative taxonomic approach**



Este capítulo foi publicado na revista Zootaxa, 4544(3): 437–445.

***Cotesia itororensis* sp. nov. from Brazilian savanna: a new reared microgastrine wasp (Hymenoptera: Braconidae) described using an integrative taxonomic approach**

BRUNO DE SOUSA-LOPES; JAMES BRYAN WHITFIELD; GERALDO
SALGADO-NETO & KLEBER DEL-CLARO

Abstract

A new species of microgastrine wasp, *Cotesia itororensis* Sousa-Lopes & Whitfield, **sp. nov.**, is described from a Brazilian savanna area in Uberlândia, Minas Gerais. This species is a koinobiont endoparasitoid recorded from caterpillars of *Oospila pallidaria* (Schaus, 1987) (Lepidoptera: Geometridae), feeding on *Mimosa setosa* var. *paludosa* (Fabaceae: Mimosoideae). Morphological, molecular, biological, ecological and geographical data are used to describe the new species and distinguish it from others formally recorded for the Neotropical region.

Keywords: Fabaceae, Geometridae, *Oospila pallidaria*, taxonomy.

Introduction

Microgastrine braconid wasps are among the most abundant endoparasitoids of lepidopterans and tend to be highly host-specific; consequently, these wasps are employed frequently as biological control agents against pest insects (Smith *et al.* 2008; Whitfield *et al.* 2009). There are ~2,700 described species worldwide (Yu *et al.* 2016), but still, many thousands of additional, undescribed ones are known to exist (Rodriguez *et al.* 2012).

Cotesia Cameron, 1891 (Braconidae: Microgastrinae) is the second largest genus of microgastrine wasps in terms of described species, encompassing roughly 300–400

described species (Shaw & Huddleston 1991; Yu *et al.* 2016). However, this number will increase in next years, since it has been estimated that nearly 1000 species of *Cotesia* exist worldwide (Mason 1981; Michel-Salzat & Whitfield 2004). This increase must be higher especially in the Neotropical region, where a relatively small number of studies recording species of *Cotesia* and their biology are available (Whitfield 1997), particularly in South America.

Cotesia is recognizable among the microgastrines by the following features: (1) forewing with second r-m vein absent, so that the small areolet is open distally; (2) propodeum coarsely sculptured, with medial longitudinal carina rather than medial areola; (3) first and second metasomal tergites usually rather quadrate in form and coarsely sculptured; and (4) ovipositor and sheaths short and barely exerted (Whitfield *et al.* 2009). These wasps have a koinobiont habit (Kankare & Shaw 2004), being recorded from caterpillars of HesperIIDae (Smith *et al.* 2008; Yu *et al.* 2016), Noctuidae (Avalos *et al.* 2016; Yu *et al.* 2016), Pieridae (Pizzato *et al.* 2016; Yu *et al.* 2016), Saturniidae, Sphingidae (Smith *et al.* 2008; Yu *et al.* 2016), and most rarely from Geometridae (Haines *et al.* 2009, Ruohomäki *et al.* 2013; Yu *et al.* 2016). As *Cotesia* species have appeared to be highly host specialized (Kankare & Shaw 2004), with cryptic species and allopatric distribution (Fiaboe *et al.* 2017), the use of an integrative taxonomic approach (combining morphological, molecular, biological and geographical data) is paramount to recognize and distinguish these parasitoid wasps (Smith *et al.*, 2008; Kaiser *et al.* 2017).

Using an integrative taxonomic approach, this paper provides a description of a new species of *Cotesia*, whose brood was produced from *Oospila pallidaria* (Schaus, 1897) (Lepidoptera: Geometridae), feeding on *Mimosa setosa* var. *paludosa* (Fabaceae:

Mimosoideae) in the Brazilian savanna (Sousa-Lopes *et al.* 2016). Additionally, we compared it with other twenty-one formally recorded species for the Neotropical region.

Materials and Methods

From January 2014 to May 2018, during visual inspections on individuals of *M. setosa* var. *paludosa* (Fig. 1a), BSL collected 210 early instars (1st and 2nd) larvae of *O. pallidaria* (Fig. 1b) in the Ecological Reserve of the Clube Caça e Pesca Itororó de Uberlândia (CCPIU), Minas Gerais, Brazil (18°59'S, 48°17'W; altitude 863 m; 640 ha).

Larvae were reared individually in plastic containers in the Behavioral Ecology and Interactions Laboratory at the Federal University of Uberlândia (12-h light and 20–30°C), until adult stage or emergence of the braconid parasitoid described below. The original host, cocoons and adult parasitoids were saved, being the first and last ones placed in 96% ethyl alcohol.

Illustrations were made using a digital camera photography setup attached to stereoscopic microscopes. Morphological terms and measurements of structures are mostly those used by Fernandez-Triana *et al.* (2014).

To check the molecular-specific characterization of the new species, the mitochondrial gene Cytochrome Oxidase I (COI) was analyzed. For the amplification of a fragment of approximately 460 bp of this gene, we used the following primer pair: COI-F (5'-GATTTTTTGGKCA YCCMGAAG-3') and COI-R (5'-CRAATACRGCTCCTATWGATAAWAC-3') (Gusmão *et al.* 2010). DNA extraction of one specimen was performed with the GenElute Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich®) and followed the manufacturer's protocol. The product was amplified via Polymerase Chain Reaction (PCR) according to the following schedule: 94°C for 2 minutes, 40 cycles of 94°C for 30 seconds, 54°C for 30 seconds,

72°C for 40 seconds and 72°C for 4 minutes. Then the PCR product was purified using polyethylene glycol precipitation (PEG; Schmitz & Riesner 2006). These samples were sequenced using the Big Dye 3.1 reagent (Life Technologies®) and 3500 xL automatic sequencer (Life Technologies®).

Results

***Cotesia itororensis* Sousa-Lopes & Whitfield, sp. nov.**

Holotype. Female, Brazil: Minas Gerais, Uberlândia, Clube Caça e Pesca Itororó de Uberlândia (18°59'00"S, 48°17'44"W – 863 m. elev.). Deposited in the Hymenoptera collection of the Museum of Zoology of the University of São Paulo (MZUSP; Carlos Brandão curator), MZSP57562, February 2018, coll. B. Sousa-Lopes, ex larva *Oospila pallidaria* on *M. setosa* var. *paludosa*.

Paratypes. 2 males, deposited in MZUSP, MZSP57563 and MZSP57564. Same dates as holotype.

Diagnosis. Antennae approximately as long as the body (head to apex of metasoma); coxae black, legs mostly honey-yellow, metasoma mostly black except pale in laterotergites; mesoscutum with distinct dense punctures, becoming smooth posteriorly anterior to scutoscutellar sulcus; wings partially pigmented with a few veins dark, but most pale; pterostigma dark greyish brown, with indistinct paler junction with C+SC; propodeum with more or less complete, but anteriorly weak medial longitudinal carina; the first tergite of metasoma rounded towards posterior margin; second tergite of metasoma almost twice as wide posteriorly as anteriorly; tergites of metasoma mostly smooth, weakly sculptured; ovipositor shorter, about one-third the length of the metasoma or hind tibia; solitary habit recorded so far from *O. pallidaria*; cocoon yellow. The above combination of characters is sufficient to separate *C. itororensis*

from all other recorded species of *Cotesia* for the Neotropical region. A detailed diagnosis one-to-one is provided below to distinguish *C. itororensis* from every other species.

Description. Female (Fig. 2). Body color: body mostly black except pale, almost whitish palpi, most of all legs distal to coxae, and laterotergites of metasoma. Antenna color: scape, pedicel and flagellum dark brown/black. Coxae color (pro-, meso-, metacoxa): black. Femora color (pro-, meso-, metafemora): honey-yellow. Tibiae color (pro-, meso-, metatibiae): honey-yellow, with slight darkening dorsally at distal end of metatabia. Tegulae color: dark brown translucent. Pterostigma color: dark greyish brown, with indistinct paler junction with C+SC. Fore wings color: partially pigmented (a few veins may be dark but most pale). Antenna length/body length: antenna approximately as long as body (head to apex of metasoma). Body in lateral view: not distinctly flattened dorso-ventrally. Body length (head to apex of metasoma): 2.0 - 2.2 mm. Fore wing length: 2.1-2.3 mm. Ocular-ocellar line/posterior ocellus diameter 1.7-1.9. Interocellar distance/posterior ocellus diameter: 2.1-2.3. Antennal flagellomere 2 length-width: 2.9-3.1. Antennal flagellomere 14 length/width 1.4-1.6. Length of flagellomere 2/length of flagellomere 14: 2.2-2.3. Tarsal claws: simple, within single basal spine-like seta. Metafemur length/width 3.2-3.3. Metatibia inner spur length/metabasitarsus length: roughly 0.5. Anteromesoscutum: anteriorly with distinct dense punctures, becoming smooth posteriorly anterior to scutoscutellar sulcus. Mesoscutellar disc: sparsely and finely but distinctly punctured; microsculpture producing satiny reflections. Number of pits in scutoscutellar sulcus: 8-10. Propodeum carina: with more or less complete but anteriorly weak medial longitudinal carina. Propodeum background sculpture: mostly very finely rugulose, almost smooth in spots. Mediotergite 1 length/width at widest point: 1.1-1.3. Mediotergite 1 shape: slightly

widening from anterior margin to 0.7 of mediotergite length (widest point), then rounding towards posterior margin. Mediotergite 1 sculpture: mostly smooth and shining with very vague sculpturing ventrally, posterolateral portions with widely scattered punctures. Mediotergite 2 width at posterior margin/length: 2.1-2.3, almost twice as wide posteriorly as anteriorly. Mediotergite 2 sculpture: mostly smooth and raised centrally, very weakly sculptured otherwise. Hypopygium: evenly sclerotized but folded medially, posteriorly forming a strongly obtuse angle in lateral view. Ovipositor thickness: tapering gradually to tip. Ovipositor sheaths: short, exposed portions less than 1/3 of hind tibia length. Length of fore wing veins 2RS/2M: 1.1-1.3. Length of fore wing veins 2M/(RS+M)b: 0.9-1.0. Pterostigma length/width 2.0-2.2. Point of insertion of vein r in pterostigma: just beyond half way point of pterostigma length. Angle of vein r with fore wing anterior margin: perpendicular. Shape of junction of veins r and 2RS in forewing: r weakly arched, junction distinctly but not strongly angled.

Male (Fig. 2). As female, but with darker distal patch on distal portions of hind tibiae and hind tarsi.

Molecular data. COI barcode deposited in GenBank (MH382197).

Host: *Oospila pallidaria* (Schaus 1897) (Lepidoptera: Geometridae).

Biology/ecology. *Cotesia itororensis* is a solitary parasitoid wasp that occurs mainly in the wet season (December–March); however, their host, *O. pallidaria*, occurs throughout the year, mainly in the dry season (May–September). It is possible that caterpillars avoid parasitism occurring at highest abundance in a temporal enemy-free space (Sousa-Lopes *et al.* 2016). Indeed, the rate of parasitism was low, representing 10.5% (22) of the caterpillars sampled during four years. Parasitism occurs between the first and second larval instar of *O. pallidaria*. Parasitized caterpillars have swollen

bodies in the posterior half (Fig. 3a), mainly in the fourth instar, when parasitoid larvae are completely developed. At this time, *O. pallidaria* moves to the abaxial side of leaves (Fig. 3b) and then *C. itororensis* leaves them near the fifth abdominal segment (Fig. 3c), builds a yellow cocoon and pupates for three days. *Cotesia itororensis* probably changes host behavior because moving to the abaxial side of leaves is an uncommon behavior for healthy caterpillars (BSL, pers. obs.). It is possible this behavior decreases vulnerability to desiccation and/or enemies in open areas where host plants and caterpillars are found by wasps.

Distribution. Known so far from Uberlândia, Minas Gerais, Brazil.

Etymology. The specific epithet (*itororensis*) is a reference, from the Tupi-Guarani *itororó* = *yy* (water) and *tororõ* (spout), water spout, also meaning noisy river and small waterfall, and *ensis* = origin, in reference to the Clube Caça e Pesca Itororó de Uberlândia, Minas Gerais, where this braconid species was found.

Detailed diagnosis. In order to facilitate future work on the group, we detail below how each of the other twenty-one species of *Cotesia* previously recorded from the Neotropical region individually differs from *C. itororensis*.

Cotesia alius (Muesebeck 1958) has the metasoma bright yellow on sides and underneath on basal half, and its metasoma is also rather stout, almost as broad as mesosoma (Muesebeck 1958); whereas *C. itororensis* has metasoma mostly black except pale in metasomal laterotergites, and narrow in comparison to mesosoma. Also, *C. alius* was recorded from a different family of Lepidoptera (Nymphalidae) in Brazil, Peru and Venezuela (Yu *et al.* 2016).

Cotesia americana (Lepeletier 1825) has antennae pale testaceous, tinged with fuscous above, the base beneath yellowish, and metasoma entirely orange-yellow (Cresson

1865); whereas *C. itororensis* has antennae dark brown/black, and metasoma black except for pale in laterotergites, mostly in segments 1 and 2. *C. americana* is recorded to parasitize different families of Lepidoptera (Pyralidae and Sphingidae), and occurs in Cuba, Dominican Republic, Guyana, Haiti, Martinique, Mexico and Puerto Rico (Yu *et al.* 2016).

Cotesia ayerza (Brethes 1920) was recorded from different species within the family Pieridae and it is known so far only from Argentina (Yu *et al.* 2016), which distinguishes it from *C. itororensis*.

Cotesia bonariensis (Brèthes 1916) has the mediotergite 2 a transverse rectangle shape, rough, slightly elevated in its middle longitudinally (Brèthes 1916); whereas *C. itororensis* has the same segment in a more triangular shape, smooth and raised centrally, very weakly sculptured otherwise. *C. bonariensis* was recorded so far from an unidentified lepidopteran in Argentina (Brèthes 1916, Yu *et al.* 2016).

Cotesia congregata (Say 1836) has a gregarious habit and cocoon whitish (Gilmore 1938); whereas *C. itororensis* is recorded so far with solitary habit and cocoon yellowish. *C. congregata* was recorded from different families of Lepidoptera (Lasiocampidae, Noctuidae, Pyralidae and Sphingidae) in Brazil, Honduras, Jamaica, Nicaragua, Peru and Puerto Rico (Yu *et al.* 2016).

Cotesia electrae (Viereck 1912) has hind femora blackish-brown, pterostigma black, and metasoma compressed and black (Viereck 1912); whereas *C. itororensis* has the hind femora yellow-honey, pterostigma dark greyish brown, with indistinct paler junction with C+SC, and metasoma compressed, but blackish with laterotergites pale. *C. electrae* was recorded so far from Saturniidae in Mexico (Yu *et al.* 2016).

Cotesia empretiae (Viereck 1913) has legs and tegulae mostly stramineous, including the fore and mid coxae rather reddish, and first and second metasomal segments

rugulose (Viereck 1913); whereas *C. itororensis* has legs mostly yellow-honey, tegulae dark brown translucent, and first and second metasomal segments mostly smooth. *C. empretiae* was recorded so far from a different family of Lepidoptera (Limacodidae) in Ecuador (Yu *et al.* 2016).

Cotesia flavipes Cameron 1981, as well as other species from the *flavipes* species group, has short antennae (much shorter than body length, usually not surpassing the length of head and mesosoma) (Fiaboe *et al.* 2017; Kaiser *et al.* 2017); whereas the antennae of *C. itororensis* is approximately as long as the body (head to apex of metasoma). Species of the *flavipes* group also show a much more highly elongate and flattened body shape than *C. itororensis*. The metasoma of *C. flavipes* is much lighter in color than *C. itororensis*. Beyond, *C. flavipes* parasitizes different hosts within the families of Lepidoptera, Crambidae, Erebidae, and Noctuidae, in Barbados, Brazil, Costa Rica, Guadeloupe, Jamaica, Mexico, Peru, Trinidad & Tobago and Venezuela (Yu *et al.* 2016).

Cotesia glomerata (Linnaeus 1758) has the plate of the first abdominal segment about two and one-half times as long as wide (=rectangular shape) (Ashmead 1906), whereas *C. itororensis* has this plate with the anterior margin slightly widening and rounded towards posterior margin. *C. glomerata* was reported parasitizing several species of lepidopterans within the families, Bombycidae, Drepanidae, Geometridae, Pieridae, Noctuidae, Nymphalidae, among others, and the coleopteran, *Trogoderma glabrum* (Herbst 1783), in Barbados and Brazil (Yu *et al.* 2016) (this last record is almost certainly erroneous).

Cotesia kraussi (Muesebeck 1958) has the disc of scutellum large, convex, sculptured like mesoscutum, first metasomal tergite entirely finely rugulose, and hind femora black (Muesebeck 1958); whereas *C. itororensis* has disc of scutellum sparsely and finely,

although distinctly punctured, first metasomal tergite mostly smooth, and femora honey-yellow. *C. kraussi* was recorded so far from *Morpheis ehrenbergi* Geyer (Lepidoptera: Cossidae) in Mexico.

Cotesia marginiventris (Cresson 1865) has hind coxae pale reddish or stramineous and wings hyaline (Cresson 1865); whereas *C. itororensis* has coxae black and wings partially pigmented, with a few veins may be dark but most pale. *C. marginiventris* was reported parasitizing different species of Noctuidae in Argentina, Bermuda, Brazil, Chile, Cuba, Nicaragua, Peru, Puerto Rico, Uruguay and Venezuela (Yu *et al.* 2016).

Cotesia marquesi (Brèthes 1924) was recorded so far parasitizing *Papilio anchisiades capys* Hübner (Lepidoptera: Papilionidae) in Argentina and Brazil (Costa-Lima 1950; Yu *et al.* 2016).

Cotesia mayaguezensis (Viereck 1913) has propodeum without a distinct median, longitudinal carina (Viereck 1913); whereas *C. itororensis* has the propodeum with a more or less complete, but anteriorly weak medial longitudinal carina. *C. mayaguezensis* was recorded so far from an unidentified host on *Cissus sicyoides* L. (Vitaceae) in Puerto Rico (Yu *et al.* 2016).

Cotesia ornatricis (Muesebeck 1958) has pro-, meso- and metacoxae piceous, yellow, and black, respectively, and mesoscutum rather uniformly punctuate (Muesebeck 1958); whereas *C. itororensis* has all coxae black, mesoscutum anteriorly with distinct dense punctures, but smooth posteriorly anterior to scutoscutellar sulcus. *C. ornatricis* was recorded so far from *Utetheisa ornatricis* Linnaeus 1758 (Erebidae: Arctiinae) in Brazil and Colombia (Muesebeck 1958; Yu *et al.* 2016).

Cotesia paphi (Schrottky 1902) has, apparently, metasoma more compressed anteroposteriorly than *C. itororensis* (Hymenoptera online, 2018). *C. paphi* was

recorded from different species within the lepidopteran families Pieridae and Sphingidae, in Argentina, Brazil, Peru and Uruguay (Yu *et al.* 2016).

Cotesia parallelis (Ashmead 1900) has ovipositor about two-thirds the length of the metasoma, and first metasomal tergite a little more than twice as long as wide, with sides parallel (Ashmead 1900); whereas *C. itororensis* has ovipositor shorter, about one-third the length of the metasoma, and first metasomal tergite as long as wide, with slightly widening from anterior margin to 0.7 of mediotergite length (widest point), then rounding towards posterior margin. *C. parallelis* was recorded so far from Saint Vincent (Yu *et al.* 2016).

Cotesia prenidis (Muesebeck 1921) has the first metasomal tergite rugulose-punctate, and the second metasomal tergite broad, almost rectangular (Muesebeck 1921); whereas *C. itororensis* has first metasomal tergite mostly smooth, and the second metasomal tergite is somewhat triangular, broadening posteriorly. *C. prenidis* was recorded so far from *Nyctelius nyctelius* (Latreille 1824) and *Panoquina nero* Fabricius (Lepidoptera: Hesperiiidae) in Puerto Rico (Yu *et al.* 2016).

Cotesia ruficrus (Haliday 1834) has legs rufous-testaceous (Cameron 1911); whereas *C. itororensis* has legs mostly yellow-honey. *C. ruficrus* was recorded from Botrichidae (Coleoptera), and lepidopterans within the families, Geometridae, Hesperiiidae, Lycaenidae, Noctuidae, Nymphalidae and Pieridae, in Trinidad & Tobago (Yu *et al.* 2016).

Cotesia schini (Muesebeck 1958) has mesoscutum uniformly covered with distinct, separated punctures, and tegulae and wing bases yellow (Muesebeck 1958); whereas *C. itororensis* has mesoscutum anteriorly with distinct dense punctures, becoming smooth posteriorly, and tegulae dark brown translucent, and wings with few veins dark, but most pale. *C. schini* was recorded so far from an unidentified caterpillar of Riodinidae

feeding on the Brazilian pepper tree or Christmas berry tree, *Schinus terebinthifolius* Raddi (Anacardiaceae) (Muesebeck 1958).

Cotesia theclae (Riley 1881) female has antennae much shorter than the body (Riley 1881); whereas females of *C. itororensis* has antennae as long as the body length. *C. theclae* was recorded from different families of Lepidoptera (Lycaenidae and Noctuidae) in Mexico (Yu *et al.* 2016).

Cotesia vestalis (Haliday 1834) has femora mostly yellowish/orangish, sometimes darker at base or tip, hind coxae mostly dark brown to black, apically slightly paler and first metasomal tergite mostly rugose-punctate in basal third (Shaw 2003); whereas *C. itororensis* has femora honey-yellow, coxae black and the first metasomal tergite mostly smooth. *C. vestalis* was recorded parasitizing several species of Lepidoptera among the families, Arctiidae, Lasiocampidae, Lymantriidae, Noctuidae, Notodontidae, Nymphalidae, Pieridae, Plutellidae, Pterophoridae, Pyralidae, and Tortricidae, in Argentina and Brazil (Yu *et al.* 2016).

Discussion

We present the first description of a new species of *Cotesia* associated with Geometridae in South America. Conversely, in other parts of the world especially Europe, there are many descriptions and records of *Cotesia* associated with Geometridae (Schumacher *et al.* 2000, Özbek & Çalmaşur 2010, Ruohomäki *et al.* 2013). Further studies in the Neotropical region will unravel new species and associations between *Cotesia* and Geometridae. However, it is important to mention that there is an unconfirmed record of *C. glomerata* parasitizing Geometridae for the Neotropical region (Yu *et al.* 2016). Furthermore, Tepe *et al.* (2014) recorded an unidentified species of *Cotesia* associated with *Eois* sp. (Geometridae) on *Piper kelleyi*

Tepe in Ecuador and Peru, and Smith *et al.* (2008) recorded, but did not describe, several new species of *Cotesia* associated with Geometridae in Costa Rica.

Cotesia itororensis appears somewhat intermediate between the closely related genera *Cotesia* and *Protapanteles*, in that the propodeum is relatively smooth, and the second metasomal tergite is somewhat triangular, broadening posteriorly (as in *Protapanteles*), but the propodeum has a complete medial carina, and the first metasomal tergite is relatively broad and broader posteriorly (true of *Cotesia*). *Protapanteles* is often reared from Geometridae, although the only species so far recorded from South America is *P. eryphanidis* (Whitfield), reared from *Eryphanis greeneyi* (Penz and Devries) (Nymphalidae) in Ecuador (Greeney *et al.* 2011). *Cotesia itororensis* is morphologically close to *C. congregata*, *C. glomerata* and *C. marginiventris*, and ecologically to *C. autumnatae*, *C. geometricae*, *C. jucunda*, although all of the three last species occur in Australia or Europe (Schumacher *et al.* 2000, Ruohomäki *et al.* 2013, Yu *et al.* 2016). With respect to molecular data, *C. itororensis* is close to *C. salebrosa*, *C. autumnatae*, *C. griffini*, *C. vestallis*, *C. meliataerum*, *C. bigneli*, *C. acuminata*, *C. glomerata*, *C. marginiventris*, *C. koebelei*, *C. rubecula*, *C. flavipes*, *C. sesamiae*, *C. urubae*, *C. nonagriae* and *C. chionis*, according to BLAST.

As species of *Cotesia* are important agents of biological control against pest insects, the correct identification of them is paramount to success in pest management programs. In this perspective, we showed here a comparative diagnosis to distinguish the species of *Cotesia* occurring in the Neotropical region. We reinforce the use of an integrative taxonomic approach (combining morphological, molecular, biological and geographical data) to avoid mistakes in identifications of *Cotesia*.

