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Condicionalidade nas interações ecológicas: características morfológicas e variações fenológicas afetando o sistema Malpighiaceae-formiga-herbívoros.

ANDRÉA ANDRADE VILELA SIQUIEROLI

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

Orientador:
Prof. Dr. Kleber Del Claro

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Banca examinadora:

Prof. Dr. Kleber Del Claro
(Titular/Orientador)

Profa. Dra. Denise Lange
(Titular)

Profa. Dra. Ariádine Cristine de Almeida
(Titular)

Prof. Dr. Vinicius Lourenço Garcia