References

- Ashmead, W.H. (1900) Report upon the Aculeate Hymenoptera of the island of St. Vincent and Grenada, with additions to the parasitic Hymenoptera and a list of the described Hymenoptera of the West Indies. *Transactions of the Entomological Society of London*, 207–367.
- Ashmead, W.H. (1906) Descriptions of new Hymenoptera from Japan. *Proceedings of the United States National Museum*, 30, 169–201.
- Avalos, D.S., Mangeaud, A. & Valladares, G.R. (2016) Parasitism and Food Web Structure in Defoliating Lepidoptera–Parasitoid Communities on Soybean. *Neotropical Entomology* 45(6), 712–717.
- Brèthes, J. (1916) Hyménoptères parasites de l'Amérique méridionale. *Anales Del Museo Nacional de Historia Natural de Buenos Aires*, 27, 401–430.
- Cameron, P. (1911) On a collection of parasitic Hymenoptera (chiefly bred) made by Mr. W.W. Froggatt, F.L.S., in New South Wales, with description of new genera and species. Part i. *Proceedings of the Linnean Society of New South Wales*, 36, 333–346.
- Costa-Lima, A.M. (1950) *Insetos do Brasil. 6º tomo. Lepidópteros. 2ª parte. Série didática. Número 8, Capítulo 28, Escola Nacional de Agronomia, Rio de Janeiro. 420 pp.*
- Fernandez-Triana, J.L., Whitfield, J.B., Rodriguez, J.J., Smith, M.A., Janzen, D.H., Hallwachs, W., Hajibabaei, M., Burns, J.M., Solis, M.A., Brown, J., Cardinal, S., Goulet, H. & Hebert, P.D.N. (2014) Review of *Apanteles* (Hymenoptera, Braconidae, Microgastrinae) from Area de Conservacion Guanacaste, Costa Rica, with keys to all described species from Mesoamerica. *ZooKeys*, 383, 15–65.
- Fiaboe, K.K.M., Fernandez-Triana, J.L., Nyamu, F.W. & Agbodzavu, K.M. (2017) *Cotesia icipe* sp. n., a new Microgastrinae wasp (Hymenoptera, Braconidae) of importance in the biological control of Lepidopteran pests in Africa. *Journal of Hymenoptera Research*, 61, 49–64.
- Gilmore, J.U. (1938) Notes on *Apanteles congregatus* (Say) as a parasite of tobacco hornworms. *Journal of Economic Entomology*, 31, 712–715.
- Greeney, H.F., Whitfield, J.B., Stireman, J.O., Penz, C.M. & Dyer, L.A. (2011) Natural History of *Eryphanis greeneyi* (Lepidoptera: Nymphalidae) and Its Enemies, With a Description of a New Species of Braconid Parasitoid and Notes on Its Tachinid Parasitoid. *Annals of the Entomological Society of America*, 104(6), 1078–1090.
- Gusmão, F.A., Harakava, R. & Campos, A.E.C. (2010) Fire-ants of the *Solenopsis saevissima* species-group (Hymenoptera: Formicidae) nesting in parks in the city of São Paulo: identification based on mtDNA sequences and morphological characters. *Sociobiology*, 56, 353–362.
- Haines, W.P., Heddle, M.L., Welton, P. & Rubinoff, D. (2009) A Recent Outbreak of the Hawaiian Koa Moth, *Scotorythra paludicola* (Lepidoptera: Geometridae), and a Review of Outbreaks between 1892 and 2003. *Pacific Science*, 63(3), 349–369.
- Hymenoptera Online (HOL) (2018) Available from: <https://hol.osu.edu/spmInfo.html?id=MZSP%2019502> (13 jun. 2018).
- Kaiser, L., Fernandez-Triana, J., Capdevielle-Dulac, C., Chantre, C., Bodet, M., Kaoula, F., Benoist, R., Calatayud, P.A., Dupas, S., Herniou, E.A., Jennette, R., Obonyo, J., Silvain, J.F. & Ru, B.L. (2017) Systematics and biology of *Cotesia typhae* sp. n. (Hymenoptera, Braconidae, Microgastrinae), a potential biological control agent

- against the noctuid Mediterranean corn borer, *Sesamia nonagrioides*. *Zookeys*, 682, 105–136.
- Kankare, M. & Shaw, M.R. (2004) Molecular phylogeny of *Cotesia* Cameron, 1891 (Insecta: Hymenoptera: Braconidae: Microgastrinae) parasitoids associated with Melitaeini butterflies (Insecta: Lepidoptera: Nymphalidae: Melitaeini). *Molecular Phylogenetics and Evolution*, 32, 207–220.
- Mason, W.R.M. (1981) The polyphyletic nature of *Apanteles* Foerster (Hymenoptera: Braconidae): a phylogeny and reclassification of Microgastrinae. *Memoirs of the Entomological Society of Canada*, 115, 1–147.
- Michel-Salzat, A. & Whitfield, J.B. (2004) Preliminary evolutionary relationships within the parasitoid wasp genus *Cotesia* (Hymenoptera: Braconidae: Microgastrinae): combined analysis of four genes. *Systematic Entomology*, 29, 371–382.
- Muesebeck, C.F.W. (1958) New Neotropical wasps of the family Braconidae (Hymenoptera) in the U.S. National Museum. *Proceedings of the United States National Museum*, 107, 405–461.
- Nixon, G.E.J. (1974) A revision of the north-western European species of the glomeratus-group of *Apanteles* Foerster (Hymenoptera: Braconidae). *Bulletin of Entomological Research*, 64, 453–524.
- Özbek, H. & Çalmaşur, Ö. (2010) Spotted ash looper, *Abraxas pantaria* (L.) (Lepidoptera: Geometridae), a new ash pest in Turkey. *Turkish Journal of Zoology*, 34, 351–358.
- Pizzato, M., Pietrowski, V., Alves, L.F.A. & Rheinheimer, A.R. (2016) Host suitability and fitness-related parameters of *Cotesia glomerata* L. (Hymenoptera: Braconidae) on different instars of *Ascia monuste orseis* Godart (Lepidoptera: Pieridae). *Arquivos do Instituto Biológico*, 83, 1–7.
- Rodriguez, J.J., Fernández-Triana, J., Smith, M.A., Janzen, D.H., Hallwachs, W., Erwin, T.L. & Whitfield, J.B. (2012) Extrapolations from field studies and known faunas converge on dramatically increased estimates of global microgastrine parasitoid wasp species richness (Hymenoptera: Braconidae). *Insect Conservation and Diversity*, 6, 530–536.
- Ruohomäki, K., Klemola, T., Shaw, M.R., Snäll, N., Sääksjärvi, I.E., Veijalanien, A. & Wahlberg, N. (2013) Microgastrinae (Hymenoptera: Braconidae) parasitizing *Epirrita autumnata* (Lepidoptera: Geometridae) larvae in Fennoscandia with description of *Cotesia autumnatae* Shaw, sp. n. *Entomologica Fennica*, 24, 65–80.
- Shaw, M.R. (2003) Revised synonymy in the genus *Cotesia* (Hymenoptera: Braconidae: Microgastrinae): the identity of *Microgaster vestalis* Haliday, 1834, as a senior synonym of *Apanteles plutellae* Kurdjumov, 1912. *Entomologist's Gazette*, 54, 187–189.
- Schmitz, A. & Riesner, D. (2006) Purification of nucleic acids by selective precipitation with polyethylene glycol 6000. *Analytical Biochemistry*, 354, 311–313.
- Schumacker, R.K., Austin, A.D. & Floyd, R.B. (2000) Parasitoids of the autumn gum moth, *Mnesampela privata* (Guenée) (Lepidoptera: Geometridae) in south-eastern Australia, with description of two new larval parasitoids. *Transactions of the Royal Society of South Australia*, 124(1), 1–15.
- Smith, M.A., Rodriguez, J.J., Whitfield, J.B., Deans, A.R., Janzen, D.H., Hallwachs, W. & Hebert, P.D.N. (2008) Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology, and

- collections. *Proceedings of the National Academy of Sciences of the United States of America*, 105(35), 12359–12364.
- Sousa-Lopes, B., Bächtold, A. & Del-Claro, K. (2016) Biology, natural history and temporal fluctuation of the geometrid *Oospila pallidaria* associated with host plant phenology. *Studies on Neotropical Fauna and Environment*, 51(2), 135–143.
- Tepe, E.J., Rodríguez-Castañeda, G., Glassmire, A.E. & Dyer, L.A. (2014) *Piper kelleyi*, a hotspot of ecological interactions and a new species from Ecuador and Peru. *PhytoKeys*, 34, 19–32.
- Viereck, H.L. (1912) Description of five new genera and twenty six new species of Ichneumon-flies. *Proceedings of the United States National Museum*, 42, 139–153.
- Viereck, H.L. (1913) Description of ten new genera and twenty-three new species of Ichneumon-flies. *Proceedings of the United States National Museum*, 44, 555–568.
- Whitfield, J.B. (1997) *Subfamily Microgastrinae*. In: Wharton, R.A., Marsh, P.M., Sharkey, M.J. editors. Identification Manual to the New World Genera of the Family Braconidae (Hymenoptera), pp. 333-364. International Society of Hymenopterists Special Publication.
- Whitfield, J.B., Rodriguez, J.J. & Masonick, P.K. (2009) Reared microgastrine wasps (Hymenoptera: Braconidae) from Yanayacu Biological Station and environs (Napo Province, Ecuador): diversity and host specialization. *Journal of Insect Science*, 9(1), article 31. <https://doi.org/10.1673/031.009.3101>
- Yu, D.S.K., Van Achterberg, C. & Horstmann, K. (2016) Taxapad, Ichneumonoidea 2015. Ottawa, Ontario, Canada. Available from: <http://www.taxapad.com>.



Fig. 1. (a) The host plant, *Mimosa setosa* var. *paludosa* (Fabaceae), and (b) the second instar larva of *Oospila pallidaria* (Geometridae) in the Brazilian savanna.

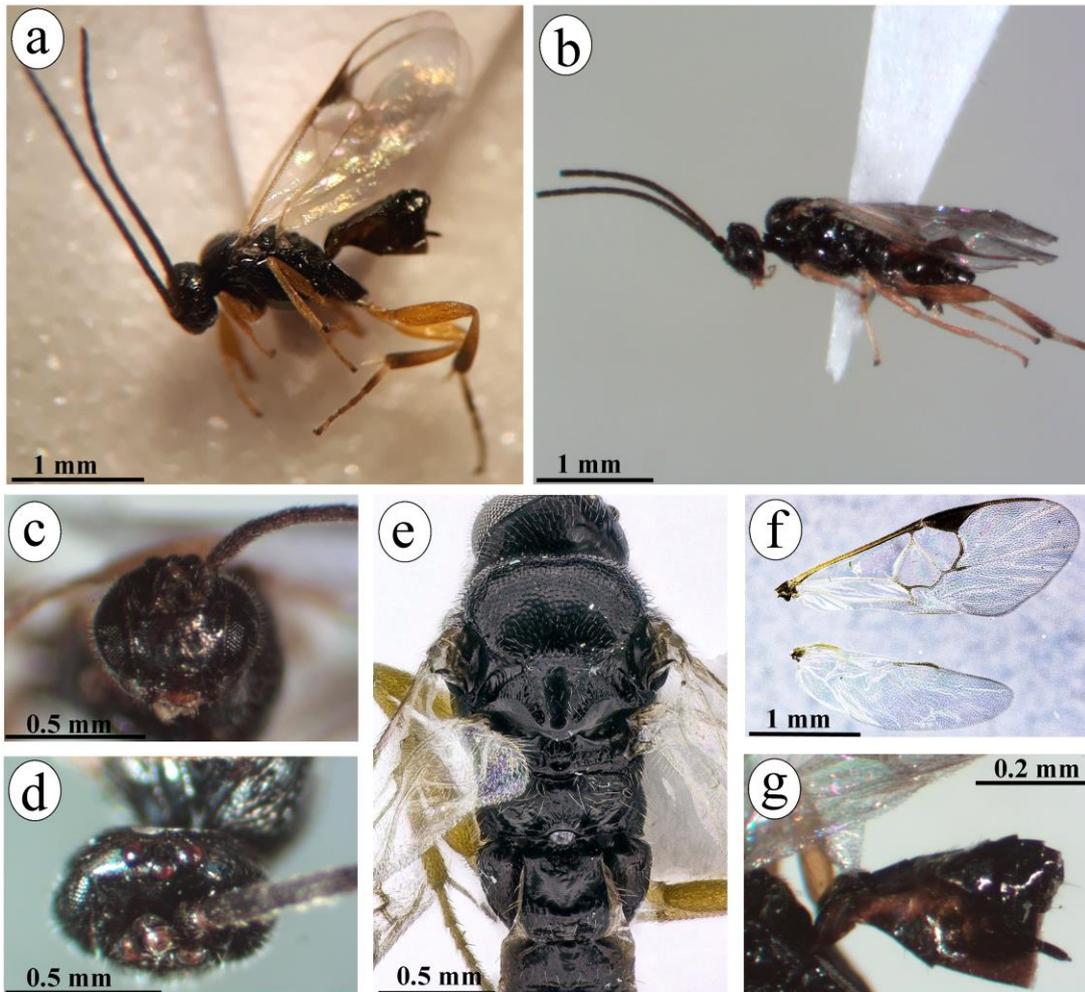


Fig. 2. (a) Lateral habitus of *Cotesia itoroensis* (Braconidae) female, (b) Lateral habitus of *C. itoroensis* male, (c) frontal view of head of *C. itoroensis* female, (d) dorsal view of head of *C. itoroensis* female, (e) mesosoma and tergites of *C. itoroensis* female, (f) wings of *C. itoroensis* female, and (g) lateral view of posterior end of metasoma of *C. itoroensis* female, showing hypopygium and ovipositor sheaths.

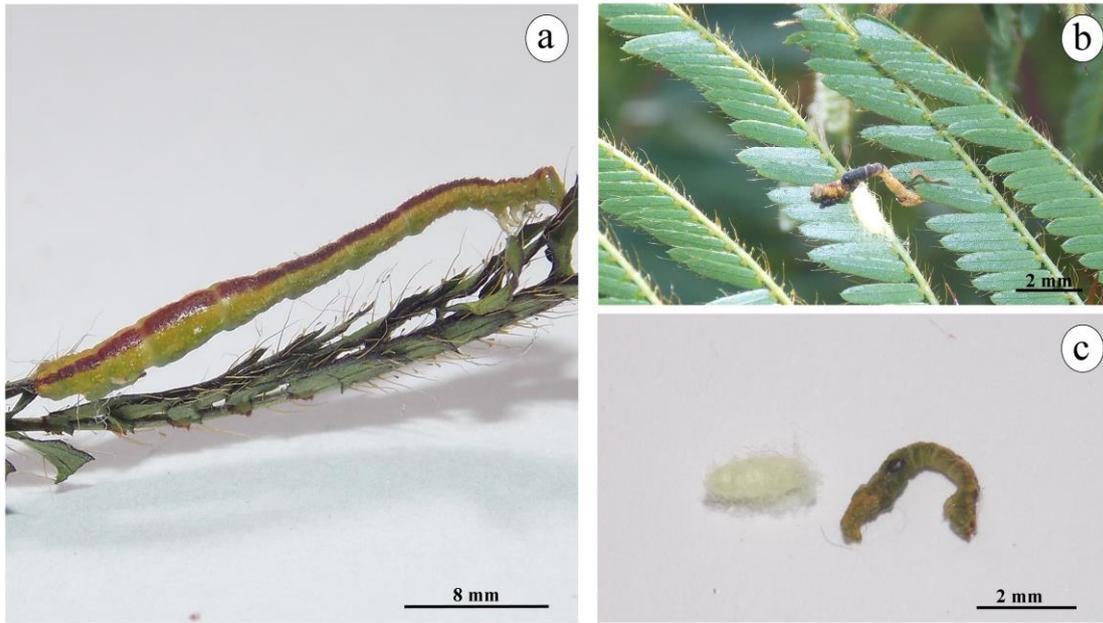


Fig. 3. (a) Parasitized larva of *Oospila pallidaria* (Geometridae) in the fourth instar, with a swollen body in the posterior half, (b) parasitized larva in the abaxial side of the leaf, and (c) dead larva and yellow cocoon of *Cotesia itororensis* (Braconidae).

CONSIDERAÇÕES FINAIS

As interações Fabaceae-insetos-predadores elucidadas nesta tese são pioneiras e sugerem: (1) baixa abundância e alta especificidade de planta hospedeira por parte dos insetos herbívoros no Cerrado, com poucas exceções (*e.g.*, Proscopiidae sp.1 teve baixa abundância, mas foi encontrado se alimentando em três das cinco espécies de plantas hospedeiras; ver Cap. 1), (2) que os insetos endofíticos são sincronizados com a fenologia das plantas hospedeiras e também os mais abundantes do sistema avaliado (*e.g.*, *A. winderi* e *A. quadridentatus*; ver Cap. 1 e 2), (3) que os traços das plantas mudam espaço-temporalmente e, conseqüentemente, afetam as características da história de vida dos insetos herbívoros, como tamanho (ver Cap. 3), e (4) que os inimigos naturais, especialmente aranhas, reduzem as taxas de herbivoria e a abundância da maior parte dos insetos herbívoros associados às plantas glandulares deste sistema (ver Cap. 1 e 4).

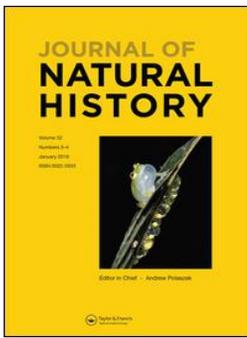
Tendo em vista que uma nova espécie de vespa endoparasitoide foi descrita e indicada a possibilidade de haver mais delas na região Neotropical (ver Cap. 5), sugere-se maior esforço amostral (coleta e criação em laboratório) tanto para parasitoides quanto para outros insetos que se alimentam internamente, como os herbívoros endofíticos. Tal esforço possivelmente resultará na descrição de novas espécies, o que contribuirá para o conhecimento da biodiversidade e/ou evidenciará novas interações ecológicas, as quais servirão como base para a ecologia teórica e, provavelmente, também para a aplicada. Todos esses tipos de contribuições são apresentados nesta tese.

Os resultados obtidos neste manuscrito sugerem que as interações inseto-planta-predador são condicionais, dependentes da identidade e guilda dos insetos herbívoros, bem como aspectos de sua história natural. Por exemplo, no mutualismo facultativo

entre as aranhas lince (*P. flava*) e as fabáceas glandulares, as aranhas são eficientes em proteger as plantas hospedeiras contra a herbivoria foliar causada por diferentes táxons exofíticos. Mas, por outro lado, as aranhas podem falhar em defender as mesmas plantas contra insetos endofíticos comedores de sementes (ver Cap. 4).

Finalmente, os resultados aqui apresentados, como alta especilização e modularidade (ver Cap. 1), sugerem que é crucial a conservação de áreas com grande diversidade de plantas. Este tipo de ação ajudaria a manter a ocorrência de diferentes espécies de insetos hervívoros e seus importantes papéis na estruturação das comunidades terrestres do Cerrado brasileiro, principalmente no atual e constante cenário de perda da biodiversidade em função das pressões antrópicas.

ANEXOS



Temporal distribution, seed damage and notes on the natural history of *Acanthoscelides quadridentatus* and *Acanthoscelides winderi* (Coleoptera: Chrysomelidae: Bruchinae) on their host plant, *Mimosa setosa* var. *paludosa* (Fabaceae: Mimosoideae), in the Brazilian Cerrado

Bruno de Sousa-Lopes, Nayane Alves-da-Silva, Cibele Stramare Ribeiro-Costa & Kleber Del-Claro

To cite this article: Bruno de Sousa-Lopes, Nayane Alves-da-Silva, Cibele Stramare Ribeiro-Costa & Kleber Del-Claro (2019) Temporal distribution, seed damage and notes on the natural history of *Acanthoscelides quadridentatus* and *Acanthoscelides winderi* (Coleoptera: Chrysomelidae: Bruchinae) on their host plant, *Mimosa setosa* var. *paludosa* (Fabaceae: Mimosoideae), in the Brazilian Cerrado, *Journal of Natural History*, 53:9-10, 611-623

To link to this article: <https://doi.org/10.1080/00222933.2019.1606358>



Published online: 30 Apr 2019.



Submit your article to this journal [↗](#)



View Crossmark data [↗](#)



Temporal distribution, seed damage and notes on the natural history of *Acanthoscelides quadridentatus* and *Acanthoscelides winderi* (Coleoptera: Chrysomelidae: Bruchinae) on their host plant, *Mimosa setosa* var. *paludosa* (Fabaceae: Mimosoideae), in the Brazilian Cerrado

Bruno de Sousa-Lopes ^a, Nayane Alves-da-Silva ^b, Cibele Stramare Ribeiro-Costa ^c and Kleber Del-Claro ^b

^aLaboratório de Ecologia Comportamental e de Interações, Pós-graduação em Entomologia, Universidade de São Paulo, Ribeirão Preto, Brazil; ^bLaboratório de Ecologia Comportamental e de Interações, Instituto de Biologia, Universidade Federal de Uberlândia, Uberlândia, Brazil; ^cLaboratório de Sistemática e Bioecologia de Coleoptera, Setor de Ciências Biológicas, Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Brazil

ABSTRACT

The seed beetles, *Acanthoscelides quadridentatus* and *Acanthoscelides winderi* are here recorded for the first time feeding on seeds of *Mimosa setosa* var. *paludosa* in the Brazilian Cerrado. Our main aims were to describe the temporal distribution, seed damage, and notes on the natural history of these two species on their host plant. We hypothesised that: (a) healthy seeds from infested fruits would have worse germination rate than healthy seeds from noninfested fruits, and (b) females of seed beetles would lay more eggs on large fruits. We made field observations and an experimental field study with the presence of seed beetles versus their exclusion on plants. Results revealed that seed beetles are synchronised with fruiting, with a temporal partitioning in occurrence. Attacked seeds did not germinate, whereas healthy seeds from infested fruits had worse germination rate than healthy seeds from noninfested fruits. Females of seed beetles laid more eggs on large fruits. These results suggest that seed beetles avoid competition through a temporal partitioning crucial for their coexistence, and select large fruits to oviposit as these fruits probably provide more food resource for their offspring. Furthermore, plants might perceive seed beetles' damage and then reduce resource allocation on infested fruits.

ARTICLE HISTORY

Received 3 September 2018
Accepted 6 April 2019

KEYWORDS

Insect–plant interactions; oviposition guild; resource allocation; seed beetles

Introduction

Pre-dispersal seed predation occurs when seeds are predated while they remain on the mother plant (Stachurska-Sawakoń et al. 2018), which may directly affect reproductive success, survival and population dynamics of plants (Janzen 1970; Harper 1977; Kolb et al. 2007). The damage varies among plant species (e.g. El Atta 1993; Fenner and Thompson 2005; Klips et al. 2005); however, it must reach $\geq 80\%$ to regulate plant populations (Van Klinken 2005; Van Klinken and Flack 2008; Rodrigues et al. 2012).

CONTACT Bruno de Sousa-Lopes  brunolopesprof@gmail.com

© 2019 Informa UK Limited, trading as Taylor & Francis Group

This happens because seed predation may only remove individuals that were doomed to die later through competition and/or other density-dependent processes (Lewis and Gripenberg 2008). Therefore, high levels of seed predation may not represent significant effects on plant population (Crawley 1992; Kolb et al. 2007).

Bruchinae (Coleoptera: Chrysomelidae) is a group of seed-feeding insects in which approximately two-thirds of the 1700 known species feed in a single seed during all larval stages (Center and Johnson 1974; Alvarez et al. 2006; Ribeiro-Costa and Almeida 2012). Some are synchronised with the fruiting, mainly in the early phase when fruits are soft and it is apparently easy for the first instar larvae to perforate the exocarp and seeds (Rossi et al. 2011). In this short time, species of bruchines may avoid competition through a temporal partitioning, with one species arriving and exploring the resources first, then another (Maia et al. 2017). This partition in foraging time is paramount for species with highly discrete internal feeding niches, such as many bruchine species, as they have little ability to switch among resources (Denno et al. 1995; Maia et al. 2017). Thus, temporal partitioning may be crucial to promoting the coexistence of species that rely strongly on common resources and would otherwise experience potentially exclusive interspecific competition (Richards 2002).

Besides synchrony with fruiting and temporal partitioning, bruchines may lay more eggs on large fruits since these fruits provide more food resources, allowing increases in offspring survival and fitness (Mitchell 1975; Cope and Fox 2003; Ostergård et al. 2007; Morales-Silva et al. 2018). Moreover, to ensure offspring survival females may also lay eggs with filaments, also called anchoring strands, which possibly help the eggs to fix on the fruit wall, avoiding detachment when fruits are growing and dehydrating (Johnson and Kingsolver 1975; Ribeiro-Costa and Costa 2002). Johnson and Romero (2004) reviewed the oviposition behaviour of 114 bruchine species and found that these beetles can oviposit in different ways. According to these authors, bruchine species can be classified as that which only oviposit on fruits while on the plant (Guild A, 77%), that which only oviposit on seeds exposed in fruits while still on the plant (Guild B, 10%), and that which only oviposit on seeds once they are exposed on the substrate (Guild C, 13%).

Larvae of bruchines bore into the seed, consume the endosperm, and when the seed embryo is widely damaged, kill the seeds (Ribeiro-Costa and Almeida 2012). Due to the high abundance of bruchines, plants lose a lot of seeds, commonly ranging from 4% to 42% (Klips et al. 2005; Rodrigues et al. 2012) but the loss can reach 90% (e.g. *Acacia nilotica*; El Atta 1993). In addition to the loss of predated seeds, healthy seeds may also suffer with fruit damage (Tomaz et al. 2007). This happens because plants may perceive seed beetles' elicitors or cues during their oviposition and/or feeding and, as a response, plants may decrease resource allocation in damaged but also in undamaged parts (e.g. Doss et al. 2000; Menezes et al. 2010; Wu and Baldwin 2010). Furthermore, it is also possible that the physical damage inflicted by seed beetles may simply change environmental conditions sufficiently (e.g. through humidity changes, the entrance of fungi, etc.) that negatively impacts the development of seeds. Thus, it is possible that healthy seeds from infested fruits have smaller resource allocation and, consequently, worse germination rate than healthy seeds from noninfested fruits.

Acanthoscelides Schilsky, 1905 is the largest American genus of Bruchinae with approximately 340 species (Nápoles and Kingsolver 2009), some of them important pests of cultivated plants (e.g. *Acanthoscelides obtectus* Say, 1831 in beans, *Phaseolus vulgaris* L.; Baier and Webster 1992). However, little is known about the natural history of some species, e.g. *Acanthoscelides winderi* Kingsolver, 1984 and *Acanthoscelides quadridentatus* (Schaeffer, 1907). For *A. winderi* there is apparently only one study with the species description and record of an unidentified species of host plant of the genus *Mimosa* L. (Fabaceae: Mimosoideae) in Brazil (Kingsolver 1984). *Acanthoscelides quadridentatus* was recorded feeding on *Mimosa pigra* L., *Mimosa invisa* Colla, and *Mimosa strigillosa* Torrey and Gray, and considered a potential agent for biological control of the weed, *M. pigra*, in Australia (Bottimer 1969; Johnson 1979; Kassulke et al. 1990).

Here we provide the first record of *A. winderi* and *A. quadridentatus* feeding in pre-dispersal seeds of *Mimosa setosa* var. *paludosa* (Benth.) Barneby. The main goals of this study were: (1) to describe the temporal distribution of *Acanthoscelides* beetles; (2) to describe the percentage of seed damage imposed by *Acanthoscelides* beetles on *M. setosa* var. *paludosa*; (3) to evaluate whether *Acanthoscelides* beetles negatively affect the germination rate of healthy seeds from infested fruits; and (4) to describe notes on the natural history of *Acanthoscelides* beetles. We tested the following hypotheses: (i) the germination rate is worse in healthy seeds from infested fruits possibly due to a decreasing in resource allocation by plants; and (ii) large fruits have a greater abundance of eggs of *Acanthoscelides* beetles possibly because females select fruits that promise more food resources for their offspring.

Material and methods

Study area

Fieldwork was carried out by BSL and NAS in the Cerrado sensu stricto vegetation of the Ecological Reserve of Clube Caça e Pesca Itororó de Uberlândia (CCPIU), Minas Gerais, Brazil. The reserve is located at 18°59'00"S, 48°17'44"W, with an elevation of 863 m and a natural area of approximately 640 ha (Del-Claro and Marquis 2015). The climate in the region is markedly seasonal, characterised by a rainy summer (October to March) which may account for up to 75% of the annual rainfall, and a dry winter (April to September) (Laboratory of Climatology, Federal University of Uberlândia; Sousa-Lopes et al. 2016).

Plant species and phenology

Mimosa setosa var. *paludosa* (Figure 1) is a swampy shrub that is endemic to Brazil and Paraguay. It reaches 1.5–5 m tall (Barneby 1991; Dutra and Garcia 2014); however, in this study area, it rarely exceeded 3 m. This species has thorns in its stems and aculeate and glandular trichomes all over the rachis, stems, inflorescences and fruits (Barneby 1991; Dutra and Garcia 2014). The fruits are red-brown coloured and partially dehiscent (craspedium fruits). Plants were found growing on the edge of a trail (3 m wide and c.1.5 km long) inside the Cerrado reserve, near a vereda, a swampy area located in the headwater of a stream (Sousa-Lopes et al. 2016). This species is important for apiarian activity and the restoration of wetlands (Pott et al. 2006); however, it is also an important weed in pasture areas as its large



Figure 1. The host plant of the seed beetles, *Mimosa setosa* var. *paludosa*, in the fruiting phase in the Brazilian Cerrado. The arrow indicates some fruits.

thorny branches can injure cattle (Lorenzi 2000; Pott et al. 2006). Despite its importance, little attention has been given to the ecological interactions of this plant species, although there is a record of the herbivore *Oospila pallidaria* (Schaus, 1897) feeding on its leaves (Sousa-Lopes et al. 2016). The plant used in this study was identified by the specialist in leguminous plants from Brazil, Dr Rubens Teixeira de Queiroz.

A total of 20 individuals of *M. setosa* var. *paludosa* were tagged and inspected once a month from January to December 2016. During inspections, the number of nearly or completely mature fruits was counted in each plant to describe the phenology and associate it with the temporal distribution of seed beetles.

Temporal distribution of *Acanthoscelides* beetles and seed damage

To assess the temporal distribution of *Acanthoscelides* beetles from March to October 2016, when fruits were available, 20 nearly or completely mature fruits were collected from each of the 20 previously tagged *M. setosa* var. *paludosa* plants ($n = 400$ fruits per month). Fruits were taken to the Behavioral Ecology and Interactions Laboratory (LECI) at the Federal University of Uberlândia and maintained for 60 days in transparent plastic pots (500 ml) covered with voile under laboratory conditions (12-h light and 20–30°C). After that, the abundance of *Acanthoscelides* beetles and damaged seeds were counted. The two species of bruchine, *A. quadridentatus* and *A. winderi*, were identified by the specialist in seed beetles, Dr Cibele Stramare Ribeiro-Costa, and deposited at the Coleção Entomológica Pe. J.S. Moure, Departamento de Zoologia, Universidade Federal do Paraná.

Germination test

To assess the germination rate of seeds, we randomly selected two flowering branches from each 10 of the previously tagged plants in February 2017. Plants were at least 5 m

apart and their branches had the same phenology and height. One branch was carefully bagged with voile to prevent infestation with *Acanthoscelides* beetles and to obtain only healthy seeds. Conversely, another branch was not bagged to allow infestation with *Acanthoscelides* beetles and to evaluate whether the larval activity of these beetles may affect resource allocation in fruits and, thus, the germination rate of healthy seeds in these same fruits. When fruits from the two branches of each plant matured, after approximately 40 days, they were collected, placed in plastic pots covered with voile, and labelled (bagged or nonbagged). Fruits were maintained for 60 days under laboratory conditions, then they were dissected and seeds were separated into three groups: (1) healthy seeds from infested fruits; (2) healthy seeds from noninfested fruits; and (3) attacked seeds. Before germination tests, the healthy seeds were scarified with sandpaper to overcome the physical dormancy (seed coat) and to allow free access to water. Attacked seeds were not scarified as *Acanthoscelides* beetles left exit holes which already allowed water to enter (Rodrigues et al. 2012). Finally, seeds were placed in filter-paper lined plastic pots (500 ml) with 10 ml of water. Each seed group had 10 replicates with 10 seeds, totalling 300 seeds. Germination was defined as the appearance of a 2 mm primary root, and the number of germinated seeds was counted daily for each group until it to reach the plateau on the fifth day of trials.

Effects of fruit size on *Acanthoscelides* beetles

To assess fruit size and correlate it with the abundance of eggs of *Acanthoscelides* beetles, in May 2017 we randomly collected another 76 fruits of the same tagged *M. setosa* var. *paludosa* plants. These fruits were taken to the laboratory and the length of each fruit was obtained using a digital calliper (mm). After that, we counted the number of eggs by using a stereomicroscope (according to Morales-Silva et al. 2018).

Data analysis

Circular statistical analysis of directional data was used to evaluate whether there was seasonality in fruit availability (mean monthly) and *Acanthoscelides* spp. abundance (monthly). In this analysis, months were converted into angles (30° intervals) and these angles were combined with the respective value of plant phenology and *Acanthoscelides* spp. abundance to describe the temporal distribution. Circular statistics provide: (1) the mean angle (μ), which is the period when a given variable (e.g. fruits or *Acanthoscelides* spp.) occurred most often; (2) the vector (r), which is a direct measure of seasonality (closer to 1 being more seasonal); and (3) the Rayleigh test (z), which indicates whether seasonality is significant (Sousa-Lopes et al. 2016). The cumulative germination rate of

Table 1. Circular statistical analysis of the seasonality of *Acanthoscelides* beetle abundance together with fruits of their host plant, *M. setosa* var. *paludosa*.

	Mean angle (μ)	Mean month	Vector (r)	Rayleigh Z test
Fruits	195.83°	July	0.643	623.34***
<i>A. winderi</i>	148.48°	May	0.925	359.67***
<i>A. quadridentatus</i>	184.80°	July	0.790	227.76***

*** $p < 0.0001$.

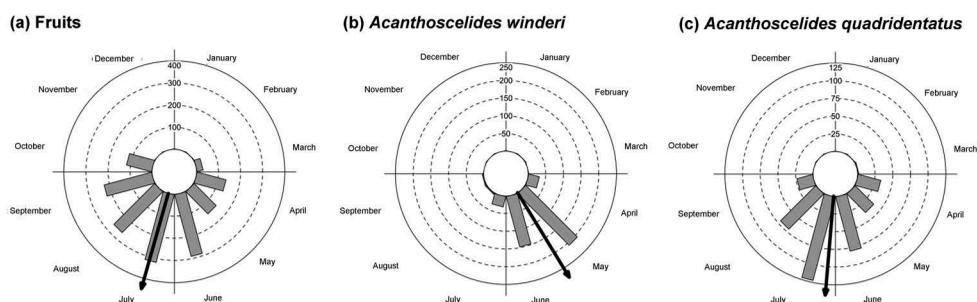


Figure 2. (a) Annual distribution of fruit availability of *M. setosa* var. *paludosa*; (b) abundance of *A. winderi*; and (c) abundance of *A. quadridentatus*. Numbers within circles are the monthly means of fruits (a) and the absolute frequency of seed beetles (b, c). Arrows are the mean month of fruits or *Acanthoscelides* beetles.

seeds from infested fruits, noninfested fruits, and attacked seeds was compared using repeated measures ANOVA followed by Bonferroni's post-hoc test. We used also a simple linear regression to evaluate the relationship between fruit size (independent variable) and abundance of eggs of *A. quadridentatus*. Oriana 4 (Kovach Computing Services, Pentraeth, Isle of Anglesey, UK), R Studio (R Development Core Team 2016) and Graph Pad Prism 5 (GraphPad Company, San Diego, CA, USA) were used to perform the analyses and graphics.

Results

We counted a total of 26,478 fruits on the 20 tagged plants throughout the year, with July being the month with the highest amount (24%) and March the month with the lowest amount (0.4%; Figure 2(a)). Statistical analyses suggest that the occurrence of fruit was seasonal (concentrated in the dry season), with the mean month being July (Figure 2(a); Table 1). We also found 420 *A. winderi* (Figure 3(a)) and 365 *A. quadridentatus* (Figure 3(b)) individuals feeding in the seeds of *M. setosa* var. *paludosa* throughout the dry season (Figure 2(b,c)). The peak infestations for *A. winderi* and *A. quadridentatus* were in May ($n = 206$) and July ($n = 122$), respectively. Circular statistical analyses showed that the occurrence of *Acanthoscelides* beetles was overlapped with the availability of fruits, with mean angle μ being very similar among them, especially between *A. quadridentatus* and fruits (Table 1). During this overlap, we found a temporal partitioning between *Acanthoscelides* beetles, with *A. winderi* being more abundant during early fruiting (April–June, 91%) and *A. quadridentatus* more abundant after that (June–August, 74%). Although we collected 785 individuals of *Acanthoscelides* beetles, we found 2000 seeds predated by these beetles in our sampling, which represented approximately 15.20% of the total counted seeds. This happened because collects were done once a month and thus many seed beetles probably completed their development among collects and left only the exit holes (Figure 3(d)).

The germination rate among attacked seeds and healthy seeds from infested and noninfested fruits differed statistically ($F_{2,4} = 87.27$, $p < 0.0001$). Attacked seeds did not germinate; whereas healthy seeds from noninfested fruits contained 94% germinated seeds, and healthy seeds from infested fruits contained 70% germinated seeds (Figure 4).

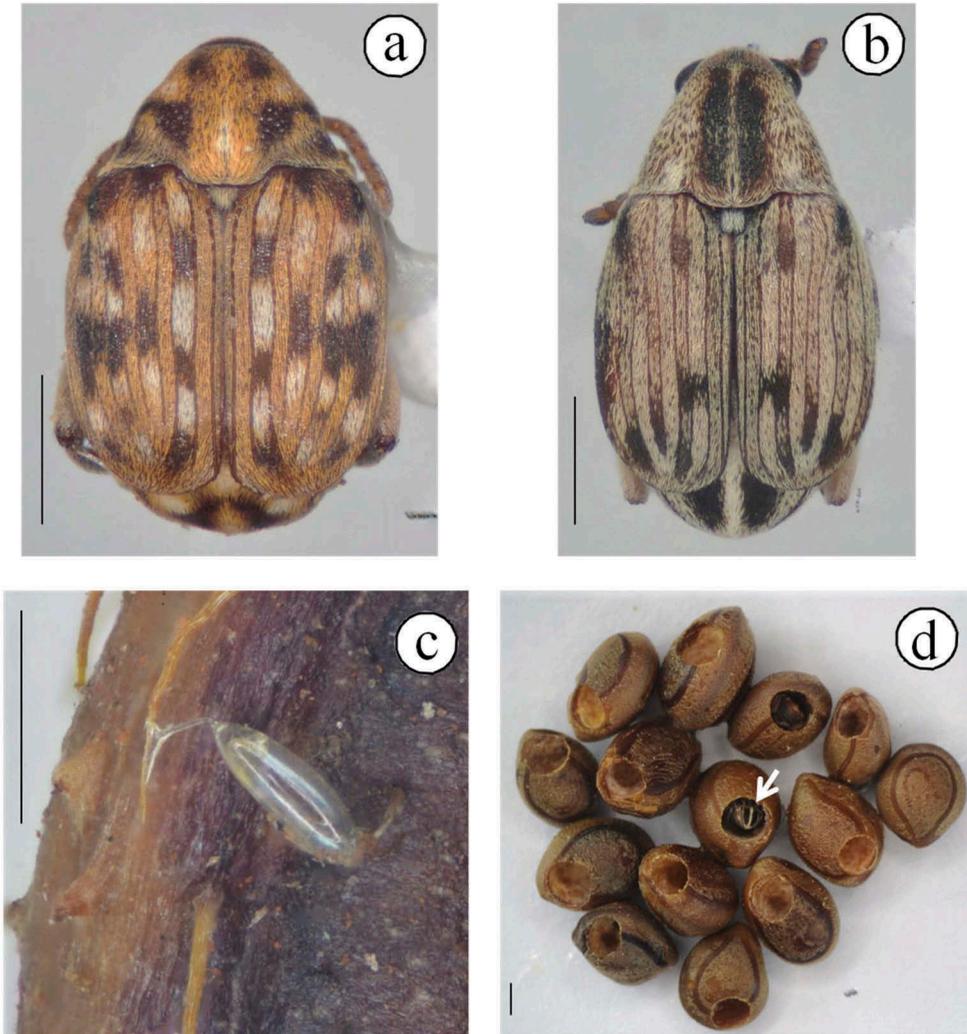


Figure 3. Seed beetles (Bruchinae) associated with *M. setosa* var. *paludosa*: (a) *A. winderi*; (b) *A. quadridentatus*; (c) seed beetle egg on the fruit surface; and (d) attacked seeds. The arrow indicates a single *Acanthoscelides* beetle inside a seed. Scale: 1 mm.

Females of both *Acanthoscelides* species laid eggs on the external surface and trichomes of fruits (Figure 3(c)). Eggs are elongated, with filaments (anchoring strands) at the anterior and posterior ends that possibly help to fix on the fruit wall and directly on trichomes (Figure 3(c)). Simple linear regression showed that the abundance of *Acanthoscelides* eggs was predicted by fruit size ($F_{1,74} = 10.40$, $p < 0.001$, $R^2 = 0.123$; Figure 5). In this analysis, we found a total of 323 eggs of *Acanthoscelides* beetles, with the highest abundance of eggs ($n = 16$) in a fruit with 56 mm length and the lowest ($n = 1$) in a fruit with 42 mm length. The mean number of eggs was $4.25 (\pm 3.24)$ and the mean size of fruits was $47.55 \text{ mm} (\pm 6.68)$. Larvae of the two species studied here developed feeding on only one seed (Figure 3(d)).

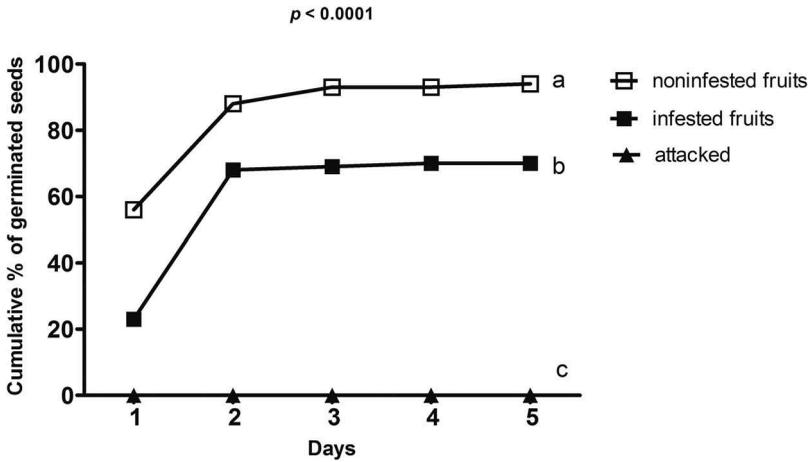


Figure 4. Cumulative percentage of germination of healthy seeds from noninfested and infested fruits, and attacked seeds of *M. setosa* var. *paludosa*. Letters indicate significant statistical difference among treatments by Bonferroni’s post-hoc test at $p < 0.0001$.

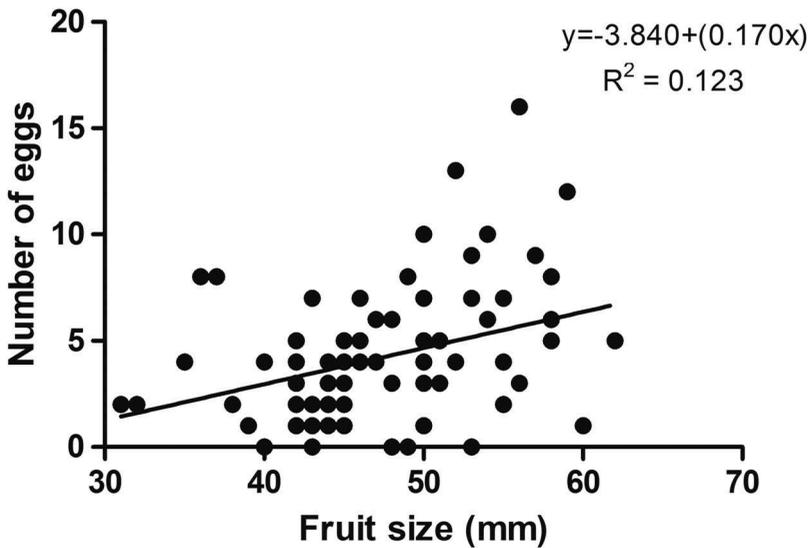


Figure 5. Simple linear regression of fruit size (independent variable) and number of *Acanthoscelides* beetle eggs ($F_{1,74} = 10.40$, $p < 0.001$).

Discussion

Our two initial hypotheses were corroborated: first, the germination rate was worse in healthy seeds from infested fruits; and second, large fruits had a greater abundance of *Acanthoscelides* eggs. Some studies have shown that the presence of insects can facilitate seed germination (Takakura 2002; Vega et al. 2011). For instance, Takakura (2002) showed that the seed beetle, *Bruchidus dorsalis* Fahraeus, plays a crucial role for *Gleditsia japonica* Miquel (Fabaceae) since it opens the hard coat of seeds and facilitates the entrance of water and, consequently, the germination. In contrast, our study showed that *Acanthoscelides* beetles negatively affected

the germination rate of *M. setosa* var. *paludosa* seeds, as none of the attacked seeds germinated. This was similar to what was found in *Leucaena leucocephala* (Lam.) de Wit, in which seeds were predated by *Acanthoscelides macrophthalmus* (Schaeffer 1907) (Rodrigues et al. 2012). Furthermore, our study also showed that *Acanthoscelides* beetles could affect unattacked seeds, as healthy seeds from infested fruits had a worse germination rate than healthy seeds from noninfested fruits, like in *Mimosa bimucronata* (DC.) Kuntze (Menezes et al. 2010). These results suggest that larval activity of *Acanthoscelides* beetles during fruit development in *M. setosa* var. *paludosa* may result in a change in resource allocation by plants. It has been shown that plants can perceive insects' elicitors or cues during their oviposition or feeding and, through electrical and/or chemical cascade events, plants may induce responses not only in damaged but also in undamaged parts (Doss et al. 2000; Wu and Baldwin 2010). For instance, Doss et al. (2000) showed that the plant *Pisum sativum* L. (Fabaceae) stimulates cell division and the formation of neoplasms when the seed beetle, *Bruchus pisorum* L., oviposits on its fruits. These neoplasms develop beneath the egg and impede larval entry into the fruits. Menezes et al. (2010) showed that healthy seeds from infested fruits of *M. bimucronata* had lower nitrogen concentration and worse germination rate than healthy seeds from noninfested fruits. These authors suggested that the damage inflicted by the beetle *Acanthoscelides schrankiae* Horn may affect the resource allocation on *M. bimucronata*. Therefore, it is also possible that *M. setosa* var. *paludosa* perceive seed beetles' attack and reduce energetic investment in infested fruits and, consequently, affect the germination rate of healthy seeds in these fruits.

Females of *Acanthoscelides* beetles laid more eggs on large fruits. Several other studies have also shown that the oviposition of seed beetles is correlated with fruit size, as large fruits have a higher availability of food resources which may positively affect offspring development and fitness (Mitchell 1975; Cope and Fox 2003; Ostergård et al. 2007; Morales-Silva et al. 2018). However, this is not a rule since the oviposition in some species of bruchines is not positively correlated with fruit size (see Ribeiro-Costa et al. 2011; Modena et al. 2012). Here, we highlight that females of *A. quadridentatus* and *A. winderi* appear to assess the fruit size before oviposition, suggesting that large fruits may be better for offspring survival and fitness.

An overlap was found between the availability of fruits of *M. setosa* var. *paludosa* and *Acanthoscelides* beetles, especially for *A. quadridentatus* and fruits whose mean month was July. Rossi et al. (2011) also found synchrony between *A. schrankiae* and fruits of *M. bimucronata*. These data reinforce that for insects with restricted diets, like *Acanthoscelides* beetles, the availability of their host plants is a crucial factor accounting for their maintenance in natural environments, especially because fruiting occurs in a short time (Bernays and Chapman 1994).

We also showed that *A. winderi* occurred mainly in the early phase of the fruiting period (April–June), whereas *A. quadridentatus* occurred in higher abundance after that (June–August). This temporal partitioning may be an adaptation to avoid direct competition when seeds are available. Maia et al. (2017) also showed that the seed beetles *Merobruchus terani* (Kingsolver 1980) and *Stator maculatopygus* (Pic 1930) can avoid competition through a temporal partitioning in *Senegalia tenuifolia* (L.) Britton & Rose (Fabaceae: Mimosoideae). In this case, the authors explained that the less abundant species, *S. maculatopygus*, arrived later probably to avoid competition with the dominant species, *M. terani*. Here, we also showed that the less abundant species, *A. quadridentatus*, arrived later than the most abundant species, *A. winderi*. Besides

avoiding competition, it is also possible that *A. quadridentatus* arrives later to avail the holes made by first instar larvae of *A. winderi*. This could happen if the eggs of *A. winderi* detach from fruits, especially when fruits are growing and dehydrating (see Ribeiro-Costa and Costa 2002), thus allowing *A. quadridentatus* to oviposit on them and first instar larvae use the same holes left by *A. winderi*. The use of pre-existing holes made on fruits is a behaviour already recorded in other species of bruchines, as for *Zabrotes interstitialis* (Chevrolat) that avails the holes made by *Pygiopachymerus lineola* (Chevrolat) on *Cassia grandis* L. (Janzen 1971). Herein, we reinforce that temporal partitioning may be crucial to avoid competition and promote the coexistence of species that rely strongly on common resources (see also Richards 2002).

Our data on seed damage (15.20%) corroborate the infestation range of other studies that focused on seed beetles (i.e. varying from 4% to 42%) (Klips et al. 2005; Rodrigues et al. 2012). This damage rate decreases seed set and abundance of *M. setosa* var. *paludosa*; however, according to Van Klinken (2005), Van Klinken and Flack (2008) and Rodrigues et al. (2012), the damage rate must reach $\geq 80\%$ to regulate plant populations. Thus, the damage imposed by *Acanthoscelides* beetles on *M. setosa* var. *paludosa* seems unable to regulate this plant population.

Regarding the natural history, we showed that *A. quadridentatus* and *A. winderi* laid eggs on the external surface of *M. setosa* var. *paludosa* fruits. This behaviour was observed in approximately 77% of all bruchine species studied (Johnson and Romero 2004), including *Acanthoscelides* species (Silva et al. 2007). According to Johnson and Romero (2004), species of seed beetles that glue their eggs to the outside of the fruit while it remains on the plants belong to the oviposition Guild A. Species belonging to this guild probably face the challenge of avoiding the detachment of eggs from the fruit wall before hatching. The strategy that seed beetles apparently developed to overcome that pressure was the use of anchoring strands, which help the eggs to fix on fruit wall when fruits are growing and dehydrating, as shown here for the two studied species (see also Ribeiro-Costa and Costa 2002). After the hatching, larvae of seed beetles from Guild A, such as *A. quadridentatus* and *A. winderi*, burrow through the fruit wall and then to seed coat, different from other guilds (B and C) that face only one barrier (seed coat). Thus, descriptions on the oviposition behaviour, including our report for *A. quadridentatus* and *A. winderi*, are paramount to understand ecological questions on seed beetle–host plant interactions.

Overall, we showed that *A. quadridentatus* and *A. winderi* decreased the seed set as well as the performance of healthy seeds from infested fruits of *M. setosa* var. *paludosa*, although they did not reach the degree of damage necessary to regulate plant population. Females of these two *Acanthoscelides* species selected large fruits to lay their eggs, and the temporal partitioning in the occurrence of *Acanthoscelides* beetles suggests a survival strategy to avoid competition and promote the coexistence between species.

Acknowledgements

We thank the staff of the Clube Caça e Pesca Itororó de Uberlândia where the study was performed, Melissa Alves Lopes for her encouragement, Aluska Tavares dos Santos for help with the identification of the seed beetles and photos, Rubens Queiroz for identification of plants, Eduardo Soares Calixto for reviewing an early version of this manuscript, and Coleção Pe. Jesus Santiago Moure that allowed us to access the collection.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (grants 142280/2016-0, 119082/2017-9, 311792/2013-8, 301605/2013-0) and Fundação de Amparo à Pesquisa de Minas Gerais.

ORCID

Bruno de Sousa-Lopes  <http://orcid.org/0000-0001-5192-3831>

Nayane Alves-da-Silva  <http://orcid.org/0000-0003-2156-1870>

Cibele Stramare Ribeiro-Costa  <http://orcid.org/0000-0001-6527-6460>

Kleber Del-Claro  <http://orcid.org/0000-0001-8886-9568>

References

- Alvarez N, Romero-Napoles J, Anton KW, Benrey B, Hossaert-McKey M. 2006. Phylogenetic relationships in the Neotropical bruchid genus *Acanthoscelides* (Bruchinae, Bruchidae, Coleoptera). *J Zool Syst Evol Res.* 44:63–74. DOI:10.1111/j.1439-0469.2005.00344.x
- Baier AH, Webster BD. 1992. Control of *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae) in *Phaseolus vulgaris* L. seed stored on small farms I. Evaluation of damage. *J Stored Prod Res.* 28:289–293. DOI:10.1016/0022-474X(92)90011-E
- Barneby CR. 1991. *Sensitivae censitae*. A description of the genus *Mimosa* L. (Mimosaceae) in the New World. *Mem N Y Bot Gard.* 65:1–835.
- Bernays EA, Chapman RF. 1994. Host-plant selection by phytophagous insects. New York (NY): Chapman & Hall Press.
- Bottimer LJ. 1969. Bruchidae associated with *Mimosa* with the description of a new species. *Can Entomol.* 101:1186–1198. DOI:10.4039/Ent1011186-11
- Center TD, Johnson CD. 1974. Coevolution of some seed beetles (Coleoptera: Bruchidae) and their hosts. *Ecology.* 55:1096–1103. DOI:10.2307/1940359
- Cope JM, Fox CW. 2003. Oviposition decisions in the seed beetle, *Callosobruchus maculatus* (Coleoptera: Bruchidae): effects of seed size on superparasitism. *J Stored Prod Res.* 39:355–365. DOI:10.1016/S0022-474X(02)00028-0
- Crawley MJ. 1992. Seed predators and plant population dynamics. In: Fenner M, editor. *Seeds, the ecology of regeneration in plant communities*. Wallingford (UK): CABI International; p. 157–191.
- Del-Claro K, Marquis RJ. 2015. Ant species identity has a greater effect than fire on the outcome of an ant protection system in Brazilian cerrado. *Biotropica.* 47:459–467. DOI:10.1111/btp.2015.47.issue-4
- Denno RF, McClure MS, Ott JR. 1995. Interspecific interactions in phytophagous insects: competition reexamined and resurrected. *Annu Rev Entomol.* 40:297–331. DOI:10.1146/annurev.en.40.010195.001501
- Doss RP, Oliver JE, Proebsting WM, Potter SW, Kuy S, Clement SL, Williamson RT, Carney JR, DeVilbiss ED. 2000. Bruchins: insect-derived plant regulators that stimulate neoplasm formation. *Pnas.* 97:6218–6223. DOI:10.1073/pnas.110054697
- Dutra VF, Garcia FCP. 2014. *Mimosa* L. (Leguminosae-Mimosoideae) dos campos rupestres de Minas Gerais, Brasil. *Iheringia Série Botânica.* 69:49–88.
- El Atta HA. 1993. The effect of *Caryedon serratus* Olivier (Col., Bruchidae) on viability and germination of seeds of *Acacia nilotica* (L. Willd. ex Del.) in the Sudan. *Forest Ecol Manag.* 57:169–177. DOI:10.1016/0378-1127(93)90170-R

- Fenner M, Thompson K. 2005. Ecology of seeds. Cambridge: Cambridge University Press.
- Harper JL. 1977. Population biology of plants. New York: Academic Press.
- Janzen DH. 1970. Herbivores and the number of tree species in tropical forests. *Am Nat.* 104:501–528. DOI:10.1086/282687
- Janzen DH. 1971. Escape of *Cassia grandis* L. beans from predators in time and space. *Ecology.* 52:964–979. DOI:10.2307/1933802
- Johnson CD. 1979. New host records for *Acanthoscelides* (Coleoptera: Bruchidae). *Pan-Pac Entomol.* 55(1):61–71.
- Johnson CD, Kingsolver JM. 1975. Ecology and redescription of the Arizona grape bruchid, *Amblycerus vitis* (Coleoptera). *Coleopt Bull.* 29(4):321–331.
- Johnson CD, Romero J. 2004. A review of evolution of oviposition guilds in the Bruchidae (Coleoptera). *Rev Bras Entomol.* 48:401–408. DOI:10.1590/S0085-56262004000300017
- Kassulke RC, Harley KLS, Maynard GV. 1990. Host specificity of *Acanthoscelides quadridentatus* and *A. puniceus* (Coleoptera: Bruchidae) for biological control of *Mimosa pigra* (with preliminary data on their biology). *Entomophaga.* 35(1):85–96. DOI:10.1007/BF02374305
- Kingsolver JM. 1984. *Acanthoscelides winderi*, new species, (Coleoptera, Bruchidae) associated with *Mimosa* spp. (Leguminosae, Mimosoideae) from Brazil. *Entomol News.* 95:87–90.
- Klips RA, Sweeney PM, Bauman EKK, Snow AA. 2005. Temporal and geographic variation in predispersal seed predation on *Hibiscus moscheutos* L. (Malvaceae) in Ohio and Maryland, USA. *Am Midl Nat.* 154:286–295. DOI:10.1674/0003-0031(2005)154[0286:TAGVIP]2.0.CO;2
- Kolb A, Ehrlén J, Eriksson O. 2007. Ecological and evolutionary consequences of spatial and temporal variation in pre-dispersal seed predation. *Perspect Plant Ecol Syst.* 9:79–100. DOI:10.1016/j.ppees.2007.09.001
- Lewis OT, Gripenberg S. 2008. Insect seed predators and environmental change. *J Appl Ecol.* 45:1593–1599. DOI:10.1111/j.1365-2664.2008.01575.x
- Lorenzi H. 2000. Plantas daninhas do Brasil: terrestres, aquáticas, parasitas e tóxicas. Nova Odessa: Instituto Plantarum, SP.
- Maia LF, Tuller J, Faria LDB. 2017. Morphological traits of two seed-feeding beetle species and the relationship to resource traits. *Neotrop Entomol.* 46:36–44. DOI:10.1007/s13744-016-0436-4
- Menezes LCCR, Klein J, Kestring J, Rossin MN. 2010. Bottom-up and top-down effects in a pre-dispersal seed predation system: are non-predated seeds damaged? *Basic Appl Ecol.* 11:126–134. DOI:10.1016/j.baae.2009.11.004
- Mitchell R. 1975. The evolution of oviposition tactics in the vean weevil, *Calosobruchus maculatus* (F.). *Ecology.* 56:696–702. DOI:10.2307/1935504
- Modena E, Pires ACV, Barônio GJ, Inforzato I, Demczuk SDB. 2012. Do fruit traits of the *Senna occidentalis* weed influence seed predation by Bruchinae? *Rev Bras Bioc.* 10:293–297.
- Morales-Silva T, Monteiro A, Faria LDB. 2018. Multitrophic web of insects associated with *Piptadenia gonoacantha* (Mart.) Macbr. (Fabaceae) and their relationship with resource traits. *Arthropod Plant Interac.* 12:553–565. DOI:10.1007/s11829-018-9602-4
- Nápoles JR, Kingsolver JM. 2009. A new species of *Acanthoscelides* Schilsky (Coleoptera: Bruchidae) from Mexico with some biological notes. *Neotrop Entomol.* 38(4):497–500. DOI:10.1590/S1519-566X2009000400009
- Ostergård H, Hambäck PA, Ehrlén J. 2007. Pre-dispersal seed predation: the role of fruit abortion and selective oviposition. *Ecology.* 88:2959–2965. DOI:10.1890/07-0346.1
- Pott A, Pott VJ, Souza TW. 2006. Plantas daninhas de pastagem na região dos Cerrados. Campo Grande (MS): EMBRAPA Gado de Corte.
- R Development Core Team. 2016. R: A language and environment for statistical computing. [accessed 2016 Mar 02]. <http://www.R-project.org>.
- Ribeiro-Costa CS, Almeida LM. 2012. Seed-Chewing Beetles (Coleoptera: Chrysomelidae, Bruchinae). In: Panizzi AR, Parra JRP, editors. *Insect bioecology and nutrition for integrated pest management*. 1st ed. Vol. 1. Boca Raton: CRC Press; p. 325–352.
- Ribeiro-Costa CS, Costa AS. 2002. Oviposition behavior of Bruchidae (Coleoptera) seed predators of *Cassia leptophylla* Vogel (Caesalpinacea), eggs morphology and description of a new species. *Rev Bras Zool.* 19:305–316. DOI:10.1590/S0101-81752002000500024

- Ribeiro-Costa CS, Sari LT, Viana JH, Manfio D. 2011. Ecologia de bruquíneos (Coleoptera: Chrysomelidae) em frutos de *Bauhinia holophylla* Steud (Caesalpinioideae) no Parque Estadual do Cerrado, Jaguariaíva, Paraná. Coletânea de Pesquisa do Parque Estadual de Vila Velha, Cerrado e Guartelá. 1:273–281.
- Richards SA. 2002. Temporal partitioning and aggression among foragers: modeling the effects of stochasticity and individual state. *Beh Ecol.* 13:427–438. DOI:10.1093/beheco/13.3.427
- Rodrigues LMS, Viana JH, Ribeiro-Costa CS, Rossi MN. 2012. The extent of seed predation by bruchine beetles (Coleoptera: Chrysomelidae: Bruchinae) in a heterogeneous landscape in southeastern Brazil. *Coleopt Bull.* 66(3):271–279. DOI:10.1649/072.066.0315
- Rossi MN, Rodrigues LMS, Ishino MN, Kestring D. 2011. Oviposition pattern and within-season spatial and temporal variation of pre-dispersal seed predation in a population of *Mimosa bimucronata* trees. *Arthropod Plant Interact.* 5:209–217. DOI:10.1007/s11829-011-9130-y
- Silva LA, Maimoni-Rodella CS, Rossi MN. 2007. A preliminary investigation of pre-dispersal seed predation by *Acanthoscelides schrankiae* Horn (Coleoptera: Bruchidae) in *Mimosa bimucronata* (DC.) Kuntze trees. *Neotrop Entomol.* 36(2):197–202. DOI:10.1590/S1519-566X2007000200005
- Sousa-Lopes B, Bächtold A, Del-Claro K. 2016. Biology, natural history and temporal fluctuation of the geometrid *Oospila pallidaria* associated with host plant phenology. *Stud Neotrop Fauna Environ.* 51:135–143.
- Stachurska-Sawakoń A, Barabasz-Krasny B, Klasa A, Palaczyk A. 2018. Reduced plant fitness by pre-dispersal seed predation in the threatened plant species *Cirsium decussatum*. *Seed Sci Res.* 28:123–130. DOI:10.1017/S0960258518000107
- Takakura K. 2002. The specialist seed predator *Bruchidius dorsalis* (Coleoptera: Bruchidae) plays a crucial role in the seed germination of its host plant, *Gleditsia japonica* (Leguminosae). *Funct Ecol.* 16:252–257. DOI:10.1046/j.1365-2435.2002.00619.x
- Tomaz CA, Kestring D, Rossi MN. 2007. Effects of the seed predator *Acanthoscelides schrankiae* on viability of its host plant *Mimosa bimucronata*. *Biol Res.* 40:281–290. DOI:10.4067/S0716-97602007000400002
- Van Klinken RD. 2005. Total annual seed loss on a perennial legume through predation by insects: the importance of within-season seed and seed feeder dynamics. *Austral Ecol.* 30:414–425. DOI:10.1111/aec.2005.30.issue-4
- Van Klinken RD, Flack LK. 2008. What limits predation rates by the specialist seed-feeder *Penthobruchus germaini* on an invasive shrub? *J Appl Ecol.* 45:1600–1611. DOI:10.1111/j.1365-2664.2008.01478.x
- Vega C, Arista M, Ortiz PL, Herrera CM, Talavera S. 2011. Endozoochory by beetles: a novel seed dispersal mechanism. *Ann Bot.* 107:629–637. DOI:10.1093/aob/mcr013
- Wu J, Baldwin IT. 2010. New insights into plant responses to the attack from insect herbivores. *Annu Rev Genet.* 44:1–24. DOI:10.1146/annurev-genet-102209-163500



ELSEVIER

Contents lists available at ScienceDirect

Acta Oecologica

journal homepage: www.elsevier.com/locate/actoec

Spatio-temporal variation in seed traits affects the occurrence and body-size pattern of a seed-feeding beetle (Chrysomelidae: Bruchinae) in Brazilian Cerrado



Bruno de Sousa-Lopes^{a,*}, Aluska Tavares dos Santos^b, Cibele Stramare Ribeiro-Costa^b, Kleber Del-Claro^a

^a Laboratório de Ecologia Comportamental e de Interações, Pós-graduação em Entomologia, Universidade de São Paulo, Avenida Bandeirantes n. 3900, CEP 14040901, Ribeirão Preto, São Paulo, Brazil

^b Laboratório de Sistemática e Bioecologia de Coleoptera, Setor de Ciências Biológicas, Departamento de Zoologia, Universidade Federal do Paraná, CEP, 81531-980, Curitiba, PR, Brazil

ARTICLE INFO

Keywords:

Acanthoscelides
Food resource quality
Life-history traits
Seed weight
Seed hardness
Mimosa setosa var. *paludosa*

ABSTRACT

Seed quality is an important bottom-up factor driving life-history traits of seed beetles (Chrysomelidae: Bruchinae). However, information on how seed traits change spatiotemporally and affect herbivores' survival is incipient in Neotropics. We evaluated how the spatio-temporal variation in seed weight, hardness, size, and water content of *Mimosa setosa* var. *paludosa* (Fabaceae: Mimosoideae) affects the occurrence and body-size pattern of the seed beetle, *Acanthoscelides quadridentatus* (Schaeffer, 1907), in Brazilian Cerrado. We collected 400 fruits from four populations of *M. setosa* var. *paludosa* in Cerrado roadsides from Goiás and Minas Gerais states and then we assessed in laboratory seed traits along with the weight and body size of *A. quadridentatus*. Additionally, we collected a total of 76 fruits (immature plus mature) in beginning and end of the fruiting phase and counted the frequencies of unhatched and hatched eggs of the seed beetle to evaluate the effect of seed hardening on beetle posture. We found greater seed damage in the population with softer, smaller and lighter seeds. Larger and heavier seeds were harder than smaller and lighter seeds; and male and female reared from larger and heavier seeds were respectively 15 and 25% larger than that reared from smaller and lighter seeds. We also found that weight and body size of *A. quadridentatus* were positively correlated with seed length, weight and water content. Unhatched eggs were found only in beginning of fruiting phase when seeds were immature and softer, suggesting the occurrence of a window of time in which females lay their eggs before seed hardening to facilitate the entry of first instar larvae. These results highlight that larger and heavier seeds are most protected against herbivory, but individuals of *A. quadridentatus* that overcome plant hardness can grow larger in size. We suggest that seed traits change across a spatio-temporal scale and affect life-history traits of *A. quadridentatus*.

1. Introduction

Host plant quality is an important bottom-up factor driving the performance and life-history traits of insect herbivores such as body-size pattern, lifespan, fecundity, and reproductive success (Janzen, 1969; Fox et al., 2007; González-Teuber et al., 2008; Price et al., 2011). In the case of seed beetles (Chrysomelidae: Bruchinae), the quality of food resource is important because many species feed in a single seed during all larval development, then any change in seed quality can affect seed-beetles' body size and other traits (Amarillo-Suárez et al., 2011; Ribeiro-Costa and Almeida, 2012). The wide phenotypic

plasticity of these beetles has been occasionally associated with seasonality (variance in humidity, precipitation, and temperature; Stillwell et al., 2007), but in most cases their plasticity is mainly related to the variation in seed quality among host plant populations (i.e., variation in weight, hardness, size, and water content; Takakura, 2004; Haga and Rossi, 2016; Canto et al., 2019).

Seeds are mature ovules containing an embryo and stored nutrients such as carbohydrates, lipids, and proteins inside a protective coat (Wall et al., 2005; Fenner and Thompson, 2005). Larger and heavier seeds may be crucial for seed beetles as they possibly contain high nutrient concentration, providing better opportunities to beetles'

* Corresponding author.

E-mail addresses: brunolopesprof@gmail.com, brunoslopes@usp.br (B.d. Sousa-Lopes), atscunha@gmail.com (A.T.d. Santos), stra@ufpr.br (C.S. Ribeiro-Costa), delclaro@ufu.br (K. Del-Claro).

<https://doi.org/10.1016/j.actao.2020.103579>

Received 25 August 2019; Received in revised form 30 March 2020; Accepted 14 April 2020

1146-609X/© 2020 Elsevier Masson SAS. All rights reserved.

development (Moegenburg, 1996; González-Teuber et al., 2008; Canto et al., 2019). For example, the seed beetles *Merobruchus terani* Kingsolver (1980) and *Stator maculatopygus* (Pic, 1930) grow to larger sizes when feeding on heavier and larger seeds of *Senegalia tenuifolia* (L.) Britton & Rose (Maia et al., 2017). In addition, the water content in seeds is crucial for seed beetles because it may facilitate nutrient assimilation, besides to represent approximately 50% of beetle's weight (Ribeiro-Costa and Almeida, 2012). Indeed, it has been suggested that variations in water content in seeds of different populations of *Leucaena leucocephala* (Lam.) de Wit (Fabaceae) explain changes in the body-size pattern of the seed beetle *Acanthoscelides macrophthalmus* (Schaeffer, 1907) (Haga and Rossi, 2016).

In comparison to other plant parts, seeds are most protected from herbivores since they are responsible for plant reproduction and therefore have a higher supply of nutrients (Bridwell, 1918; Wall et al., 2005). According to the Optimal Defense Theory (ODT), plants should allocate more defenses to structures in relation to their value and probability of attack (McKey, 1974, 1979). In this sense, we expect that seeds (especially the large ones) may be most protected against herbivory with a very thick seed coat which may affect the entry and survival of beetles' larvae of the first instar larvae (Howe and Currie, 1964; Janzen, 1969; Seifelnasr, 1991). Therefore, seed beetles can face a trade-off between the quality (e.g., weight and size of seeds) and defenses of host plants (e.g., hardness; Fox, 2000; Fox and Czesak, 2006).

In order to find food sources of superior quality and also avoid plant defenses (e.g., seed hardening), many seed beetle species synchronize their occurrence with the host-plant phenology (temporal pattern), especially in beginning of the fruiting phase, when the seeds are soft thus favoring the entry by first instar larvae that perforate the exocarp (Szentesi, 2006; Rossi et al., 2011). For instance, *Acanthoscelides schrankiae* (Horn, 1873) presents higher abundance in the field before the fruit maturation of *Mimosa bimucronata* (DC) Kuntze probably because larvae fail to penetrate the tough inner lining of the mature fruit wall (Rossi et al., 2011). However, species of seed beetles may also develop other behavioral strategies to overcome the natural hardening of seeds that apparently become a plant defense. Female seed beetles of the multivoltine species *Bruchidius dorsalis* (Fähræus, 1839) can regulate egg size according to seasonal changes in hardness of host-plant seeds (Takakura, 2004). In this case, females lay larger eggs in harder seeds in the overwintering generation, which allow larvae to drill farther into these seeds reducing larval mortality and providing large adult beetles (Takakura, 2004).

As female seed beetles face substantial fecundity selection for egg size shaped by seed traits (e.g., hardness), they generally are larger and respond faster to host plant changes in comparison to males (Fox et al., 2007). This is observed in *A. macrophthalmus* on *L. leucocephala*, in which females are commonly larger than males when collected in a wide spatial scale from Northern to South from Brazil (Minas Gerais to Rio Grande do Sul states; Haga and Rossi, 2016). However, exceptions include males of a few species of *Stator* and *M. terani* can be larger than females (Fox et al., 2007; Maia et al., 2017). In addition, it has been observed that female varied more in size than male among plant populations due to their greater sensitivity to seed traits such as water content (Haga and Rossi, 2016). Therefore, food resource quality plays a major role on the life-history traits of herbivore insects such as seed beetles, with fundamental implications in the ecology and evolution of these animals (Blanckenhorn and Demont, 2004; Kolb et al., 2007), especially on females.

Since the last century, the attention on how the variation in seed traits among plant populations affects life-history traits of seed beetles has increased exponentially (Szentesi and Jermy, 1995; Messina, 2004; Yang et al., 2006; Stotz et al., 2013; Maia et al., 2017). However, the large Neotropical genus *Acanthoscelides* Schilsky, 1905 has been relatively poorly explored (but see Kestring et al., 2009; Haga and Rossi, 2016; Sousa-Lopes et al., 2019a), although this genus has

approximately 300 species (Johnson, 1981; Nápoles and Kingsolver, 2009; Morse, 2014) and contains important pest species of stored legumes (Southgate, 1979; Baier and Webster, 1992). This lack of ecological information is mainly related to species that are not associated with stored seeds such as beans (*Phaseolus vulgaris* L.).

In order to provide more ecological information about *Acanthoscelides*, our main goals are: (1) to describe the damage rate inflicted by *Acanthoscelides quadridentatus* (Schaeffer, 1907) among populations of the host plant, *Mimosa setosa* var. *paludosa* (Benth.) Barneby (Fabaceae: Mimosoideae), in a spatial scale encompassing Goiás and Minas Gerais states (Brazil); (2) to analyze how the variation in seed traits (i.e., weight, size, and water content) among populations of *M. setosa* var. *paludosa* affects the weight and body-size pattern of *A. quadridentatus* (spatial scale); (3) to analyze whether possible high quality (i.e., larger and heavier seeds) are most protected in hardness than possible low quality seeds (i.e., smaller and lighter seeds); and (4) to evaluate if postures of *A. quadridentatus* are synchronized with the beginning of fruiting phase, possibly avoiding mature fruits and harder seeds (temporal scale). We hypothesized that: (i) the variation in seed traits among plant populations, that probably changes seed quality in a spatial scale (Stillwell et al., 2007; Haga and Rossi, 2016), explains the variation in the body-size pattern of *A. quadridentatus*, where plant population with larger and heavier seeds will provide larger seed beetles; (ii) larger and heavier seeds have also a harder seed coat since they are most expensive than smaller and lighter seeds, and according to ODT they must be most protected (McKey, 1974, 1979); (iii) *A. quadridentatus* egg laying is synchronized with the beginning of fruiting phase possibly to avoid seed hardening (temporal scale) (Rossi et al., 2011); and (iv) females are larger than males as shown for the major part of seed beetle species, with few exceptions (e.g. Fox et al., 2007), since they must be able to lay larger eggs for the first instar larvae overcome seed coat barrier.

2. Materials and methods

2.1. Study system

Mimosa setosa var. *paludosa* is an endemic shrub to Brazil and Paraguay, and it has been recorded in several Brazilian states including Acre, Bahia, Ceará, Goiás, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Rio de Janeiro, São Paulo and Tocantins (Pott et al., 2006; Dutra and Garcia, 2014). This species occurs predominantly clumped in floodplains, margin of rivers, lakes, and roadsides, especially in the Brazilian Cerrado (Lorenzi, 2000; Dutra and Garcia, 2014). The fruits are red-brown colored, dehiscent (craspedium fruits), and found mainly in the dry season (April–September). Immature fruits are bright red-brown with soft and green seeds, whereas mature fruits are dark red-brown with hard and brown seeds (Sousa-Lopes pers. obs.). This plant is important for the restoration of wetlands, but it is also an important weed in pasture areas as its large thorny branches can injure cattle (Lorenzi, 2000; Pott et al., 2006). Recently, this plant species has been extensively studied as to its ecological interactions in Brazilian Cerrado with relevant implications to the knowledge of trophic interactions (Sousa-Lopes et al., 2016, 2019a; 2019b, 2019c).

Acanthoscelides quadridentatus and *Acanthoscelides winderi* Kingsolver (1984) are American species of seed beetles recorded only for the genus *Mimosa* L. (Kingsolver, 1984; Bottimer, 1969; Kassulke et al., 1990; Escobar-Domínguez et al., 2018). Individuals develop feeding in a single seed which they kill the embryo and left exit holes to adult emergence (Sousa-Lopes et al., 2019a). In *M. setosa* var. *paludosa*, these beetles inflicted a seed damage of about 15%, representing a decrease in seed set and therefore on plant fitness (Sousa-Lopes et al., 2019a). However, further studies on seed beetles are needed to better understand how ecological attributes such as the spatio-temporal scale influence the trophic interactions in which these beetles are involved.

In this study, we analyze the effect of host plant quality in a spatio-

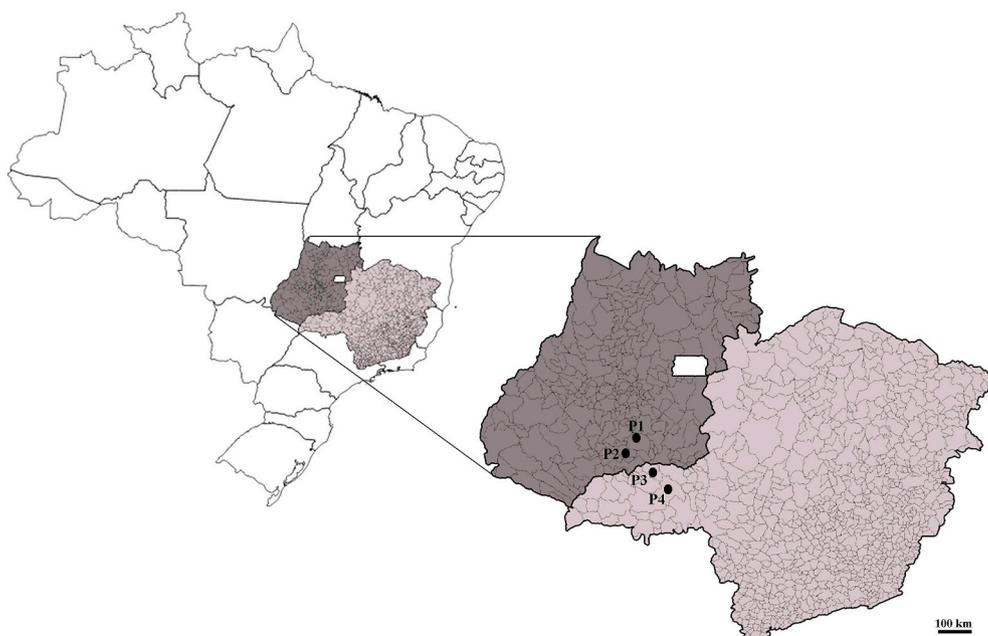


Fig. 1. Spatial distribution of the populations (P1 = Morrinhos-GO, P2 = Goiatuba-GO, P3 = Tupaciguara-MG and P4 = Uberlândia-MG) of *Mimosa setosa* var. *paludosa* (Fabaceae: Mimosoideae) from which *Acanthoscelides quadridentatus* (Chrysomelidae: Bruchinae) individuals were collected. Dark and light grey colors indicate the Brazilian states Goiás and Minas Gerais, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

temporal scale on *A. quadridentatus* since we found this species in all sampled plant populations and always with the highest abundance. This may be related to time of collection, since most fruits were collected at the end of the fruiting phase (September), and also to the temporal partitioning already recorded between *A. quadridentatus* and *A. winderi* (Sousa-Lopes et al., 2019a). Individuals were deposited at Coleção Entomológica Padre Santiago Jesus Moure (DZUP), Departamento de Zoologia, Universidade Federal do Paraná.

2.2. Assessment of fruits and beetles

In September 2017, we randomly collected 100 mature fruits in each of the four populations of *M. setosa* var. *paludosa* distributed in roadsides from the Brazilian Cerrado in the states of Goiás (GO) and Minas Gerais (MG), near the cities of Morrinhos-GO (P1 = 17° 38' 01.17" S, 49° 10' 32.26" W), Goiatuba-GO (P2 = 18° 08' 28.13" S, 49° 17' 17.59" W), Tupaciguara-MG (P3 = 18° 39' 38.39" S, 48° 39' 42.73" W) and Uberlândia-MG (P4 = 18° 58' 10.38" S, 48° 15' 55.91" W) (Fig. 1). The minimum distance among populations was 60 km (P1–P2) and the maximum distance was 240 km (P1–P4). We selected at least three different plants at least 1 m distant from each other to randomly collect the fruits of each population. After collecting, we set the fruits individually into transparent plastic containers (500 ml) covered with voile fabric. Containers were taken to the Behavioral Ecology and Interactions Laboratory at the Federal University of Uberlândia and maintained for 150 days under laboratory conditions (12-h light and 20–30 °C) until all adult emerged and posteriorly were analyzed. Damaged and undamaged seeds and *Acanthoscelides* individuals were counted.

2.3. Assessment of seed traits

To assess seed size we randomly selected 50 undamaged (without exist holes and no wither) seeds from each population of *M. setosa* var. *paludosa* and photographed them by using a stereomicroscope with scale nearest 0.1 mm (adapted from Kestring et al., 2009). Then, we used the program Image J (Schneider et al., 2012) to obtain individual seed measurements (length and width, mm). To assess seed weight (mg) we randomly chose another 50 undamaged seeds from each population of *M. setosa* var. *paludosa* and weighted them individually with a precision analytical balance (M 163 II Meter Bel Mark Balance; Monza,

Milan, Italy; mg). To assess water content, we used the same data from seed weight (above) as fresh weight, and then we dried seeds at 60 °C for 48 h. After drying all seeds, we reweighed individual seeds and water content was determined as the following: % = (fresh weight – dry weight) x 100/fresh weight (according to Haga and Rossi, 2016). Finally, to assess seed hardness we randomly selected another 50 undamaged seeds from each population and used the fruit hardness tester (Instrutherm PTR-300; São Paulo, Brazil) to measure the need strength (Newtons) to perforate individually each seed surface (adapted from Constant et al., 1996).

2.4. Assessment of weight and body size of *Acanthoscelides quadridentatus*

To assess the weight (mg) and body size (mm) of *A. quadridentatus* we randomly selected 50 individuals from each population of *M. setosa* var. *paludosa* and carefully dried those at 60 °C for 48 h, then we weighted them using a precision analytical balance (according to Maia et al., 2017). After, we measured the length (mm) of pronotum, left and right elytra through photos made with a digital camera coupled to a stereomicroscope at a standard magnification (1.5×) and with scale nearest 0.1 mm (Fig. 2). Measurements were obtained individually analyzing photos by the software Image J. The lengths of traits (pronotum, left and right elytra) of each *A. quadridentatus* were summed to obtain the body size and evaluate its relationship with seed traits (adapted from Szentesi and Jermy, 1995; Kestring et al., 2009). Male and female beetles were separated by examining the shape of the last one abdominal ventrite, which is slightly emarginated in males whereas it is straight in females. Differences between sexes can also be seen in the pygidium, which is laterally more convex in males in comparison to females (Ribeiro-Costa and Almeida, 2012). Individuals of *A. quadridentatus* in which the sex determination was difficult and/or doubtful by analysis of the last one abdominal ventrite were excluded from the assessment of weight and body size.

2.5. Assessment of seed beetles' eggs on fruits

To assess the number of seed beetles' eggs on fruits we avail another 76 fruits of *M. setosa* var. *paludosa* collected in September 2017 ($n = 20$) and April 2018 ($n = 56$) in Uberlândia-MG that have not been used in other analyses. We used fruits only from Uberlândia since that was the single locality in which we were able to collect in both dry and

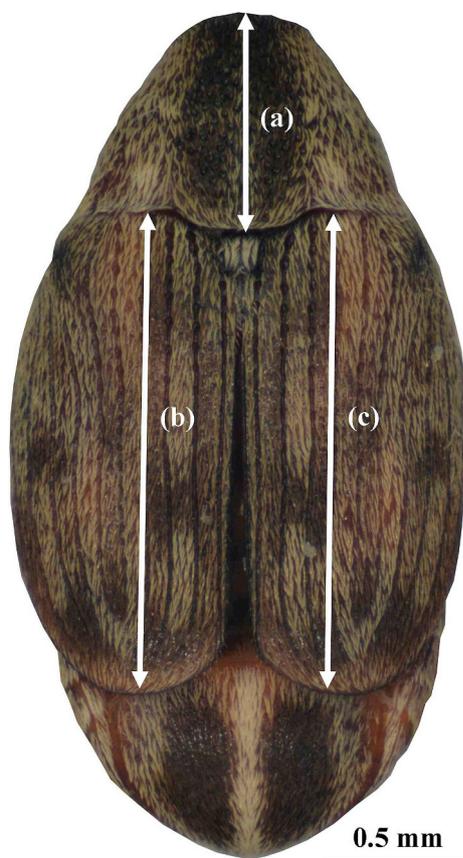


Fig. 2. Body traits of *Acanthoscelides quadridentatus* (Chrysomelidae: Bruchinae) taken to estimate its body-size pattern among populations (P1 = Morrinhos-GO, P2 = Goiatuba-GO, P3 = Tupaciguara-MG and P4 = Uberlândia-MG). The arrows indicate: (a) pronotum, (b) left elytra, and (c) right elytra.

wet season. When these fruits were taken to the laboratory we computed their stage (immature or mature; see characteristic of these fruits in the section Study system), and counted the number of hatched (*i.e.*, opaque after larval emergence; Ribeiro-Costa and Almeida, 2012) and unhatched eggs (*i.e.*, with visible chorion) with a stereomicroscope. Since it was not possible to differentiate the eggs of *A. quadridentatus* and *A. winderi*, we counted all eggs of seed beetles independently of species.

2.6. Data analysis

We used the Chi-square test (χ^2) to evaluate whether frequencies of damaged and undamaged seeds differed from the expected frequencies among populations of *M. setosa* var. *paludosa* and to evaluate whether frequencies of unhatched and hatched eggs differed between immature and mature fruits. To test whether there was difference among means of seeds and *A. quadridentatus* traits among populations we used Analyses of Variance (one-way ANOVA) followed by Tukey's post-hoc test. The unpaired *t*-test was used to evaluate whether there was a difference in body size between male and female, and also between the means of damaged seeds of Minas Gerais in comparison to Goiás. Since the conjunct of data from the spatial scale (all populations together) did not meet the assumptions of normality and homoscedasticity, we used Spearman's correlations to test which seed traits influenced weight and body size of *A. quadridentatus*. Analyses were performed in R 3.1.2 and package "PerformanceAnalytics" (R Development Core, 2016) at the 5% probability level. We also used GraphPad Prism 5 (GraphPad Company, San Diego, CA, USA) to generate graphics.

Table 1

Damaged and undamaged seeds (total number and percentage) for each population of *Mimosa setosa* var. *paludosa* (Fabaceae: Mimosoideae) in roadsides from Brazilian Cerrado. P1 = Morrinhos-GO, P2 = Goiatuba-GO, P3 = Tupaciguara-MG and P4 = Uberlândia-MG. Observed frequencies differed statistically from the expected frequencies by the Chi-square test at $P < 0.0001$.

Population	Damaged seeds		Undamaged seeds	
	Nº	%	Nº	%
P1	246	32	518	68
P2	306	36	547	64
P3	260	52	241	48
P4	316	58	229	42

3. Results

We found 314 individuals of *Acanthoscelides* associated with the four populations of *M. setosa* var. *paludosa*, being 82% ($n = 258$) of them belonging to *A. quadridentatus*. Regarding seed predation, we found a difference in frequencies of damaged and undamaged seeds among populations of *M. setosa* var. *paludosa* ($\chi^2 = 120.1$, $P < 0.0001$). The highest percentage of damage occurred in P4 (Minas Gerais), whereas the smallest was in P1 (Goiás, Table 1), although there was no significant difference between the means of damaged seeds of Minas Gerais in comparison to Goiás ($t = -1.14$, $P = 0.29$).

We found significant differences in seed traits among populations, with highest mean values in P1 followed by P2, P3 and P4, respectively (hardness: $F = 52.36$, $P < 0.0001$; Length: $F = 54.49$, $P < 0.0001$; weight: $F = 92.99$, $P < 0.001$; Width: $F = 84.80$, $P < 0.0001$). The exception occurred for water content in which P3 had higher amount compared with other populations ($F = 26.06$, $P < 0.0001$; Table 2). There was a positive and significant relationship between seed length, weight, hardness, and water content (Fig. 3), indicating that larger and heavier seeds possess a higher amount of water, but they are also most protected in hardness.

Beetles were larger in P1 followed by P2, P3 and P4, although a significant difference was found only between males of P1 and P4 ($F = 36.86$, $P < 0.001$, Tukey post-hoc test = $P < 0.01$), and females of P1, P2 and P3 in comparison to P4 ($F = 26.49$, $P < 0.0001$, Tukey post-hoc test = $P < 0.01$). Males and females from P1 were approximately 15 and 25% larger than P4, respectively. In general, females were significantly larger than males ($t = 2.16$, $P = 0.03$), although in P4 the mean value of female's body size was lower than male's (Table 3). On the other hand, we did not find difference in weight of male ($F = 2.51$, $P > 0.05$) and female ($F = 4.48$, $P > 0.05$) among populations (Table 3). We also found a positive and significant correlation between seed length, weight, hardness, and water content with body size of male and female, and weight of female *A. quadridentatus* (Fig. 3).

Unhatched eggs were found only in immature fruits collected in April 2018, and it was found a significant difference in frequencies of unhatched and hatched eggs between immature and mature fruits ($\chi^2 = 32.35$, $P < 0.0001$; Fig. 4).

4. Discussion

Our initial hypotheses were corroborated: first, changes in seed traits explained the variation in the body-size pattern of *A. quadridentatus* among populations; second, larger and heavier seeds were harder than smaller and lighter seeds; third, *A. quadridentatus* egg laying was synchronized with the beginning of fruiting phase; and fourth, female *A. quadridentatus* were larger than male among populations (with the exception of P4). We also found a higher percentage of seed damage in *M. setosa* var. *paludosa* population (P4) which seeds were softer. Although we showed some evidence of synchronicity

Table 2

Seed traits (mean ± SD) from each population of *Mimosa setosa* var. *paludosa* (Fabaceae: Mimosoideae) in roadsides from Brazilian Cerrado. P1 = Morrinhos-GO, P2 = Goiatuba-GO, P3 = Tupaciguara-MG and P4 = Uberlândia-MG. Different letters represent that means differed statistically by Tukey's post-hoc test at $P < 0.05$.

Population	Hardness(N)	Length(mm)	Water content (%)	Weight(mg)	Width (mm)
P1	154.13(± 27.84)a	3.27(± 0.32)a	37(± 20)a	0.028(± 0.030)ab	2.37(± 0.23)a
P2	151.91(± 37.46)a	3.20(± 0.47)a	37(± 25)a	0.024(± 0.005)a	2.38(± 0.31)a
P3	144.37(± 39.31)a	3.18(± 0.50)a	68(± 19)b	0.021(± 0.005)a	2.34(± 0.38)a
P4	72.41(± 45.95)b	2.34(± 0.36)b	32(± 27)a	0.016(± 0.017)ac	1.55(± 0.30)b

between beetle egg laying and immature fruit availability, we suggest that this phenomenon must be carefully evaluated. It is because we were able to assess egg laying only in one population and long-term studies are still necessary to confirm this pattern.

Seed weight, size, and water content proved to be important factors influencing weight and body size of seed beetles (Moegenburg, 1996; Maia et al., 2017). For example, the weight of *Caryobruchus gleditsiae* (Linnaeus, 1763) was positively correlated with the weight of seed consumed, which was also correlated with total seed biomass of *Sabal palmetto* (Walter) Lodd. ex Schult. & Schult. f. (Moegenburg, 1996). It was also shown that larger individuals of *M. terani* and *S. maculatopygus* were found feeding on larger and heavier seeds of *S. tenuifolia* (Maia et al., 2017). In addition, water content was the factor that most explained variation in body size of *A. macrophthalmus* in a spatial scale (Haga and Rossi, 2016). We also found that seed length, weight, and water content were positively correlated with the weight and body size of *A. quadridentatus*, being seed weight one of the most important factors affecting the body-size pattern of this beetle species. Indeed, in P1, where we found heavier seeds, we also found larger beetles, whereas, in P4, where we found lighter seeds, we found smaller beetles. In this sense, it is possible that heavier seeds might be more nutritious because they contain a higher proportion of carbohydrates, lipids, and proteins as well as water content which provides more available resources to seed beetles, and thus affecting their body-size pattern, survival, and

reproductive success (Mitchell, 1975; Messina, 2004; Fox et al., 2007; Amarillo-Suárez et al., 2011).

We found greater seed hardness in P1 and lower seed hardness in P4 and, consequently, P1 had lower seed damage than P4. This result fits in the ODT (McKey, 1974, 1979; Rhoades, 1979), suggesting that expensive resources (i.e., larger and heavier seeds) are also most protected and therefore less damaged by seed beetles. However, individuals of *A. quadridentatus* that had overcome seed hardness in P1 could enter into the seeds, intake high amount of nutrients and then grow to larger sizes. Seed hardness was the reason that Seifelnas (1991) provided for the nondevelopment of some individuals of *Callosobruchus maculatus* (Fabricius, 1775) in seeds of *P. vulgaris*. Nevertheless, as shown here, there is no indication that large seeds with harder seed coat are physically inimical to seed beetles' development since some individuals can overcome this plant defense (Janzen, 1969).

Other studies on the tropical region suggested that seed beetles seem to have developed a temporal strategy to avoid seed hardness by synchronizing their life cycle with the beginning of fruiting phase (e.g., Brazil; Sari et al., 2005). In this way, when females lay their eggs on immature seeds and fruits that therefore have softer fruit/seed surface, they probably facilitate the penetration of first instar larvae in fruits and seeds. For instance, it was shown that *A. schrankiae* and *Sennius crudelis* Ribeiro-Costa and Reynaud (1998) occur in higher abundance in the field before maturation of *M. bimucronata* and *Senna multijuga*

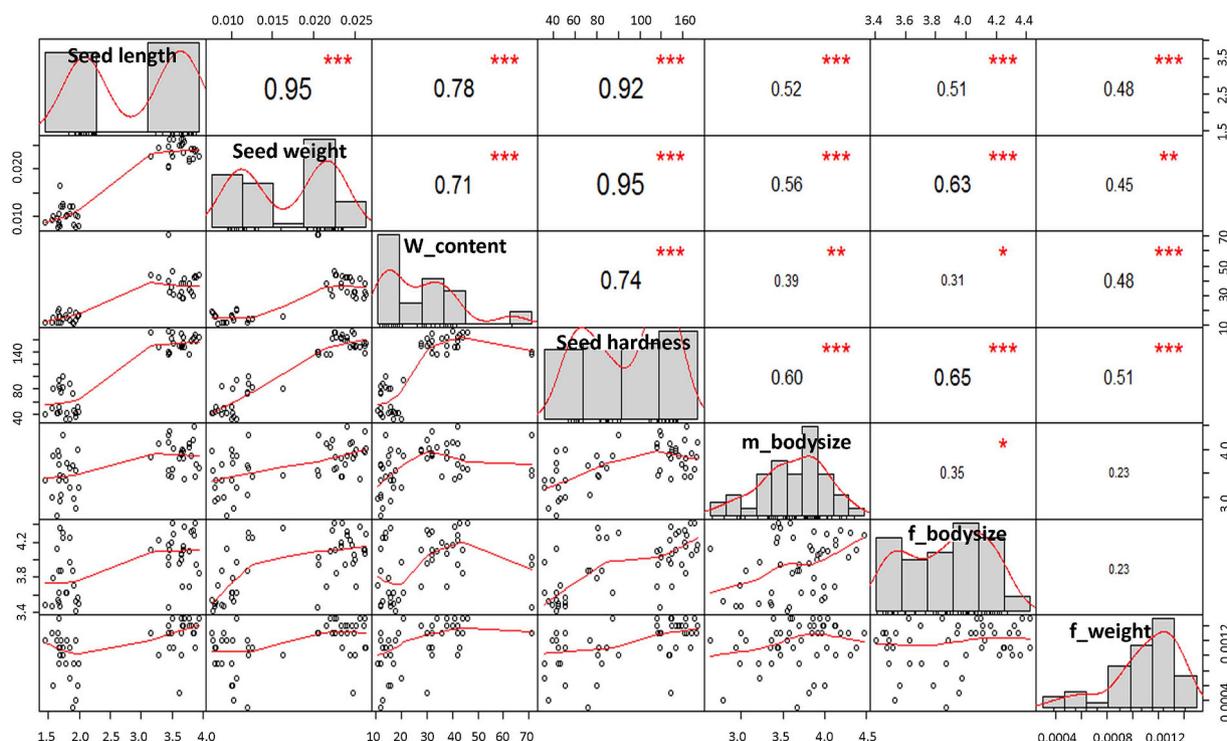


Fig. 3. Correlation matrix between traits of the host plant *Mimosa setosa* var. *paludosa* (seed length, weight, water content, and hardness) and *Acanthoscelides quadridentatus* (male body size, and female body size and weight). Data are presented in bars, scatter plots and with Spearman correlation values for each relationship. Analyses must be performed taking into account lines versus columns. * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$.

Table 3

Weight (mean \pm SD) and body size (mean \pm SD) of *Acanthoscelides quadridentatus* (Chrysomelidae: Bruchinae) according to the population of *Mimosa setosa* var. *paludosa* (Fabaceae: Mimosoideae) where they were collected. P1 = Morrinhos-GO, P2 = Goiatuba-GO, P3 = Tupaciguara-MG and P4 = Uberlândia-MG; M = male and F = female. Body size is the sum of left elytra, pronotum, and right elytra. Different letters represent that means differed statistically by Tukey's post-hoc test at $P < 0.05$.

Population	Weight (mg)		Body size (mm)	
	M	F	M	F
P1	0.0011(\pm 0.0003)a	0.0013(\pm 0.0003)a	3.92(\pm 0.30)ab	4.28(\pm 0.28)a
P2	0.0009(\pm 0.0003)a	0.0012(\pm 0.0002)a	3.84(\pm 0.23)a	3.96(\pm 0.14)a
P3	0.0009(\pm 0.0002)a	0.0010(\pm 0.0004)a	3.77(\pm 0.44)a	3.96(\pm 0.31)a
P4	0.0009(\pm 0.0003)a	0.0009(\pm 0.0004)a	3.44(\pm 0.42)ac	3.39(\pm 0.47)b

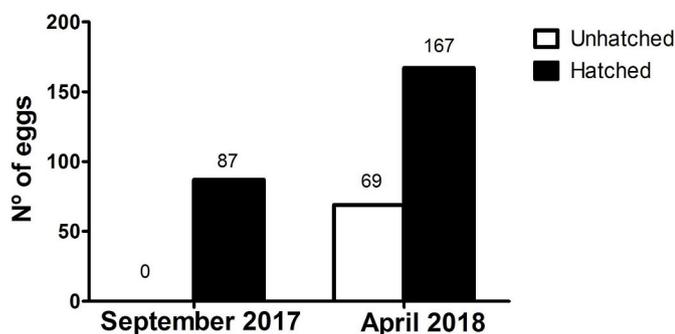


Fig. 4. Frequency of unhatched and hatched eggs of *Acanthoscelides* beetles (Chrysomelidae: Bruchinae) in the end (September 2017) and beginning of fruiting phase (April 2018) on fruits of *Mimosa setosa* var. *paludosa* (Fabaceae: Mimosoideae).

(Rich.) H. S. Irwin & Barneby fruits, respectively. That apparently occurs because it is easier for the first instar larvae to penetrate fruits' wall (Rossi et al., 2011; Sari et al., 2005). In this study we found unhatched eggs only in the beginning of the fruiting phase, which suggests that females can make postures before fruit ripping for first instar larvae avoid seed hardening. Thus, seed hardness is an important bottom-up factor which plays a crucial role on the occurrence and survival of *A. quadridentatus*, although further long-term studies are still necessary to confirm that. Other plant traits such as age, size and defensive chemical compounds may also change among populations of *M. setosa* var. *paludosa* and play an important role (spatial scale), although unexplored here (see Janzen, 1969; Sari et al., 2005; Kergoat et al., 2005; Ribeiro-Costa and Almeida, 2012). Our data suggest that earliest arriving females may found a window of time with better opportunities (i.e., softer seeds) to lay their eggs.

In general, females of seed beetles are larger than males, with few exceptions such as in *M. terani* and *Stator* spp. (Fox et al., 2007; Maia et al., 2017). Here we also found that females of *A. quadridentatus* are larger than males, although in P4 males were larger than females. It is possible that the selective pressure for fecundity is higher in females from populations with harder seeds (P1, P2, and P3) since they must lay larger eggs to enable big larvae to overcome seed hardness. Indeed, it has been showed that female of the seed beetle, *B. dorsalis*, can regulate egg size according to the hardness of host-plant seeds (Takakura, 2004). On the other hand, as P4 seeds are softer, the selective pressure to females oviposit larger eggs may be weak compared to other populations, which explains smaller females. In this case, it is possible that stronger selective pressure occurs in males. For example, in many species of seed beetles males transfer nuptial gifts to females in the form of a large volume of seminal fluid, in many cases contributing with 6–10% of their weight to females during mating (Savalli and Fox, 1998; Fox et al., 2007). Thus, in poor seeds as that from P4, it is possible that females need male nuptial gifts to survive and produce eggs, which would justify a male-biased size in this population. Nevertheless, we neither address fecundity questions or measure egg size in this study then

further studies on these subjects are still necessary to better understand the ecology of *A. quadridentatus*.

5. Conclusion

Overall, we showed that the variation in seed traits in the spatial scale (~240 km), especially weight, affected the body-size pattern of *A. quadridentatus*, highlighting a phenotypic plasticity in this beetle among host plant populations. Additionally, we showed that the temporal variation in seed hardness during the fruiting phase is another important bottom-up factor influencing postures and possibly the survival of *A. quadridentatus*. This study reports new information about the ecology of *A. quadridentatus*, and the results found here suggest that the spatio-temporal scale is an important ecological component driving variations in seed traits and consequently life-history traits of the associated seed beetle. However, large scale studies including questions about synchrony, fecundity and egg size of *A. quadridentatus*, and other host plant traits such as age, size and amount of chemical compounds will contribute to a deeper understanding on the ecology and evolution of this interaction.

Acknowledgements

We thank Nayane Alves da Silva and Melissa Alves Lopes for their encouragement and help with fieldwork, and Samara Andrade for reviewing the English of this manuscript. We also thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (grants 142280/2016-0, 119082/2017-9, 311792/2013-8, 301605/2013-0) and Fundação de Amparo à Pesquisa de Minas Gerais for funding (KDC-PM).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.actao.2020.103579>.

Author's contribution

BSL and KDC conceived the ideas, designed methodology, collected and analyzed the data. BSL, ATS, CSRC and KDC led the writing of the manuscript, contributing critically to the drafts and gave final approval for publication.

References

- Amarillo-Suárez, A.R., Stillwell, R.C., Fox, C.W., 2011. Natural selection on body size is mediated by multiple interacting factors: a comparison of beetle populations varying naturally and experimentally in body size. *Ecol. Evol.* 1, 1–14. <https://doi.org/10.1002/ece3.1>.
- Baier, A.H., Webster, B.D., 1992. Control of *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae) in *Phaseolus vulgaris* L. seed stored on small farms I. Evaluation of damage. *J. Stored Prod. Res.* 28, 289–293. [https://doi.org/10.1016/0022-474X\(92\)90011-E](https://doi.org/10.1016/0022-474X(92)90011-E).
- Blanckenhorn, W.U., Demont, M., 2004. Bergmann and converse Bergmann latitudinal clines in arthropods: two ends of a continuum? *Integr. Comp. Biol.* 44, 413–424. <https://doi.org/10.1093/icb/44.6.413>.
- Bottimer, L.J., 1969. Bruchidae associated with *Mimosa* with the description of a new

- species. *Can. Entomol.* 101, 1186–1198. <https://doi.org/10.4039/Ent1011186-11>.
- Bridwell, J.C., 1918. Notes on the Bruchidae and their parasites in the Hawaiian Islands. *Proc. Hawaii. Entomol. Soc.* 3, 465–505.
- Canto, A., Rodríguez, R., Reyes-Navelo, E., 2019. Relationship between the weights of seed beetles of the genus *Megacerus* Fähræus, 1839 (Coleoptera: Chrysomelidae: Bruchinae) and their host seeds of the family Convolvulaceae. *Sci. Rep.* 9, 8438. <https://doi.org/10.1038/s41598-019-44761-8>.
- Constant, B., Grenier, S., Febvay, G., Bonnot, G., 1996. Host plant hardness in oviposition of *Macrolophus caliginosus* (Hemiptera: Miridae). *J. Econ. Entomol.* 89, 1446–1452. <https://doi.org/10.1093/jee/89.6.1446>.
- Dutra, V.F., Garcia, F.C.P., 2014. *Mimosa* L. (Leguminosae-Mimosoideae) dos campos rupestres de Minas Gerais, Brasil. *Iheringia Ser. Bot.* 69, 49–88.
- Escobar-Domínguez, A.A., Romero-Nápoles, J., Equihua-Martínez, A., Carrilo-Sánchez, J.L., Ramírez-Alarcón, S., 2018. Bruchids (Coleoptera: Bruchidae) associated with seeds of species of *Mimosa* L. genus (Leguminosae: Mimosoideae). *Acta Zool. Mex.* 34, 1–17. <https://doi.org/10.21829/azm.2018.3412149>.
- Fenner, M., Thompson, K., 2005. *The Ecology of Seeds*. Cambridge University Press, Cambridge.
- Fox, C.W., 2000. Natural selection on seed-beetle eggs size in nature and the laboratory: variation among environments. *Ecology* 81 (11), 3029–3035. [https://doi.org/10.1890/0012-9658\(2000\)081\[3029:NSOSBE\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2000)081[3029:NSOSBE]2.0.CO;2).
- Fox, C.W., Czesak, M.E., 2006. Selection on body size and sexual size dimorphism differs between host species in a seed-feeding beetle. *J. Evol. Biol.* 19 (4), 1167–1174. <https://doi.org/10.1111/j.1420-9101.2006.01092.x>.
- Fox, C.W., Stillwell, R.C., Moya-Laraño, J., 2007. Variation in selection, phenotypic plasticity, and ecology of sexual size dimorphism in two seed-feeding beetles. In: Fairbairn, D.J., Blanckenhorn, W.U., Székely, T. (Eds.), *Sex, Size and Gender Roles: Evolutionary Studies of Sexual Size Dimorphism*. Oxford University Press, Oxford, NY, pp. 88–96.
- González-Teuber, M., Segovia, R., Gianoli, E., 2008. Effects of maternal diet and host quality on oviposition patterns and offspring performance in a seed beetle (Coleoptera: Bruchidae). *Naturwissenschaften* 95, 609–615. <https://doi.org/10.1007/s00114-008-0361-4>.
- Haga, E.B., Rossi, M.N., 2016. The effect of seed traits on geographic variation in body size and sexual size dimorphism of the seed-feeding beetle *Acanthoscelides macrophthalmus*. *Ecol. Evol.* 6 (19), 6892–6905. <https://doi.org/10.1002/ece3.2364>.
- Horn H., G., 1873. Revision of the Bruchidae of the United States. *Transactions of the American Entomological Society* 4, 311–342.
- Howe, R.W., Currie, J.E., 1964. Some laboratory observations on the rates of development, mortality and oviposition of several species of Bruchidae breeding in stored pulses. *Bull. Entomol. Res.* 55, 437–477. <https://doi.org/10.1017/S0007485300049580>.
- Janzen, D.H., 1969. Seed-eaters versus seed size, number, toxicity and dispersal. *Evolution* 23 (1), 1–27. <https://doi.org/10.2307/2406478>.
- Johnson, C.D., 1981. Interactions between bruchid (Coleoptera) feeding guilds and behavioral patterns of fruits of the Leguminosae. *Environ. Entomol.* 10, 249–253. <https://doi.org/10.1093/ee/10.2.249>.
- Kassulke, R.C., Harley, K.L.S., Maynard, G.V., 1990. Host specificity of *Acanthoscelides quadridentatus* and *A. puniceus* (Col.: Bruchidae) for biological control of *Mimosa pigra* (with preliminary data on their biology). *Entomophaga* 35 (1), 85–96. <https://doi.org/10.1007/BF02374305>.
- Kergoat, G.J., Delobel, A., Fédère, G., Rü, B.L., Silvain, J.-F., 2005. Both host-plant phylogeny and chemistry have shaped the African seed-beetle radiation. *Mol. Phylogenet. Evol.* 35 (3), 602–611. <https://doi.org/10.1016/j.ympev.2004.12.024>.
- Kestring, D., Menezes, L.C.R., Tomaz, C.A., Lima, G.P.P., Rossi, M.N., 2009. Relationship among phenolic contents, seed predation, and physical seed traits in *Mimosa bimucronata* plants. *J. Plant Biol.* 52, 569–576. <https://doi.org/10.1007/s12374-009-9073-3>.
- Kingsolver M., J., 1980. Eighteen new species of Bruchidae, principally from Costa Rica, with host records and distributional notes (Insecta: Coleoptera). *Proceedings of the Biological Society of Washington* 93, 229–283.
- Kingsolver, J.M., 1984. *Acanthoscelides winderi*, new species, (Coleoptera, Bruchidae) associated with *Mimosa* spp. (Leguminosae, Mimosoideae) from Brazil. *Entomol. News* 95, 87–90.
- Kolb, A., Ehrlén, J., Eriksson, O., 2007. Ecological and evolutionary consequences of spatial and temporal variation in pre-dispersal seed predation. *Perspect. Plant Ecol. Syst.* 9, 79–100. <https://doi.org/10.1016/j.ppees.2007.09.001>.
- Lorenzi, H., 2000. Plantas daninhas do Brasil: terrestres, aquáticas, parasitas e tóxicas. Instituto Plantarum, Nova Odessa, SP.
- Maia, L.F., Tuller, J., Faria, L.D.B., 2017. Morphological traits of two seed-feeding beetle species and the relationship to resource traits. *Neotrop. Entomol.* 46, 36–44. <https://doi.org/10.1016/j.rbe.2017.04.003>.
- McKey, D., 1974. Adaptive patterns in alkaloid physiology. *Am. Nat.* 108 (961), 305–320.
- McKey, D., 1979. The distribution of plant secondary compounds within plants. In: Rosenthal, G.A., Janzen, D.H. (Eds.), *Herbivores: Their Interactions with Secondary Plant Metabolites*. Academic press, New York, pp. 55–133.
- Mitchell, R., 1975. The evolution of oviposition tactics in the vean weevil, *Callosobruchus maculatus* (F.). *Ecology* 56, 696–702.
- Messina, F.J., 2004. Predictable modification of body size and competitive ability following a host shift by a seed beetle. *Evolution* 58, 2788–2797. <https://doi.org/10.1554/04-372>.
- Moegenburg, S.M., 1996. *Sabal palmetto* seed size: causes of variation, choices of predators, and consequences for seedlings. *Oecologia* 106 (4), 539–543. <https://doi.org/10.1007/BF00329713>.
- Morse, G.E., 2014. Bruchinae Latreille, 1802. In: Leschen, R.A.B., Beutel, R.G. (Eds.), *Handbook of Zoology, Coleoptera Volume 3: Morphology and Systematics* (Phytophaga). Walter de Gruyter, Berlin, pp. 189–198.
- Nápoles, J.R., Kingsolver, J.M., 2009. A new species of *Acanthoscelides* Schilsky (Coleoptera: Bruchidae) from Mexico with some biological notes. *Neotrop. Entomol.* 38 (4), 497–500. <https://doi.org/10.1590/S1519-566X2009000400009>.
- Pott, A., Pott, V.J., Souza, T.W., 2006. Plantas daninhas de pastagem na região dos Cerrados. EMBRAPA Gado de Corte, Campo Grande, MS.
- Price, P.W., Denno, R.F., Eubanks, M.D., Finke, D.L., Kaplan, I., 2011. *Insect Ecology: Behavior, Populations and Communities*. Cambridge University Press, New York, pp. 764p.
- R Development Core Team, 2016. R: a language and environment for statistical computing. Available form: <http://www.R-project.org>.
- Rhoades, D.F., 1979. Evolution of plant defense against herbivores. In: Rosenthal, G.A., Janzen, D.H. (Eds.), *Herbivores: Their Interactions with Secondary Plant Metabolites*. Academic press, New York, pp. 1–55.
- Ribeiro-Costa, C.S., Almeida, L.M., 2012. Seed-chewing beetles (Coleoptera: Chrysomelidae, Bruchinae). In: Panizzi, A.R., Parra, J.R.P., Org (Eds.), *Insect Bioecology and Nutrition for Integrated Pest Management*. Ied. CRC Press, Boca Raton, pp. 325–352.
- Ribeiro-Costa S., C., Reynaud T., D., 1998. Bruchids from *Senna multijuga* (Rich) I. & B. (Caesalpinaceae) in Brazil with descriptions of two new species. *Coleopterists Bulletin* 52 (3), 245–252.
- Rossi, M.N., Rodrigues, L.M.S., Ishino, M.N., Kestring, M., 2011. Oviposition pattern and within-season spatial and temporal variation of pre-dispersal seed predation in a population of *Mimosa bimucronata* trees. *Arthropod Plant Interact.* 5, 209–217. <https://doi.org/10.1007/s11829-011-9130-y>.
- Sari, L.T., Ribeiro-Costa, C.S., Roper, J.J., 2005. Dinâmica populacional de bruquíneos (Coleoptera, Chrysomelidae) em *Senna multijuga* (Rich.) H. S. Irwin & Barneby (Caesalpinaceae). *Rev. Bras. Zool.* 22 (1), 169–174. <https://doi.org/10.1590/S0101-81752005000100019>.
- Savalli, U.M., Fox, C.W., 1998. Genetic variation in paternal investment in a seed beetle. *Anim. Behav.* 56 (4), 953–961. <https://doi.org/10.1006/anbe.1998.0853>.
- Schaeffer, C., 1907. New Bruchidae with notes on known species and list of species known to occur at Brownsville, Texas, and in the Huachuca Mountains, Arizona. *Bulletin Inst Arts Sci Brooklyn* 1907 (10), 291–306.
- Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9 (7), 671–675. <https://doi.org/10.1038/nmeth.2089>.
- Seifelnar, Y.E., 1991. The role of asparagine and seed coat thickness in resistance of *Phaseolus vulgaris* (L.) to *Callosobruchus maculatus* (F.) (Col., Bruchidae). *J. Appl. Entomol.* 111, 412–417. <https://doi.org/10.1111/j.1439-0418.1991.tb00342.x>.
- Stillwell, R.C., Morse, G.E., Fox, C.W., 2007. Geographic variation in body size and sexual size dimorphism of a seed-feeding beetle. *Am. Nat.* 170, 358–369.
- Stotz, G.C., Suarez, L.H., Gonzales, W.L., Gianoli, E., 2013. Local host adaptation and use of a novel host in the seed beetle *Megacerus eulophus*. *PLoS One* 8, e53892. <https://doi.org/10.1371/journal.pone.0053892>.
- Szentesi, A., 2006. Pre-dispersal seed predation by *Bruchidius villosus* (Coleoptera, Bruchidae) in *Laburnum anagyroides* (Fabaceae, Genisteae). *Community Ecol.* 7, 13–22. <https://doi.org/10.1556/ComEc.7.2006.1.2>.
- Szentesi, A., Jermy, T., 1995. Predispersal seed predation in leguminous species: seed morphology and bruchid distribution. *Oikos* 73, 23–32.
- Sousa-Lopes, B., Bächtold, A., Del-Claro, K., 2016. Biology, natural history and temporal fluctuation of the geometrid *Oospila pallidaria* associated with host plant phenology. *Stud. Neotrop. Fauna Environ.* 51, 135–143. <https://doi.org/10.1080/01650521.2016.1199140>.
- Sousa-Lopes, B., Alves-da-Silva, N., Ribeiro-Costa, C.S., Del-Claro, K., 2019a. Temporal distribution, seed damage and notes on the natural history of *Acanthoscelides quadridentatus* and *Acanthoscelides winderi* (Coleoptera: Chrysomelidae: Bruchinae) on their host plant, *Mimosa setosa* var. *paludosa* (Fabaceae), in the Brazilian Cerrado. *J. Nat. Hist.* 53 (9–10), 611–623. <https://doi.org/10.1080/00222933.2019.1606358>.
- Sousa-Lopes, B., Alves-da-Silva, N., Alves-Martins, F., Del-Claro, K., 2019b. Antiherbivore protection and plant selection by the lynx spider *Peucetia flava* (Araneae: Oxyopidae) in the Brazilian Cerrado. *J. Zool.* 308 (2), 121–127. <https://doi.org/10.1111/jzo.12662>.
- Sousa-Lopes, B., Whitfield, J.B., Salgado-Neto, G., Del-Claro, K., 2019c. *Cotesia itororensis* sp. nov. from Brazilian savanna: a new reared microgasterinae wasp described using an integrative taxonomic approach. *Zootaxa* 4544 (3), 437–445. <https://doi.org/10.11646/zootaxa.4544.3.9>.
- Southgate, B.J., 1979. Biology of the Bruchidae. *Annu. Rev. Entomol.* 24, 449–473. <https://doi.org/10.1146/annurev.en.24.010179.002313>.
- Takakura, K., 2004. Variation in egg size within and among generations of the bean weevil, *Bruchidius dorsalis* (Coleoptera: Bruchidae): effects of host plant quality and paternal nutritional investment. *Ann. Entomol. Soc. Am.* 97, 346–352. [https://doi.org/10.1603/0013-8746\(2004\)097.\[0346:VIESWA\]2.0.CO;2](https://doi.org/10.1603/0013-8746(2004)097.[0346:VIESWA]2.0.CO;2).
- Wall, S.B.V., Forget, P.M., Lambert, J.E., Hulme, P.E., 2005. Seed fate pathways: filling the gap between parent and offspring. In: Forget, P.M., Lambert, J.E., Hulme, P.E., Wall, S.B.V. (Eds.), *Seed Fate: Predation, Dispersal and Seedling Establishment*. CABI Publishing, Wallingford, pp. 1–8.
- Yang, R.L., Fushing, H., Horng, S.B., 2006. Effects of search experience in a resource-heterogeneous environment on the oviposition decisions of the seed beetle, *Callosobruchus maculatus* (F.). *Ecol. Entomol.* 31 (4), 285–293. <https://doi.org/10.1111/j.1365-2311.2006.00696.x>.

Antiherbivore protection and plant selection by the lynx spider *Peucetia flava* (Araneae: Oxyopidae) in the Brazilian Cerrado

B. de Sousa-Lopes¹, N. Alves-da-Silva², F. Alves-Martins³ & K. Del-Claro² 

¹ Laboratório de Ecologia Comportamental e de Interações, Programa de Pós-Graduação em Entomologia, Universidade de São Paulo, São Paulo, Brazil

² Laboratório de Ecologia Comportamental e de Interações, Universidade Federal de Uberlândia, Minas Gerais, Brazil

³ Museo Nacional de Ciencias Naturales, Madrid, España

Keywords

entrapped carrion; Fabaceae; facultative mutualisms; herbivore damage; *Mimosa*; spider-plant interactions; tri-trophic interactions.

Correspondence

Kleber Del-Claro, Laboratório de Ecologia Comportamental e de Interações, Universidade Federal de Uberlândia, Rua Ceará, s/n. Bloco 2D-Campus Umuarama, 38400902 Uberlândia, Minas Gerais, Brazil.
Email: delclaro@ufu.br

Editor: Gabriele Uhl

Received 5 July 2018; revised 16 January 2019; accepted 6 February 2019

doi:10.1111/jzo.12662

Abstract

Spiders are ubiquitous in most vegetation, however very little empirical data are available on specific spider–plant interactions and their reciprocal outcomes. In the Brazilian Cerrado, the plant *Mimosa setosa* var. *paludosa*, (Fabaceae) has glandular trichomes in its leaves and stems, commonly entrapping insects (i.e. carrion) as well as hosting the lynx spider, *Peucetia flava*. We hypothesized that: (1) the damage inflicted by exophytic (leaves) and endophytic (seeds) herbivore insects that overcome the glandular trichomes is lower in plants where the lynx spider is present; and (2) the presence of this predator is positively related to food availability (live insects and/or carrion) and plant size. We performed field observations and an experimental field study in terms of the spider's presence versus absence on the Fabaceae plants. Our results showed that the proportion of damaged foliolules on the Fabaceae plants differed between the spiders-present and spiders-absent treatments, and that the absence of spiders led to a 3.3-fold increase in the number of damaged foliolules. However, there was no significant difference in the proportion of seeds taken by endophytic herbivores from branches with and without spiders. We also found that the presence of *Peucetia flava* was positively related to the presence of entrapped carrion on plants, and that there was a positive and marginally significant effect of increasing abundance of the spiders on taller plants. The results of this study suggest that it is more common to find lynx spiders interacting with *M. setosa* var. *paludosa* in larger plants with higher carrion abundance (food source), and that *P. flava* and *M. setosa* var. *paludosa* interact in a facultative mutualism, in which plants provide entrapped carrion for spiders to feed on and possibly facilitate prey manipulation. In return, lynx spiders decrease the damage inflicted by exophytic, but not by endophytic herbivores. These results also contribute to a better understanding of which ecological factors may affect plant selection by lynx spiders and what the influence of this predator is on the structure of food webs in glandular plants.

Introduction

Plants represent a complex of microhabitats hosting several animals and giving place to different ecological interactions, which vary from antagonisms to mutualisms (Janzen, 1967; Heil, 2008; Price *et al.*, 2011). Spiders are among the most ubiquitous arthropods found on plants (Foelix, 1996), and in some cases, they play an important role as plant guards into facultative mutualisms (Whitney, 2004; Vasconcellos-Neto *et al.*, 2017). In these relationships, plants provide suitable microhabitats for spiders forage, breed and shelter (Romero & Vasconcellos-Neto, 2005); spiders, in return, can benefit the host plant by preying on herbivores and

decreasing damage in plant tissues (Morais-Filho & Romero, 2010). However, very few empiric data are available in the literature regarding specific spider–plant interactions and which plant traits affect plant selection by spiders (Vasconcellos-Neto *et al.*, 2017), despite the fact that their impact on the reproductive success of plants have received increased attention in recent years (Del-Claro *et al.*, 2016).

The plant selection by spiders may be strongly influenced by the availability of live arthropods on plants, which represent a food source for spiders (Morais-Filho & Romero, 2008). However, increasing the abundance of several predators including spiders may be also related to the abundance of dead

insects adhered to plant surfaces (hereafter: carrion) (Krimmel & Pearce, 2014; LoPresti, Krimel & Pearce, 2018). The carrion would represent an accessible food source and without costs associated with attacking and manipulating live preys, making that predators remain on plants with reliable carrion sources (LoPresti, Pearce & Charles, 2015). On the other hand, plant size is also another important factor influencing plant selection by spiders (Lawton, 1983; Halaj, Ross & Moldenke, 2000; Langellotto & Denno, 2004). Large plants are more conspicuous and attractive for insects from different herbivore guilds (e.g. endophytic and exophytic herbivores) due to their wide range of microhabitats and resources (Schlinkert *et al.*, 2015), thus providing more abundant and variable food sources for spiders (Romero & Vasconcellos-Neto, 2005). Indeed, spiders can select the host plant according to the plant height (Morais-Filho & Romero, 2008).

The lynx spider *Peucetia flava* Keyserling 1877 (Araneae: Oxyopidae; Fig. 1a) is commonly found on glandular trichome-bearing plants worldwide (Vasconcellos-Neto *et al.*, 2007; Morais-Filho and Romero, 2009). These plants have sticky hairs that, in addition to the defensive functions against herbivores, facilitate prey capture and provide carrion as a food source for lynx spiders, and thereby increasing spider fidelity to the host plant (Romero, Souza & Vasconcellos-Neto, 2008). Indeed, lynx spiders have been recorded feeding on carrion (scavenging behavior; Romero *et al.*, 2008), although the effect of the carrion abundance on plant selection by lynx spiders is poorly understood. Lynx spiders, in return to the benefits given by the host plant, may reduce the damage inflicted by herbivores that overcome the glandular trichomes on leaves and floral buds (Vasconcellos-Neto *et al.*, 2017). For instance, the damage on leaves and floral buds in *Rhynchanthera dichotoma* (Ness) C.B. Clarke (Melastomataceae) was reported to decrease in 74% and 85%, respectively, in the presence of the lynx spiders (Morais-Filho & Romero, 2010). However, the effect of lynx spiders as plant guards may be taxon and guild specific (Romero *et al.*, 2008). If on the one hand, these spiders may be effective in protecting the *R. dichotoma* plants against exophytic herbivores (e.g. Miridae sp. and Cicadellidae sp.) (Morais-Filho & Romero, 2010). On the other hand, lynx spiders may not be effective against endophytic herbivores, as it was reported for the endophytic dipterans (e.g. *Melanogromyza* spp.; Agromyzidae) on *Trichogoniopsis adenantha* (DC.) King & Rob (Asteraceae) (Romero *et al.*, 2008). Indeed, endophytic herbivores can be protected against some predators as they feed inside plant tissues (Alves-Silva *et al.*, 2014).

Spiders and ants together exert a positive and complementary effect on the reproductive success of extrafloral nectaried plants by reducing or deterring herbivores (Nahas, Gonzaga & Del-Claro, 2012, 2017; Stefani *et al.*, 2015; Del-Claro *et al.*, 2013, 2017). Glandular trichome-bearing plants also have received more attention currently, especially Asteraceae, Melastomataceae, and Solanaceae (Romero & Vasconcellos-Neto, 2012). However, little attention has been paid to the relationship between spiders and plant species of the large family Fabaceae and its role in structuring food webs. In order to fill these gaps, we record here the impact of the presence of the lynx spider, *P. flava*, on *Mimosa setosa* var. *paludosa*

(Benth.) Barneby (Fabaceae: Mimosoideae; Fig. 1b), a glandular trichome-bearing plant commonly found in the Brazilian Cerrado, in which lynx spiders interact with entrapped carrion and live insects of the exophytic and endophytic guild (Fig. 1c,d,h,i).

Our main aims were as follows: (1) to evaluate the effectiveness of lynx spiders as a plant guard against exophytic and endophytic herbivores; and (2) to evaluate the effects of insect abundance (live and carrion) and *M. setosa* var. *paludosa* size on the presence and abundance of lynx spiders. We hypothesized that: (1) the damage inflicted by exophytic (leaves) and endophytic (seeds) herbivores will be lower in plants where the lynx spider is present; and (2) the presence of this predator will be positively related to the food availability (live insects and/or entrapped carrion) and plant size.

Materials and methods

Study site and plant species

The experiments were performed between February and July 2017 and December 2017 and February 2018, in the Ecological Reserve of Clube Caça e Pesca Itororó de Uberlândia (CCPIU), Minas Gerais state, southeastern Brazil (18°59'00"S, 48°17'44" W). The climate in the region is markedly seasonal, characterized by a rainy summer (October to April) which may account for up to 75% of the annual rainfall, and a dry winter (May to September) (Laboratory of Climatology, Federal University of Uberlândia; Sousa-Lopes, Bächtold & Del-Claro, 2016).

Mimosa setosa var. *paludosa* is a shrub that rarely exceeds a height of 3 m, has compound leaves, craspedium fruits, glandular trichomes over all its parts (Dutra & Garcia, 2014), and occurs on the edge of CCPIU near a vereda, a swampy area located in the headwater of a stream (Sousa-Lopes *et al.*, 2016). The glandular trichomes present a sticky substance that entraps insects (Fig. 1c,d) but not lynx spiders, which are adapted to glandular plants and use them to feed on, breed and shelter (Vasconcellos-Neto *et al.*, 2007). The plant produces leaves all year round, (Sousa-Lopes *et al.*, 2016). Flowering and fruiting season is from February to May and March to October, respectively (B. Sousa-Lopes, pers. obs.).

Experimental manipulations

To test whether lynx spiders would have a negative impact on the damage inflicted by exophytic herbivores, we tagged 20 plants with similar height and phenology and separated them into two treatments, 10 plants in spiders-present treatment and 10 plants in spiders-absent treatment. On the spiders-present treatment, we kept one individual of *P. flava*, similarly to what we used to find on plants (Sousa-Lopes, pers. obs.), whereas spiders were removed on plants belonging to spiders-absent treatment. Every 3 days, we inspected all plants and spiders, and after 15 days of trials (five visits), we randomly selected 10 leaves of the 20 plants, and we counted the number of foliolules damaged (chewed) and undamaged (not chewed). Then, we calculated the proportion of foliolules damaged relative to the total number of foliolules found in the 10 selected leaves

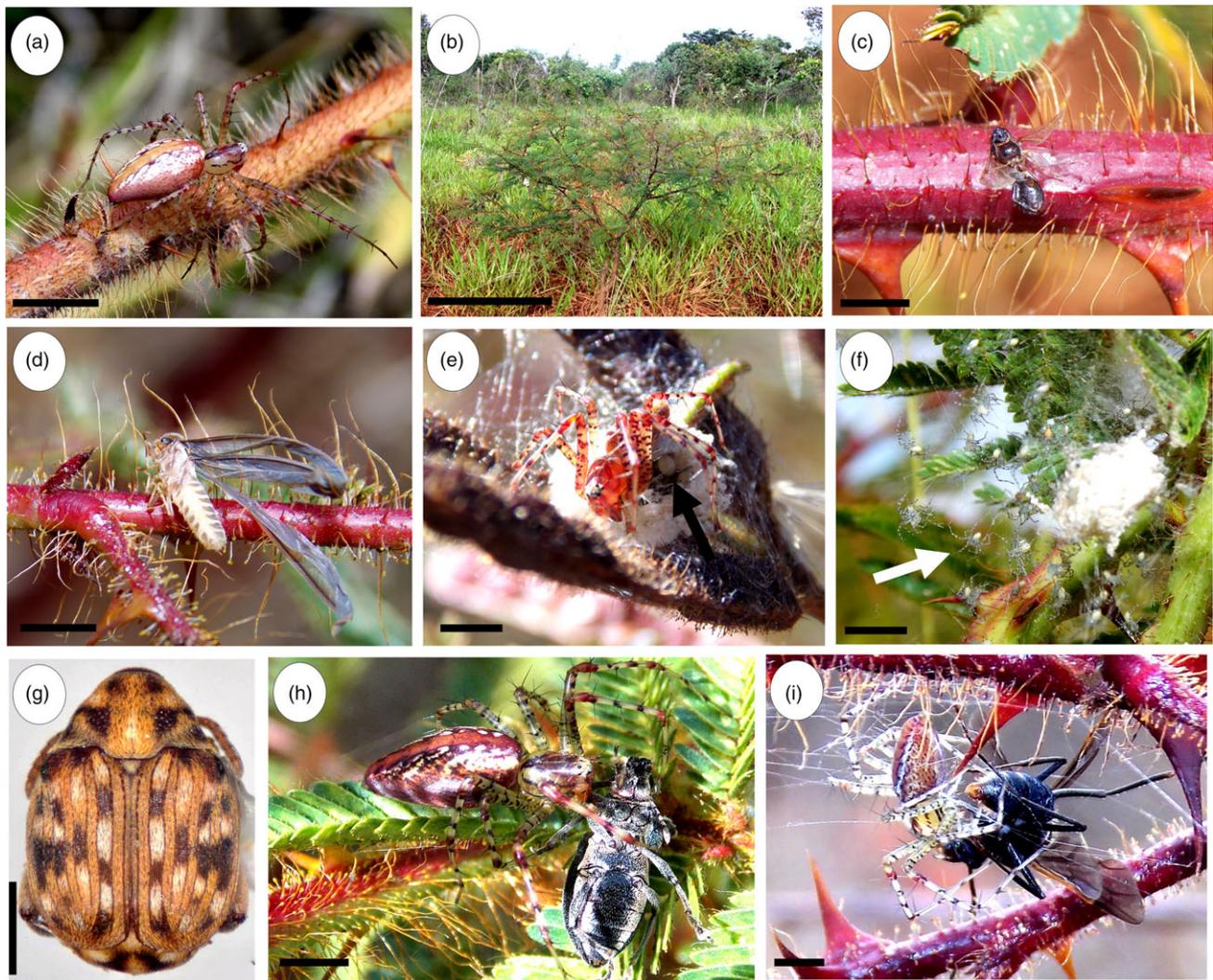


Figure 1 Lynx spider–herbivore interactions on *Mimosa setosa* var. *paludosa* in the Brazilian Cerrado. (a) The lynx spider, *Peucetia flava* Keyserling 1877; (b) *Mimosa setosa* var. *paludosa*; (c) glandular trichomes with an hymenopteran entrapped; (d) glandular trichomes with an isopteran entrapped; (e) *P. flava* with egg sac on fruit surface (black arrow), offering parental care; (f) juveniles of *P. flava* dispersing (white arrow); (g) the endophytic seed beetle, *Acanthoscelides winderi* Kingsolver, 1984; (h) *P. flava* preying on the exophytic herbivore, *Naupactus* sp.; (i) *Peucetia flava* preying on an unidentified hymenopteran entrapped on the host plant. Scale bar: 1 mm, except in (b) with 1 m.

per plant for both spiders-present and spiders-absent treatments (sensu Romero *et al.*, 2008).

To test whether lynx spiders would have a negative impact on the damage inflicted by endophytic herbivores, we tagged another 13 plants with similar height and phenology. At the beginning of the flowering (February 2017), we carefully bagged two branches of each plant with a polyester net ($n = 26$ branches) to allow free access for the most abundant endophytic seed feeder, the seed beetle *Acanthoscelides winderi* Kingsolver, 1984 (Chrysomelidae: Bruchinae; Fig. 1g), and to prevent damage by exophytic herbivores such as lepidopterans (see Sousa-Lopes *et al.*, 2016). We used a polyester net with small holes (3×3 mm) so that seed beetles could come in and lynx spiders could not come out. In each plant, we randomly chose one of the two bagged branches

and put one spider inside the net ($n = 13$), while the other branch had no spider ($n = 13$). We inspected the branches every 3 days to check for spider survival, and after 60 days of trials (20 visits) we removed the nets, collected fruits, and took them to the laboratory in plastic containers (500 mL), where we counted the number of damaged and healthy seeds. Finally, we calculated the proportion of seeds damaged relative to the total number of seeds produced per plant per treatment (sensu Romero *et al.*, 2008).

Assessing the abundance of insects, carrion, lynx spiders and plant traits

In the field, we performed visual inspections lasting 20 min in another 53 randomly selected *M. setosa* var. *paludosa* plants,

in which we counted the abundance of carrion, live insects and lynx spiders by examining all plant structures. Then, we measured the maximum height of branches with leaves, the maximum canopy diameter, and the perpendicular length to this diameter in each of the 53 plants. Measures were transformed in cubic meters to estimate a plant's real size (sensu Jacobucci *et al.*, 2009). Finally, we randomly collected one branch in each individual plant and took it to the Behavioral Ecology and Interactions Laboratory (LECI) at the Federal University of Uberlândia, where we counted all the trichomes present in a 4 mm² area on the stem, using a stereo microscope.

Spider–herbivore interactions

To evaluate the effectiveness of lynx spiders as predators of the main endophytic seed feeder, *A. winderi*, we staged 10 encounters between a lynx spider and an endophytic beetle (totaling 10 spiders and 10 seed beetles). We performed encounters under laboratory conditions (12-h light and 20–30°C), and we used transparent plastic containers (500 mL) to facilitate the observations. For acclimation, all lynx spiders were maintained in the containers omit 24 h before the trials. The observation period started after the first physical encounter, when spiders touched seed beetles, and lasted 10 min. We recorded the duration of encounters in seconds. In addition, we conducted 30 h of *ad libitum* sense observation (Altmann, 1974) in sessions lasting 20 min in each plant ($n = 90$ plants) in field, in order to record interactions between *P. flava* and other herbivores.

Statistical analyses

We used a hurdle model approach with a zero-truncated Poisson regression to assess whether the presence and abundance of *P. flava* is increased by plant traits (size and trichomes), carrion abundance, and live insect's abundance. In short, the hurdle model approach separates zero and non-zero data and allows modeling of both presence/absence and abundance in an integrated framework (Potts & Elith, 2006). The predictors were log-transformed to improve model fit. We used the paired *t*-test to compare the proportion of damaged foliolules and the proportion of predated seeds between spiders-present and spiders-absent treatments. Statistical analyses were performed in R Studio and package lme4 (R Development Core Team, 2016). We used Graph Pad Prism 5 (GraphPad Company, San Diego, CA, USA) to generate the graphics.

Results

The lynx spiders provided protection for plants against exophytic, but not against endophytic herbivory. On the one hand, the proportion of foliolules damaged differed between the spiders-present (0.10 ± 0.08) and spiders-absent treatments (0.43 ± 0.10 ; $t = 7.96$, d.f. = 18, $P < 0.001$) and the absence of spiders led to a 3.3-fold increase in the number of damaged foliolules (Fig. 2). On the other hand, there was no significant statistical difference between the proportion of seeds damaged

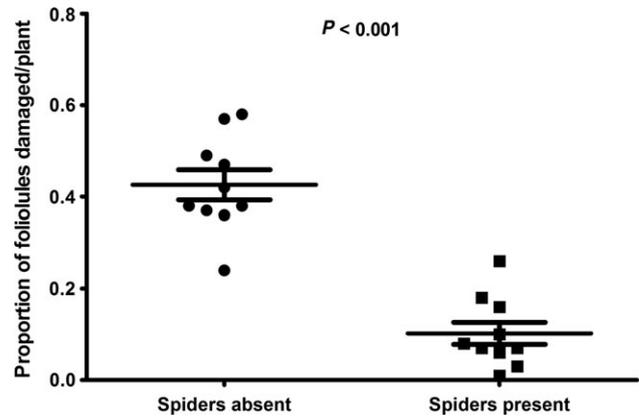


Figure 2 Mean proportion (\pm SE) of foliolules damaged in spiders-absent and spiders-present treatments on *Mimosa setosa* var. *paludosa*. Note a significant statistical difference at paired *t*-test ($P < 0.0001$, $n = 10$ plants). The total number of foliolules damaged on spiders absent and spiders present were 1012 (30%) and 305 (10%), respectively.

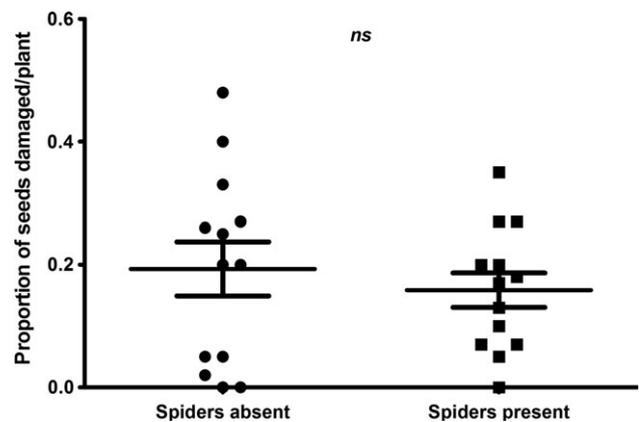


Figure 3 Mean proportion (\pm SE) of seeds damaged on *Mimosa setosa* var. *paludosa* by *Acanthoscelides winderi* in *Peuceetia flava* absence or presence. Note a non-significant statistical difference at paired *t*-test ($P = 0.34$, $n = 13$ plants, 26 samples). The total of seeds damaged on spiders absent and spiders present were 54 (19%) and 43 (15%), respectively.

in the spiders-present (0.16 ± 0.10) and spiders-absent treatments (0.19 ± 0.16 ; $t = 0.98$, d.f. = 12, $P = 0.34$; Fig. 3).

During our field observations, we recorded 23 *P. flava* spiders on *M. setosa* var. *paludosa* plants, in individuals larger than 0.0840 m³ (plant size varied from 0.0019 to 4.1231 m³, $X = 0.4911$, $SD = 0.7942$). Overall, we found only one spider per plant, except in four cases when we found two ($n = 3$) and four ($n = 1$) spiders on plants with 0.5152, 0.8114, 1.1609, and 3.8250 m³, respectively. The hurdle regression model showed that the presence of *P. flava* increases with increasing carrion abundance ($P < 0.050$; Table 1), and spiders showed a trend (positive, marginally significant effect) in choosing taller plants ($P = 0.057$, in the Zero hurdle model). The results also indicated a trend (positive, marginally

Table 1 Hurdle regression model between the presence and abundance of *Peucetia flava* and plant traits (size, number of trichomes), carrion abundance and abundance of live insects on *Mimosa setosa* var. *paludosa*

	Estimate	SE	z value	Pr(> z)
Zero hurdle model coefficients (binomial with logit links)				
(Intercept)	-6.9540	4.6349	-1.500	0.1335
Plant_size	1.9112	1.0040	1.904	<i>0.0570</i>
Trichome	1.3136	1.5238	0.862	0.3887
Carrion	1.5714	0.7182	2.188	0.0287
Insect_count	0.9837	0.5569	1.766	<i>0.0773</i>
Count model coefficients (truncated poisson with log link)				
(Intercept)	0.7397	6.2375	0.119	0.9060
Plant_size	2.3373	1.2379	1.888	<i>0.0590</i>
Trichome	-1.0235	2.1321	-0.480	0.6310
Carrion	-0.4889	0.5904	-0.828	0.4080
Insect_count	0.3111	0.6781	0.459	0.6460

The zero hurdle model (above) accounts for the effect of the predictors (first column) on the presence of *P. flava*. The count model (below) accounts for the effect of the predictors (first column) on the abundance of *P. flava*. In bold, significant result. Marginally significant results are italicized.

significant effect) in increasing the abundance of lynx spiders in taller plants ($P = 0.059$; Table 1).

In our field observations, we could also notice that *P. flava* lay egg sacs on the fruit surface (from September to October) and stay near them offering parental care (Fig. 1e) until the plant produces new leaves, and then juvenile spiders disperse towards the leaves (October; Fig. 1f). We also observed *P. flava* feeding two times on the carrion of an unidentified species of isopteran and preying on the herbivore species *Oospila pallidaria* (Schaus, 1897) (Lepidoptera: Geometridae; $n = 1$) and *Naupactus* sp. (Coleoptera: Curculionidae; $n = 1$; Fig. 1h), and two unidentified species of hymenopterans ($n = 1$ for each species; Fig. 1i).

In our laboratory observations, we found that lynx spiders spent on average 28 s (± 14) to encounter and attack *A. winderi*, and interactions lasted 115 s (± 22 ; $n = 10$). Spiders could not prey on any of *A. winderi* individuals since these beetles protected their head and legs under the pronotum and elytra and stayed immobile until spiders stopped the attack.

Discussion

Our results corroborate the hypotheses: (1) the damage inflicted by exophytic herbivores was lower in lynx spider presence since there was a decrease in foliolules damaged on plants with spiders; and (2) the presence of lynx spiders on plants is positively related to the food availability (i.e. carrion). We also showed a tendency in spiders choosing taller plants, possibly because large plants provide more suitable sites for spiders to find carrion and preys, and also sheltering and breeding.

Other studies focused on lynx spiders showed that their presence decreased the leaf herbivory on plants, but the influence of these spiders was guild-dependent (Romero *et al.*, 2008; Morais-Filho & Romero, 2010). The spiders decreased

the abundance and damage inflicted by several exophytic herbivores, but had no effect on some herbivores, particularly those that are endophytic. For example, Romero *et al.* (2008) showed that lynx spiders decreased by 16-fold the proportion of damage inflicted by an exophytic Geometridae larvae (Lepidoptera). However, spiders did not affect the endophytic dipterans *Melanogromyza* since their larvae feed inside plant tissues and the adults have little movements on plants, making them less vulnerable to spider attack. Similarly, our study showed that the endophytic seed beetles *A. winderi* are less vulnerable to *P. flava* attack as these beetles feed inside plant tissue (seeds) during immature stages (Sousa-Lopes, pers. obs.), and adults avoid spider attack by the physical protection provided by their elytra (see also Linz *et al.*, 2016). Therefore, we suggest that *P. flava* is an important plant guard for *M. setosa* var. *paludosa*, but the outcomes of this interaction may be dependent on the taxon and herbivore guilds. This is true since the defensive behavior in some taxonomic groups (e.g. seed beetles) and the habit of feeding inside plant tissues (i.e. endophytic guild; immature stages of seed beetles) can make these herbivores less vulnerable to spider attack.

We found that the presence of *P. flava* is positively related to the carrion abundance as well as showed by other studies focused on several predators on glandular trichome-bearing plants (LoPresti *et al.*, 2015, 2018). Since the scavenging behavior has been recorded for lynx spiders (Romero *et al.*, 2008; and herein), it is possible they take into account the carrion abundance before choosing their host plants. Higher carrion abundance would be advantageous for spiders because they represent an accessible food source free of costs associated with attacking and manipulating live preys. Thus, the presence of *P. flava* may be not only related to the abundance of live arthropods (Morais-Filho & Romero, 2008) but also carrion, which reinforce that these spiders choose sites of high food availability, independently if the food sources are alive or dead.

Morais-Filho & Romero (2008) and Jacobucci *et al.* (2009) showed that lynx spiders might select their host plants based on size. For instance, the abundance of *P. flava* was positively related to the size of *Solanum thomasiifolium* Sendtner (Solanaceae), and authors suggested that larger plants are more suitable for spiders forage (Jacobucci *et al.*, 2009), and possibly for breeding and shelter. Indeed, large plants are more conspicuous and attractive for arthropods due to the wide range of microhabitats and resources, including preys for predators such as lynx spiders (Lawton, 1983; Schlinkert *et al.*, 2015) and sites for hiding of natural enemies (Foelix, 1996). Although we found a marginally significant effect in choosing and increasing the abundance of *P. flava* on taller plants, we suggest that these spiders can take into account the plant size prior to select their host plant.

The results of this study suggest that it is more common to find lynx spiders interacting with *M. setosa* var. *paludosa* in larger plants with higher carrion abundance (food source). *Peucetia flava* and *M. setosa* var. *paludosa* interact in a facultative mutualism, in which plants provide entrapped carrion for spiders to feed on and possibly facilitate prey manipulation. In return, lynx spiders decrease the damage inflicted by exophytic, but not by endophytic herbivores. Our findings

contribute to a better understanding of which ecological factors may affect plant selection by lynx spiders and what the influence of this predator is on the structure of the food webs in glandular plants.

Acknowledgements

We thank the staff of Clube Caça e Pesca Itororó de Uberlândia where the study was performed, Melissa Lopes for her encouragement, Isamara Mendes Silva for help with part of the fieldwork, Cibele Stramare Ribeiro-Costa for help with seed beetle identification, Aluska Tavares dos Santos for seed beetle photo and Luis M. Carrascal for statistical advice.

Conflict of interest

The authors declare that they have no potential conflict of interest.

Funding information

BSL thanks the Brazilian CNPq (Proc.142280/2016-0), NAS thanks the Brazilian CNPq (Proc. 119082/2017-9), FAM thanks the Brazilian CAPES (Proc. 88881.120147/2016-01), and KDC thanks the Brazilian CNPq (Proc. 301605/2013-0) and also thanks a FAPEMIG research grant.

References

- Altmann, J. (1974). Observational study of behavior: sampling methods. *Behaviour* **49**, 227–266.
- Alves-Silva, E., Bächtold, A., Barônio, G.J., Torezan-Silingardi, H.M. & Del-Claro, K. (2014). Ant-herbivore interactions in an extrafloral nectaried plant: are ants good plant guards against curculionids beetles? *J. Nat. Hist.* **49**, 841–851.
- Del-Claro, K., Stefani, V., Lange, D., Vilela, A.A., Nahas, L., Velasques, M. & Torezan-Silingardi, H.M. (2013). The importance of natural history studies for a better comprehension of animal-plant interactions networks. *Biosci. J.* **29**, 439–448.
- Del-Claro, K., Rico-Gray, V., Torezan-Silingardi, H.M., Alves-Silva, E., Fagundes, R., Lange, D., Dáttilo, W., Vilela, A.A., Aguirre, A. & Rodriguez-Morales, D. (2016). Loss and gains in ant-plant interactions mediated by extrafloral nectar: fidelity, cheats, and lies. *Insectes Soc.* **63**, 207–221.
- Del-Claro, K., Stefani, V., Nahas, L. & Torezan-Silingardi, H.M. (2017). Spiders as plant partners: complementing ant services to plants with extrafloral nectaries. In *Behaviour and ecology of spiders: contribution from the Neotropical region*: 215–226. Viera, C. & Gonzaga, M.O. (Eds). Gewerbestrasse: Springer.
- Dutra, V.F. & Garcia, F.C.P. (2014). Mimosoideae (Leguminosae-Mimosoideae) dos campos rupestres de Minas Gerais, Brasil. *Iheringia* **69**, 49–88.
- Foelix, R.F. (1996). *Biology of spiders*. 2nd edn: 330. Oxford, UK: Oxford University Press.
- Halaj, J., Ross, D.W. & Moldenke, A.R. (2000). Importance of habitat structure to arthropod food-webs in Douglas-fir canopies. *Oikos* **90**, 139–152.
- Heil, M. (2008). Indirect defence via tritrophic interactions. *New Phytol.* **178**, 41–61.
- Jacobucci, G.B., Medeiros, L., Vasconcellos-Neto, J. & Romero, G.Q. (2009). Habitat selection and potential antiherbivore effects of *Peucea flava* (Oxyopidae) on *Solanum thomasiifolium* (Solanaceae). *J. Arachnol.* **37**, 365–367.
- Janzen, D. (1967). Interaction of the bull's-horn acacia (*Acacia cornigera* L.) with an ant inhabitant (*Pseudomyrmex ferruginea* F. Smith) in eastern Mexico. *Univ. Kans. Sci. Bull.* **47**, 315–558.
- Krimmel, B.A. & Pearse, I.S. (2014). Generalist and sticky plant specialist predators suppress herbivores on a sticky plant. *Arthropod Plant Interact.* **8**, 403–410.
- Langellotto, G.A. & Denno, R.F. (2004). Responses of invertebrate natural enemies to complex-structured habitats: a meta-analytical synthesis. *Oecologia* **139**, 1–10.
- Lawton, J.H. (1983). Plant Architecture and the Diversity of Phytophagous Insects. *Annu. Rev. Entomol.* **28**, 23–39.
- Linz, D.M., Hu, A.W., Sitvarin, M.I. & Tomoyasu, Y. (2016). Functional value of elytra under various stresses in the red flour beetle, *Tribolium castaneum*. *Sci. Rep.* **6**, 34813.
- LoPresti, E.F., Pearse, I.S. & Charles, G.K. (2015). The siren of a sticky plant: columbines provision mutualist arthropods by attracting and killing passerby insects. *Ecology* **96**, 2862–2869.
- LoPresti, E.F., Krimel, B. & Pearse, I.S. (2018). Entrapped carrion increases indirect plant residence and intra-guild predation on a sticky tarweed. *Oikos* **127**, 1033–1044.
- Morais-Filho, J.C. & Romero, G.Q. (2008). Microhabitat use by *Peucea flava* (Oxyopidae) on the glandular plant *Rhynchanthera dichotoma* (Melastomataceae). *J. Arachnol.* **36**, 374–378.
- Morais-Filho, J.C. & Romero, G.Q. (2009). Natural history of *Peucea flava* (Araneae: Oxyopidae): seasonal density fluctuation, phenology and sex ratio on the glandular plant *Rhynchanthera dichotoma* (Melastomataceae). *J. Nat. Hist.* **43**, 701–711.
- Morais-Filho, J.C. & Romero, G.Q. (2010). Plant glandular trichomes mediate protective mutualism in a spider-plant system. *Ecol. Entomol.* **35**, 485–494.
- Nahas, L., Gonzaga, M.O. & Del-Claro, K. (2012). Emergent impacts of ant spider interactions: herbivory reduction in a tropical savanna tree. *Biotropica* **44**, 498–505.
- Nahas, L., Gonzaga, M.O. & Del-Claro, K. (2017). Wandering and web spiders feeding on the nectar from extrafloral nectaries in neotropical savanna. *J. Zool.* **301**, 125–132.
- Potts, J. & Elith, J. (2006). Comparing species abundance models. *Ecol. Model.* **199**, 153–163.
- Price, P.W., Denno, R.F., Eubanks, M.D., Finke, D.I. & Kaplan, I. (2011). *Insect ecology, behavior, populations and communities*. New York: Cambridge University Press.
- R Development Core Team (2016). *R: a language and environment for statistical computing*. <http://www.R-project.org>. Accessed 02 March 2016.

- Romero, G.Q. & Vasconcellos-Neto, J. (2005). The effects of plant structure on the spatial and microspatial distribution of a bromeliad-living jumping spider (Salticidae). *J. Anim. Ecol.* **74**, 12–21.
- Romero, G.Q., and Vasconcellos-Neto, J., (2012). Interações entre aranhas e plantas: associações específicas e mutualismos. In *Ecologia das interações plantas-animais: uma abordagem ecológico-avaliativa*: 241–256. Del-Claro, K., & Torezan-Silingardi, H.M., (Eds). Rio de Janeiro: Technical Books.
- Romero, G.Q., Souza, J.C. & Vasconcellos-Neto, J. (2008). Anti-herbivore protection by mutualistic spiders and the role of plant glandular trichomes. *Ecology* **89**, 3105–3115.
- Schlinkert, H., Westphal, C., Clough, Y., László, Z., Ludwig, M. & Tschantke, T. (2015). Plant size as determinant of species richness of herbivores, natural enemies and pollinators across 21 Brassicaceae species. *PLoS ONE*, **10**, e0135928.
- Sousa-Lopes, B., Bächtold, A. & Del-Claro, K. (2016). Biology, natural history and temporal fluctuation of the geometrid *Oospila pallidaria* associated with host plant phenology. *Stud. Neotrop. Fauna Environ.* **51**, 135–143.
- Stefani, V., Pires, T.L., Torezan-Silingardi, H.M. & Del-Claro, K. (2015). Beneficial effects of ants and spiders on the reproductive value of *Eriotheca gracilipes* (Malvaceae) in a tropical savanna. *PLoS ONE* **10**, e0131843.
- Vasconcellos-Neto, J., Romero, G.Q., Santos, A.J. & Dippenaar-Schoeman, A.S. (2007). Associations of spiders of the genus *Peucetia* (Oxyopidae) with plants bearing glandular hairs. *Biotropica* **39**, 221–226.
- Vasconcellos-Neto, J., Messas, Y.F., Souza, H.S., Villanueva-Bonilla, G.A., and Romero, G.Q., (2017). Spider–plant interactions: an ecological approach. In *Behaviour and ecology of spiders: contribution from the neotropical region*: 165–214. Viera, C. and Gonzaga, M.O. (Eds). Gewerbestrasse: Springer.
- Whitney, K.D. (2004). Experimental evidence that both parties benefit in a facultative plant-spider mutualism. *Ecology* **6**, 1642–1650.

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/330355051>

Cotesia itororensis sp. Nov. From Brazilian savanna: A new reared microgastrine wasp (Hymenoptera: Braconidae) described using an integrative taxonomic approach

Article in *Zootaxa* · January 2019

DOI: 10.11646/zootaxa.4544.3.9

CITATION

1

READS

259

4 authors:



Bruno de Sousa-Lopes

University of São Paulo

18 PUBLICATIONS 9 CITATIONS

SEE PROFILE



James B Whitfield

University of Illinois, Urbana-Champaign

225 PUBLICATIONS 5,443 CITATIONS

SEE PROFILE



Geraldo Salgado-Neto

Universidade Federal de Santa Maria

57 PUBLICATIONS 46 CITATIONS

SEE PROFILE



Kleber Del-Claro

Universidade Federal de Uberlândia (UFU)

248 PUBLICATIONS 3,313 CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:



Influência da fenologia e estrutura da vegetação sobre as propriedades de redes de interações multitróficas formiga-planta-polinizador [View project](#)



Evolutionary ecology in social Pseudoscorpions [View project](#)



<https://doi.org/10.11646/zootaxa.4544.3.9>

<http://zoobank.org/urn:lsid:zoobank.org:pub:3DE4D618-BDD7-4978-9ED4-E3CFC4405F83>

***Cotesia itororensis* sp. nov. from Brazilian savanna: a new reared microgastrine wasp (Hymenoptera: Braconidae) described using an integrative taxonomic approach**

BRUNO DE SOUSA-LOPES¹, JAMES BRYAN WHITFIELD², GERALDO SALGADO-NETO³ & KLEBER DEL-CLARO⁴

¹Laboratório de Ecologia Comportamental e de Interações, Pós-graduação em Entomologia, Universidade de São Paulo, Avenida Bandeirantes n. 3900, 14040901, Ribeirão Preto, SP, Brazil. E-mail: brunoslopes@usp.br

²Department of Entomology, 320 Morrill Hall, 505 South Goodwin Ave., University of Illinois at Urbana-Champaign, Urbana, IL 61801, U.S.A. E-mail: jwhitfie@life.illinois.edu

³Pós-graduação em Agronomia, Departamento de Defesa Fitossanitária, Universidade Federal de Santa Maria, 97105-900, Santa Maria, RS, Brazil. E-mail: gsalgado@bol.com.br

⁴Laboratório de Ecologia Comportamental e de Interações, Instituto de Biologia, Universidade Federal de Uberlândia, Rua Ceará, s/n. Bloco 2D-Campus Umuarama, 38400902, Uberlândia, MG, Brazil. E-mail: delclaro@ufu.br

Abstract

A new species of microgastrine wasp, *Cotesia itororensis* Sousa-Lopes & Whitfield, **sp. nov.**, is described from a Brazilian savanna area in Uberlândia, Minas Gerais. This species is a koinobiont endoparasitoid recorded from caterpillars of *Oospila pallidaria* (Schaus, 1897) (Lepidoptera: Geometridae), feeding on *Mimosa setosa* var. *paludosa* (Fabaceae: Mimosoideae). Morphological, molecular, biological, ecological and geographical data are used to describe the new species and distinguish it from others formally recorded for the Neotropical region.

Key words: Fabaceae, Geometridae, *Oospila pallidaria*, taxonomy

Introduction

Microgastrine braconid wasps are among the most abundant endoparasitoids of lepidopterans and tend to be highly host-specific; consequently, these wasps are employed frequently as biological control agents against pest insects (Smith *et al.* 2008; Whitfield *et al.* 2009). There are ~2,700 described species worldwide (Yu *et al.* 2016), but still, many thousands of additional, undescribed ones are known to exist (Rodriguez *et al.* 2012).

Cotesia Cameron, 1891 (Braconidae: Microgastrinae) is the second largest genus of microgastrine wasps in terms of described species, encompassing roughly 300–400 described species (Shaw & Huddleston 1991; Yu *et al.* 2016). However, this number will increase in the coming years, since it has been estimated that nearly 1000 species of *Cotesia* exist worldwide (Mason 1981; Michel-Salzat & Whitfield 2004). This increase must be higher especially in the Neotropical region, where a relatively small number of studies recording species of *Cotesia* and their biology are available (Whitfield 1997), particularly in South America.

Cotesia is recognizable among the microgastrines by the following features: (1) forewing with second r-m vein absent, so that the small areolet is open distally; (2) propodeum coarsely sculptured, with medial longitudinal carina rather than medial areola; (3) first and second metasomal tergites usually rather quadrate in form and coarsely sculptured; and (4) ovipositor and sheaths short and barely exerted (Whitfield *et al.* 2009). These wasps have a koinobiont habit (Kankare & Shaw 2004), being recorded from caterpillars of Hesperidae (Smith *et al.* 2008; Yu *et al.* 2016), Noctuidae (Avalos *et al.* 2016; Yu *et al.* 2016), Pieridae (Pizzato *et al.* 2016; Yu *et al.* 2016), Saturniidae, Sphingidae (Smith *et al.* 2008; Yu *et al.* 2016), and most rarely from Geometridae (Haines *et al.* 2009, Ruohomäki *et al.* 2013; Yu *et al.* 2016). As *Cotesia* species have appeared to be highly host specialized

(Kankare & Shaw 2004), with many cryptic species and allopatric distributions (Fiaboe *et al.* 2017), the use of an integrative taxonomic approach (combining morphological, molecular, biological and geographical data) is paramount for recognizing and distinguishing these parasitoid wasps (Smith *et al.* 2008; Kaiser *et al.* 2017).

Using an integrative taxonomic approach, this paper provides a description of a new species of *Cotesia*, whose brood was produced from *Oospila pallidaria* (Schaus, 1897) (Lepidoptera: Geometridae), feeding on *Mimosa setosa* var. *paludosa* (Fabaceae: Mimosoideae) in the Brazilian savanna (Sousa-Lopes *et al.* 2016). Additionally, we compared it with other twenty-one formally recorded species for the Neotropical region.

Materials and methods

From January 2014 to May 2018, during visual inspections on individuals of *M. setosa* var. *paludosa* (Fig. 1a), BSL collected 210 early instars (1st and 2nd) larvae of *O. pallidaria* (Fig. 1b) in the Ecological Reserve of the Clube Caça e Pesca Itororó de Uberlândia (CCPIU), Minas Gerais, Brazil (18°59'S, 48°17'W; altitude 863 m; 640 ha).

Larvae were reared individually in plastic containers in the Behavioral Ecology and Interactions Laboratory at the Federal University of Uberlândia (12-h light and 20–30°C), until adult stage or emergence of the braconid parasitoid described below. The original host, cocoons and adult parasitoids were saved, being the first and last ones placed in 96% ethyl alcohol.

Illustrations were made using a digital camera photography setup attached to stereoscopic microscopes. Morphological terms and measurements of structures are mostly those used by Fernandez-Triana *et al.* (2014).

To check the molecular-specific characterization of the new species, the mitochondrial gene Cytochrome Oxidase I (COI) was analyzed. For the amplification of a fragment of approximately 460 bp of this gene, we used the following primer pair: COI-F (5'-GATTTTTTGGKCA YCCMGAAG-3') and COI-R (5'-CRAATACRGCTCCTATWGATAAWAC-3') (Gusmão *et al.* 2010). DNA extraction of one specimen was performed with the GenElute Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich®) and followed the manufacturer's protocol. The product was amplified via Polymerase Chain Reaction (PCR) according to the following schedule: 94°C for 2 minutes, 40 cycles of 94°C for 30 seconds, 54°C for 30 seconds, 72°C for 40 seconds and 72°C for 4 minutes. Then the PCR product was purified using polyethylene glycol precipitation (PEG; Schmitz & Riesner 2006). These samples were sequenced using the Big Dye 3.1 reagent (Life Technologies®) and 3500 xL automatic sequencer (Life Technologies®).



FIGURE 1. (a) The host plant, *Mimosa setosa* var. *paludosa* (Fabaceae), and (b) the second instar larva of *Oospila pallidaria* (Geometridae) in the Brazilian savanna

Results

Cotesia itororensis Sousa-Lopes & Whitfield, sp. nov.

Holotype. Female, Brazil: Minas Gerais, Uberlândia, Clube Caça e Pesca Itororó de Uberlândia (18°59'00"S, 48°17'44"W—863 m. elev.). Deposited in the Hymenoptera collection of the Museum of Zoology of the University of São Paulo (MZUSP; Carlos Brandão curator), number MZSP57562, February 2018, coll. B. Sousa-Lopes, ex larva *Oospila pallidaria* on *M. setosa* var. *paludosa*.

Paratypes. 2 males, deposited in MZUSP, numbers MZSP57563 and MZSP57564. Same data as holotype.

Diagnosis. Antennae approximately as long as the body (head to apex of metasoma); coxae black, legs mostly honey-yellow, metasoma mostly black except pale in laterotergites; mesoscutum with distinct dense punctures, becoming smooth posteriorly anterior to scutoscutellar sulcus; wings partially pigmented with a few veins dark, but most pale; pterostigma dark greyish brown, with indistinct paler junction with C+SC; propodeum with more or less complete, but anteriorly weak medial longitudinal carina; the first tergite of metasoma rounded towards posterior margin; second tergite of metasoma almost twice as wide posteriorly as anteriorly; tergites of metasoma mostly smooth, weakly sculptured; ovipositor shorter, about one-third the length of the metasoma or hind tibia; solitary habit recorded so far from *O. pallidaria*; cocoon yellow. The above combination of characters is sufficient to separate *C. itororensis* from all other recorded species of *Cotesia* for the Neotropical region. A detailed diagnosis one-to-one is provided below to distinguish *C. itororensis* from every other species.

Description. Female (Fig. 2). Body color: body mostly black except pale, almost whitish palpi, most of all legs distal to coxae, and laterotergites of metasoma. Antenna color: scape, pedicel and flagellum dark brown/black. Coxae color (pro-, meso-, metacoxa): black. Femora color (pro-, meso-, metafemora): honey-yellow. Tibiae color (pro-, meso-, metatibiae): honey-yellow, with slight darkening dorsally at distal end of metatibiae. Tegulae color: dark brown translucent. Pterostigma color: dark greyish brown, with indistinct paler junction with C+SC. Fore wings color: partially pigmented (a few veins may be dark but most pale). Antenna length/body length: antenna approximately as long as body (head to apex of metasoma). Body in lateral view: not distinctly flattened dorso-ventrally. Body length (head to apex of metasoma): 2.0–2.2 mm. Fore wing length: 2.1–2.3 mm. Ocular-ocellar line/posterior ocellus diameter 1.7–1.9. Interocellar distance/posterior ocellus diameter: 2.1–2.3. Antennal flagellomere 2 length-width: 2.9–3.1. Antennal flagellomere 14 length/width 1.4–1.6. Length of flagellomere 2/length of flagellomere 14: 2.2–2.3. Tarsal claws: simple, within single basal spine-like seta. Metafemur length/width 3.2–3.3. Metatibia inner spur length/metabasitarsus length: roughly 0.5. Anteromesoscutum: anteriorly with distinct dense punctures, becoming smooth posteriorly anterior to scutoscutellar sulcus. Mesoscutellar disc: sparsely and finely but distinctly punctured; microsculpture producing satiny reflections. Number of pits in scutoscutellar sulcus: 8–10. Propodeum carina: with more or less complete but anteriorly weak medial longitudinal carina. Propodeum background sculpture: mostly very finely rugulose, almost smooth in spots. Mediotergite 1 length/width at widest point: 1.1–1.3. Mediotergite 1 shape: slightly widening from anterior margin to 0.7 of mediotergite length (widest point), then rounding towards posterior margin. Mediotergite 1 sculpture: mostly smooth and shining with very vague sculpturing ventrally, posterolateral portions with widely scattered punctures. Mediotergite 2 width at posterior margin/length: 2.1–2.3, almost twice as wide posteriorly as anteriorly. Mediotergite 2 sculpture: mostly smooth and raised centrally, very weakly sculptured otherwise. Hypopygium: evenly sclerotized but folded medially, posteriorly forming a strongly obtuse angle in lateral view. Ovipositor thickness: tapering gradually to tip. Ovipositor sheaths: short, exposed portions less than 1/3 of hind tibia length. Length of fore wing veins 2RS/2M: 1.1–1.3. Length of fore wing veins 2M/(RS+M)b: 0.9–1.0. Pterostigma length/width 2.0–2.2. Point of insertion of vein r in pterostigma: just beyond half way point of pterostigma length. Angle of vein r with fore wing anterior margin: perpendicular. Shape of junction of veins r and 2RS in forewing: r weakly arched, junction distinctly but not strongly angled.

Male (Fig. 2). As female, but with darker distal patch on distal portions of hind tibiae and hind tarsi.

Molecular data. COI barcode deposited in GenBank (MH382197).

Host: *Oospila pallidaria* (Schaus, 1897) (Lepidoptera: Geometridae).

Biology/ecology. *Cotesia itororensis* is a solitary parasitoid wasp that occurs mainly in the wet season (December–March); however, their host, *O. pallidaria*, occurs throughout the year, mainly in the dry season (May–September). It is possible that caterpillars avoid parasitism occurring at highest abundance in a temporal

enemy-free space (Sousa-Lopes *et al.* 2016). Indeed, the rate of parasitism was low, representing 10.5% (22) of the caterpillars sampled during four years. Parasitism occurs between the first and second larval instar of *O. pallidaria*. Parasitized caterpillars have swollen bodies in the posterior half (Fig. 3a), mainly in the fourth instar, when parasitoid larvae are completely developed. At this time, *O. pallidaria* moves to the adaxial side of leaves (Fig. 3b) and then *C. itororensis* leaves them near the fifth abdominal segment (Fig. 3c), builds a yellow cocoon and pupates for three days. *Cotesia itororensis* probably changes host behavior because moving to the adaxial side of leaves is an uncommon behavior for healthy caterpillars (BSL, pers. obs.). It is possible this behavior decreases vulnerability to desiccation and/or enemies in open areas where host plants and caterpillars are found by wasps.

Distribution. Known so far from Uberlândia, Minas Gerais, Brazil.

Etymology. The specific epithet (*itororensis*) is a reference, from the Tupi-Guarani *i-tororó* = *yy* (water) and *tororõ* (spout), water spout, also meaning noisy river and small waterfall, and *ensis* = origin, in reference to the Clube Caça e Pesca Itororó de Uberlândia, Minas Gerais, where this braconid species was found.

Detailed diagnosis. In order to facilitate future work on the group, we detail below how each of the other twenty-one species of *Cotesia* previously recorded from the Neotropical region individually differs from *C. itororensis*.

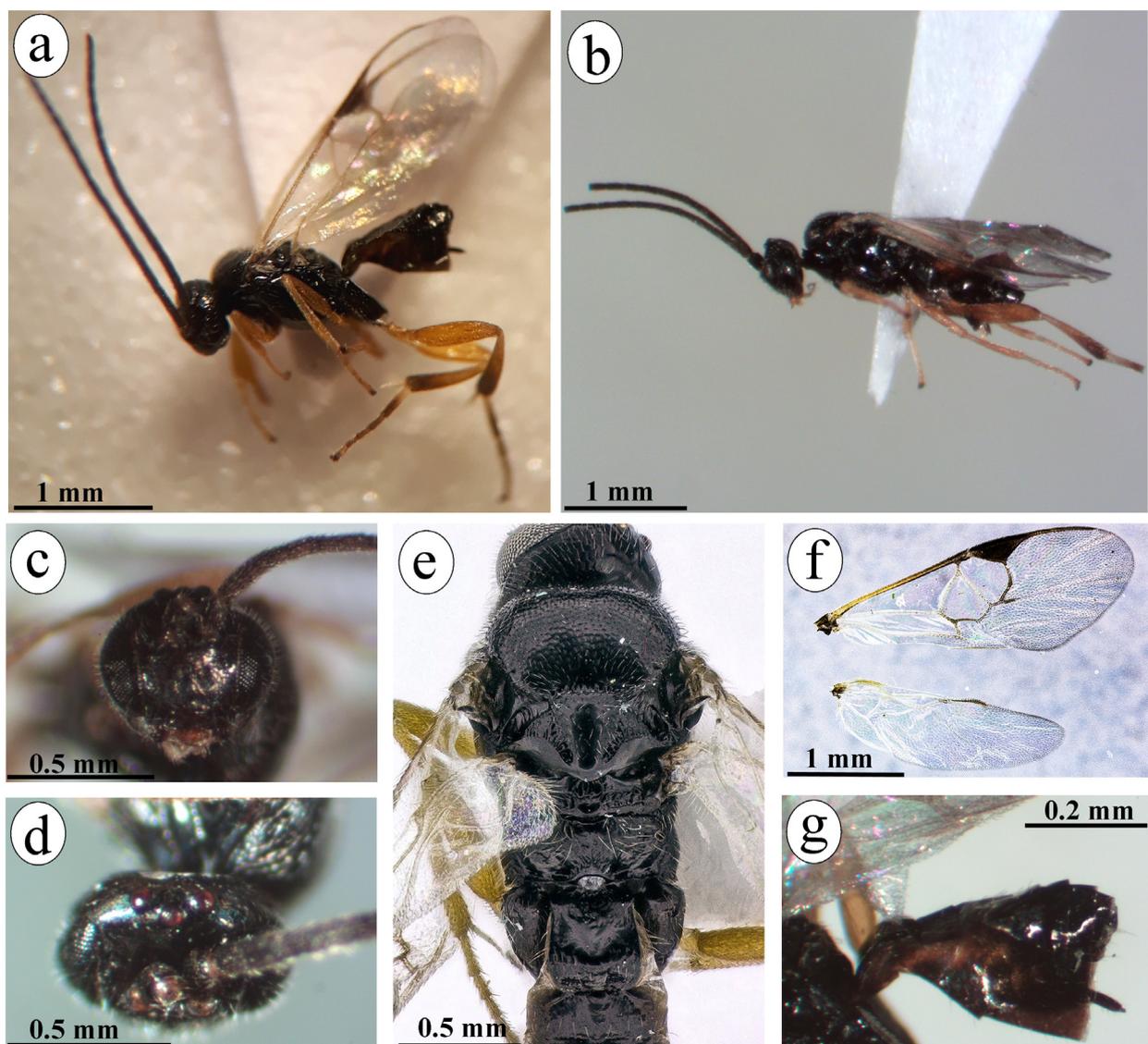


FIGURE 2. (a) Lateral habitus of *Cotesia itororensis* (Braconidae) female, (b) Lateral habitus of *C. itororensis* male, (c) frontal view of head of *C. itororensis* female, (d) dorsal view of head of *C. itororensis* female, (e) mesosoma and tergites of *C. itororensis* female, (f) wings of *C. itororensis* female, and (g) lateral view of posterior end of metasoma of *C. itororensis* female, showing hypopygium and ovipositor sheaths

Cotesia alius (Muesebeck 1958) has the metasoma bright yellow on sides and underneath on basal half, and its metasoma is also rather stout, almost as broad as mesosoma (Muesebeck 1958); whereas *C. itororensis* has metasoma mostly black except pale in metasomal laterotergites, and narrow in comparison to mesosoma. Also, *C. alius* has been recorded from a different family of Lepidoptera (Nymphalidae) in Brazil, Peru and Venezuela (Yu *et al.* 2016).

Cotesia americana (Lepeletier 1825) has antennae pale testaceous, tinged with fuscous above, the base beneath yellowish, and metasoma entirely orange-yellow (Cresson 1865); whereas *C. itororensis* has antennae dark brown/black, and metasoma black except for pale in laterotergites, mostly in segments 1 and 2. *Cotesia americana* is recorded to parasitize different families of Lepidoptera (Pyralidae and Sphingidae), and occurs in Cuba, Dominican Republic, Guyana, Haiti, Martinique, Mexico and Puerto Rico (Yu *et al.* 2016).

Cotesia ayerza (Brethes 1920) has been recorded from different species within the family Pieridae and it is known so far only from Argentina (Yu *et al.* 2016), which distinguishes it from *C. itororensis*.

Cotesia bonariensis (Brèthes 1916) has the mediotergite 2 a transverse rectangle shape, rough, slightly elevated in its middle longitudinally (Brèthes 1916); whereas *C. itororensis* has the same segment in a more triangular shape, smooth and raised centrally, very weakly sculptured otherwise. *Cotesia bonariensis* has been recorded so far from an unidentified lepidopteran in Argentina (Brèthes 1916, Yu *et al.* 2016).

Cotesia congregata (Say 1836) has a gregarious habit and cocoon whitish (Gilmore 1938); whereas *C. itororensis* is recorded so far with solitary habit and cocoon yellowish. *Cotesia congregata* has been recorded from different families of Lepidoptera (Lasiocampidae, Noctuidae, Pyralidae and Sphingidae) in Brazil, Honduras, Jamaica, Nicaragua, Peru and Puerto Rico (Yu *et al.* 2016).

Cotesia electrae (Viereck 1912) has hind femora blackish-brown, pterostigma black, and metasoma compressed and black (Viereck 1912); whereas *C. itororensis* has the hind femora yellow-honey, pterostigma dark greyish brown, with indistinct paler junction with C+SC, and metasoma compressed, but blackish with laterotergites pale. *Cotesia electrae* has been recorded so far from Saturniidae in Mexico (Yu *et al.* 2016).

Cotesia empreitiae (Viereck 1913) has legs and tegulae mostly stramineous, including the fore and mid coxae rather reddish, and first and second metasomal segments rugulose (Viereck 1913); whereas *C. itororensis* has legs mostly yellow-honey, tegulae dark brown translucent, and first and second metasomal segments mostly smooth. *Cotesia empreitiae* has been recorded so far from a different family of Lepidoptera (Limacodidae) in Ecuador (Yu *et al.* 2016).

Cotesia flavipes Cameron 1981, as well as other species from the *flavipes* species group, has short antennae (much shorter than body length, usually not surpassing the length of head and mesosoma) (Fiaboe *et al.* 2017; Kaiser *et al.* 2017); whereas the antennae of *C. itororensis* is approximately as long as the body (head to apex of metasoma). Species of the *flavipes* group also show a much more highly elongate and flattened body shape than *C. itororensis*. The metasoma of *C. flavipes* is much lighter in color than *C. itororensis*. Beyond, *C. flavipes* parasitizes different hosts within the families of Lepidoptera, Crambidae, Erebidae, and Noctuidae, in Barbados, Brazil, Costa Rica, Guadeloupe, Jamaica, Mexico, Peru, Trinidad & Tobago and Venezuela (Yu *et al.* 2016).

Cotesia glomerata (Linnaeus 1758) has the plate of the first abdominal segment about two and one-half times as long as wide (=rectangular shape) (Ashmead 1906), whereas *C. itororensis* has this plate with the anterior margin slightly widening and rounded towards posterior margin. *Cotesia glomerata* has been reported parasitizing several species of lepidopterans within the families, Bombycidae, Drepanidae, Geometridae, Pieridae, Noctuidae, Nymphalidae, among others, and the coleopteran, *Trogoderma glabrum* (Herbst 1783), in Barbados and Brazil (Yu *et al.* 2016) (this last record is almost certainly erroneous).

Cotesia kraussi (Muesebeck 1958) has the disc of scutellum large, convex, sculptured like mesoscutum, first metasomal tergite entirely finely rugulose, and hind femora black (Muesebeck 1958); whereas *C. itororensis* has disc of scutellum sparsely and finely, although distinctly punctured, first metasomal tergite mostly smooth, and femora honey-yellow. *Cotesia kraussi* has been recorded so far from *Morpheis ehrenbergi* Geyer (Lepidoptera: Cossidae) in Mexico.

Cotesia marginiventris (Cresson 1865) has hind coxae pale reddish or stramineous and wings hyaline (Cresson 1865); whereas *C. itororensis* has coxae black and wings partially pigmented, with a few veins may be dark but most pale. *Cotesia marginiventris* has been reported parasitizing different species of Noctuidae in Argentina, Bermuda, Brazil, Chile, Cuba, Nicaragua, Peru, Puerto Rico, Uruguay and Venezuela (Yu *et al.* 2016).

Cotesia marquesi (Brèthes 1924) has been recorded so far parasitizing *Papilio anchisiades capys* Hübner (Lepidoptera: Papilionidae) in Argentina and Brazil (Costa-Lima 1950; Yu *et al.* 2016).

Cotesia mayaguezensis (Viereck 1913) has propodeum without a distinct median, longitudinal carina (Viereck 1913); whereas *C. itororensis* has the propodeum with a more or less complete, but anteriorly weak medial longitudinal carina. *Cotesia mayaguezensis* has been recorded so far from an unidentified host on *Cissus sicyoides* L. (Vitaceae) in Puerto Rico (Yu *et al.* 2016).

Cotesia ornatrix (Muesebeck 1958) has pro-, meso- and metacoxae piceous, yellow, and black, respectively, and mesoscutum rather uniformly punctate (Muesebeck 1958); whereas *C. itororensis* has all coxae black, mesoscutum anteriorly with distinct dense punctures, but smooth posteriorly anterior to scutoscutellar sulcus. *Cotesia ornatrix* has been recorded so far from *Utetheisa ornatrix* Linnaeus 1758 (Erebidae: Arctiinae) in Brazil and Colombia (Muesebeck 1958; Yu *et al.* 2016).

Cotesia paphi (Schrottky 1902) has, apparently, metasoma more compressed anteroposteriorly than *C. itororensis* (Hymenoptera online, 2018). *Cotesia paphi* has been recorded from different species within the lepidopteran families Pieridae and Sphingidae, in Argentina, Brazil, Peru and Uruguay (Yu *et al.* 2016).

Cotesia parallelis (Ashmead 1900) has ovipositor about two-thirds the length of the metasoma, and first metasomal tergite a little more than twice as long as wide, with sides parallel (Ashmead 1900); whereas *C. itororensis* has ovipositor shorter, about one-third the length of the metasoma, and first metasomal tergite as long as wide, with slight widening from anterior margin to 0.7 of mediotergite length (widest point), then becoming rounder towards posterior margin. *Cotesia parallelis* has been recorded so far from Saint Vincent (Yu *et al.* 2016).

Cotesia prenidis (Muesebeck 1921) has the first metasomal tergite rugulose-punctate, and the second metasomal tergite broad, almost rectangular (Muesebeck 1921); whereas *C. itororensis* has first metasomal tergite mostly smooth, and the second metasomal tergite is somewhat triangular, broadening posteriorly. *Cotesia prenidis* has been recorded so far from *Nyctelius* (Latreille 1824) and *Panoquina nero* Fabricius (Lepidoptera: Hesperidae) in Puerto Rico (Yu *et al.* 2016).

Cotesia ruficrus (Haliday 1834) has legs rufous-testaceous (Cameron 1911); whereas *C. itororensis* has legs mostly yellow-honey. *Cotesia ruficrus* has been recorded from Bostrichidae (Coleoptera), and lepidopterans within the families, Geometridae, Hesperidae, Lycaenidae, Noctuidae, Nymphalidae and Pieridae, in Trinidad & Tobago (Yu *et al.* 2016).



FIGURE 3. (a) Parasitized larva of *Oospila pallidaria* (Geometridae) in the fourth instar, with a swollen body in the posterior half, (b) parasitized larva in the abaxial side of the leaf, and (c) dead larva and yellow cocoon of *Cotesia itororensis* (Braconidae)

Cotesia schini (Muesebeck 1958) has mesoscutum uniformly covered with distinct, separated punctures, and tegulae and wing bases yellow (Muesebeck 1958); whereas *C. itororensis* has mesoscutum anteriorly with distinct dense punctures, becoming smooth posteriorly, and tegulae dark brown translucent, and wings with few veins dark, but most pale. *Cotesia schini* has been recorded so far from an unidentified caterpillar of Riodinidae feeding on the Brazilian pepper tree or Christmas berry tree, *Schinus terebinthifolius* Raddi (Anacardiaceae) (Muesebeck 1958).

Cotesia theclae (Riley 1881) female has antennae much shorter than the body (Riley 1881); whereas females of *C. itororensis* has antennae as long as the body length. *Cotesia theclae* has been recorded from different families of Lepidoptera (Lycaenidae and Noctuidae) in Mexico (Yu *et al.* 2016).

Cotesia vestalis (Haliday 1834) has femora mostly yellowish/orangish, sometimes darker at base or tip, hind coxae mostly dark brown to black, apically slightly paler and first metasomal tergite mostly rugose-punctate in basal third (Shaw 2003); whereas *C. itororensis* has femora honey-yellow, coxae black and the first metasomal tergite mostly smooth. *Cotesia vestalis* has been recorded parasitizing several species of Lepidoptera among the families Arctiidae, Lasiocampidae, Lymantriidae, Noctuidae, Notodontidae, Nymphalidae, Pieridae, Plutellidae, Pterophoridae, Pyralidae, and Tortricidae, in Argentina and Brazil (Yu *et al.* 2016).

Discussion

We present the first description of a new species of *Cotesia* associated with Geometridae in South America. Conversely, in other parts of the world especially Europe, there are many descriptions and records of *Cotesia* associated with Geometridae (Schumacher *et al.* 2000; Özbek & Çalmaşur 2010; Ruohomäki *et al.* 2013). Further studies in the Neotropical region will likely reveal new species and associations between *Cotesia* and Geometridae. However, it is important to mention that there is an unconfirmed record of *C. glomerata* parasitizing Geometridae for the Neotropical region (Yu *et al.* 2016). Furthermore, Tepe *et al.* (2014) recorded an unidentified species of *Cotesia* associated with *Eois* sp. (Geometridae) on *Piper kelleyi* Tepe in Ecuador and Peru, and Smith *et al.* (2008) recorded, but did not describe, several new species of *Cotesia* associated with Geometridae in Costa Rica.

Cotesia itororensis appears somewhat intermediate between the closely related genera *Cotesia* and *Protapanteles*, in that the propodeum is relatively smooth, and the second metasomal tergite is somewhat triangular, broadening posteriorly (as in *Protapanteles*), but the propodeum has a complete medial carina, and the first metasomal tergite is relatively broad and broader posteriorly (true of *Cotesia*). *Protapanteles* is often reared from Geometridae, although the only species so far recorded from South America is *P. eryphanidis* (Whitfield), reared from *Eryphanis greeneyi* (Penz and Devries) (Nymphalidae) in Ecuador (Greeney *et al.* 2011). *Cotesia itororensis* is morphologically close to *C. congregata*, *C. glomerata* and *C. marginiventris*, and ecologically to *C. autumnatae*, *C. geometricae*, *C. jucunda*, although all of the three last species occur in Australia or Europe (Schumacher *et al.* 2000; Ruohomäki *et al.* 2013; Yu *et al.* 2016). With respect to molecular data, *C. itororensis* is close to *C. salebrosa*, *C. autumnatae*, *C. griffini*, *C. vestallis*, *C. meliataerum*, *C. bigneli*, *C. acuminata*, *C. glomerata*, *C. marginiventris*, *C. koebelei*, *C. rubecula*, *C. flavipes*, *C. sesamiae*, *C. urubae*, *C. nonagriae* and *C. chionis*, according to BLAST.

As species of *Cotesia* are important agents of biological control against pest insects, the correct identification of them is paramount to success in pest management programs. In this perspective, we provided here a comparative diagnosis to distinguish the species of *Cotesia* occurring in the Neotropical region. We reinforce the use of an integrative taxonomic approach (combining morphological, molecular, biological and geographical data) to avoid mistakes in identifications of *Cotesia*.

Acknowledgments

We are grateful to Lizzy Dabek for some photography of specimens, Dr Ricardo Harakava of Instituto Biológico de São Paulo for analyses of DNA barcode, and the staff of Clube Caça e Pesca Itororó de Uberlândia, where the study was performed. BSL would like to thank Nayane Alves da Silva for support in field and laboratory and Melissa Alves Lopes for her encouragement. BSL thanks the Brazilian CNPq (Proc.142280/2016-0), KDC thanks the Brazilian CNPq (Proc. 301605/2013-0) and also thanks to a FAPEMIG research grant.

References

- Ashmead, W.H. (1900) Report upon the Aculeate Hymenoptera of the island of St. Vincent and Grenada, with additions to the parasitic Hymenoptera and a list of the described Hymenoptera of the West Indies. *Transactions of the Entomological Society of London*, 1900, 207–367.
- Ashmead, W.H. (1906) Descriptions of new Hymenoptera from Japan. *Proceedings of the United States National Museum*, 30, 169–201.
<https://doi.org/10.5479/si.00963801.30-1448.169>
- Avalos, D.S., Mangeaud, A. & Valladares, G.R. (2016) Parasitism and food web structure in defoliating Lepidoptera–parasitoid communities on soybean. *Neotropical Entomology* 45 (6), 712–717.
<https://doi.org/10.1007/s13744-016-0416-8>
- Brèthes, J. (1916) Hyménoptères parasites de l'Amérique méridionale. *Anales Del Museo Nacional de Historia Natural de Buenos Aires*, 27, 401–430.
- Cameron, P. (1911) On a collection of parasitic Hymenoptera (chiefly bred) made by Mr. W.W. Froggatt, F.L.S., in New South Wales, with description of new genera and species. Part i. *Proceedings of the Linnean Society of New South Wales*, 36, 333–346.
<https://doi.org/10.5962/bhl.part.21902>
- Costa-Lima, A.M. (1950) *Insetos do Brasil. 6º tomo. Lepidópteros. 2ª parte. Série didática. Número 8. Capítulo 28*. Escola Nacional de Agronomia, Rio de Janeiro, 420 pp.
- Cresson, E.T. (1865) On the Hymenoptera of Cuba. *Proceedings of the Entomological Society of Philadelphia*, 4, 1–200.
- Fernandez-Triana, J.L., Whitfield, J.B., Rodriguez, J.J., Smith, M.A., Janzen, D.H., Hallwachs, W., Hajibabaei, M., Burns, J.M., Solis, M.A., Brown, J., Cardinal, S., Goulet, H. & Hebert, P.D.N. (2014) Review of *Apanteles* (Hymenoptera, Braconidae, Microgastrinae) from Area de Conservacion Guanacaste, Costa Rica, with keys to all described species from Mesoamerica. *ZooKeys*, 383, 15–65.
<https://doi.org/10.3897/zookeys.383.6418>
- Fiaboe, K.K.M., Fernandez-Triana, J.L., Nyamu, F.W. & Agbodzavu, K.M. (2017) *Cotesia icipe* sp. n., a new Microgastrinae wasp (Hymenoptera, Braconidae) of importance in the biological control of Lepidopteran pests in Africa. *Journal of Hymenoptera Research*, 61, 49–64.
<https://doi.org/10.3897/jhr.61.21015>
- Gilmore, J.U. (1938) Notes on *Apanteles congregatus* (Say) as a parasite of tobacco hornworms. *Journal of Economic Entomology*, 31, 712–715.
<https://doi.org/10.1093/jee/31.6.712>
- Greeney, H.F., Whitfield, J.B., Stireman, J.O., Penz, C.M. & Dyer, L.A. (2011) Natural History of *Eryphanis greeneyi* (Lepidoptera: Nymphalidae) and Its Enemies, With a Description of a New Species of Braconid Parasitoid and Notes on Its Tachinid Parasitoid. *Annals of the Entomological Society of America*, 104 (6), 1078–1090.
<https://doi.org/10.1603/AN10064>
- Gusmão, F.A., Harakava, R. & Campos, A.E.C. (2010) Fire-ants of the *Solenopsis saevissima* species-group (Hymenoptera: Formicidae) nesting in parks in the city of São Paulo: identification based on mtDNA sequences and morphological characters. *Sociobiology*, 56, 353–362.
- Haines, W.P., Heddle, M.L., Welton, P. & Rubinoff, D. (2009) A Recent Outbreak of the Hawaiian Koa Moth, *Scotorythra paludicola* (Lepidoptera: Geometridae), and a Review of Outbreaks between 1892 and 2003. *Pacific Science*, 63 (3), 349–369.
<https://doi.org/10.2984/049.063.0305>
- Hymenoptera Online (HOL) (2018) Available from: <https://hol.osu.edu/spmInfo.html?id=MZSP%2019502> (accessed 13 June 2018)
- Kaiser, L., Fernandez-Triana, J., Capdevielle-Dulac, C., Chantre, C., Bodet, M., Kaoula, F., Benoist, R., Calatayud, P.A., Dupas, S., Herniou, E.A., Jennette, R., Obonyo, J., Silvain, J.F. & Ru, B.L. (2017) Systematics and biology of *Cotesia typhae* sp. n. (Hymenoptera, Braconidae, Microgastrinae), a potential biological control agent against the noctuid Mediterranean corn borer, *Sesamia nonagrioides*. *Zookeys*, 682, 105–136.
<https://doi.org/10.3897/zookeys.682.13016>
- Kankare, M. & Shaw, M.R. (2004) Molecular phylogeny of *Cotesia* Cameron, 1891 (Insecta: Hymenoptera: Braconidae: Microgastrinae) parasitoids associated with Melitaeini butterflies (Insecta: Lepidoptera: Nymphalidae: Melitaeini). *Molecular Phylogenetics and Evolution*, 32, 207–220.
<https://doi.org/10.1016/j.ympev.2003.11.013>
- Mason, W.R.M. (1981) The polyphyletic nature of *Apanteles* Foerster (Hymenoptera: Braconidae): a phylogeny and reclassification of Microgastrinae. *Memoirs of the Entomological Society of Canada*, 115, 1–147.
<https://doi.org/10.4039/entm113115fv>
- Michel-Salzat, A. & Whitfield, J.B. (2004) Preliminary evolutionary relationships within the parasitoid wasp genus *Cotesia* (Hymenoptera: Braconidae: Microgastrinae): combined analysis of four genes. *Systematic Entomology*, 29, 371–382.
<https://doi.org/10.1111/j.0307-6970.2004.00246.x>
- Muesebeck, C.F.W. (1921) A revision of the North American species of ichneumon-flies belonging to the genus *Apanteles*.

- Proceedings of the United States National Museum*, 58, 483–576.
<https://doi.org/10.5479/si.00963801.2349.483>
- Muesebeck, C.F.W. (1958) New Neotropical wasps of the family Braconidae (Hymenoptera) in the U.S. National Museum. *Proceedings of the United States National Museum*, 107, 405–461.
<https://doi.org/10.5479/si.00963801.108-3389.405>
- Özbek, H. & Çalmaşur, Ö. (2010) Spotted ash looper, *Abraxas pantaria* (L.) (Lepidoptera: Geometridae), a new ash pest in Turkey. *Turkish Journal of Zoology*, 34, 351–358.
- Pizzato, M., Pietrowski, V., Alves, L.F.A. & Rheinheimer, A.R. (2016) Host suitability and fitness-related parameters of *Cotesia glomerata* L. (Hymenoptera: Braconidae) on different instars of *Ascia monuste orseis* Godart (Lepidoptera: Pieridae). *Arquivos do Instituto Biológico*, 83, 1–7.
- Riley, C.V. (1881) Notes on North American Microgasters, with descriptions of new species. *Transactions of the Academy of Science of St. Louis*, 4, 296–315.
- Rodriguez, J.J., Fernández-Triana, J., Smith, M.A., Janzen, D.H., Hallwachs, W., Erwin, T.L. & Whitfield, J.B. (2012) Extrapolations from field studies and known faunas converge on dramatically increased estimates of global microgastrine parasitoid wasp species richness (Hymenoptera: Braconidae). *Insect Conservation and Diversity*, 6, 530–536.
<https://doi.org/10.1111/icad.12003>
- Ruohomäki, K., Klemola, T., Shaw, M.R., Snäll, N., Sääksjärvi, I.E., Veijalanien, A. & Wahlberg, N. (2013) Microgastrinae (Hymenoptera: Braconidae) parasitizing *Epirrita autumnata* (Lepidoptera: Geometridae) larvae in Fennoscandia with description of *Cotesia autumnatae* Shaw, sp. n. *Entomologica Fennica*, 24, 65–80.
- Shaw, M.R. (2003) Revised synonymy in the genus *Cotesia* (Hymenoptera: Braconidae: Microgastrinae): the identity of *Microgaster vestalis* Haliday, 1834, as a senior synonym of *Apanteles plutellae* Kurdjumov, 1912. *Entomologist's Gazette*, 54, 187–189.
- Shaw, M.R. & Huddleston, T. (1991) Classification and biology of braconid wasps (Hymenoptera, Braconidae). Handbooks for the Identification of British Insects. *Royal Entomological Society of London*, 7, 1–126.
- Schmitz, A. & Riesner, D. (2006) Purification of nucleic acids by selective precipitation with polyethylene glycol 6000. *Analytical Biochemistry*, 354, 311–313.
<https://doi.org/10.1016/j.ab.2006.03.014>
- Schumacker, R.K., Austin, A.D. & Floyd, R.B. (2000) Parasitoids of the autumn gum moth, *Mnesampela privata* (Guenée) (Lepidoptera: Geometridae) in south-eastern Australia, with description of two new larval parasitoids. *Transactions of the Royal Society of South Australia*, 124 (1), 1–15.
- Smith, M.A., Rodriguez, J.J., Whitfield, J.B., Deans, A.R., Janzen, D.H., Hallwachs, W. & Hebert, P.D.N. (2008) Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology, and collections. *Proceedings of the National Academy of Sciences of the United States of America*, 105 (35), 12359–12364.
<https://doi.org/10.1073/pnas.0805319105>
- Sousa-Lopes, B., Bächtold, A. & Del-Claro, K. (2016) Biology, natural history and temporal fluctuation of the geometrid *Oospila pallidaria* associated with host plant phenology. *Studies on Neotropical Fauna and Environment*, 51 (2), 135–143.
<https://doi.org/10.1080/01650521.2016.1199140>
- Tepe, E.J., Rodríguez-Castañeda, G., Glassmire, A.E. & Dyer, L.A. (2014) *Piper kelleyi*, a hotspot of ecological interactions and a new species from Ecuador and Peru. *PhytoKeys*, 34, 19–32.
<https://doi.org/10.3897/phytokeys.34.6376>
- Viereck, H.L. (1912) Description of five new genera and twenty six new species of Ichneumon-flies. *Proceedings of the United States National Museum*, 42, 139–153.
<https://doi.org/10.5479/si.00963801.1888.139>
- Viereck, H.L. (1913) Description of ten new genera and twenty-three new species of Ichneumon-flies. *Proceedings of the United States National Museum*, 44, 555–568.
<https://doi.org/10.5479/si.00963801.1968.555>
- Whitfield, J.B. (1997) Subfamily Microgastrinae. In: Wharton, R.A., Marsh, P.M., Sharkey, M.J. (Eds.), *Identification Manual to the New World Genera of the Family Braconidae (Hymenoptera)*. *International Society of Hymenopterists Special Publication*. International Society of Hymenopterists, Washington, D.C., pp. 333–364.
- Whitfield, J.B., Rodriguez, J.J. & Masonick, P.K. (2009) Reared microgastrine wasps (Hymenoptera: Braconidae) from Yanayacu Biological Station and environs (Napo Province, Ecuador): diversity and host specialization. *Journal of Insect Science*, 9 (1), article 31, 1–22.
<https://doi.org/10.1673/031.009.3101>
- Yu, D.S.K., Van Achterberg, C. & Horstmann, K. (2016) *Taxapad, Ichneumonoidea 2015*. Ottawa, Ontario. Available from: <http://www.taxapad.com> (accessed 11 December 2018)

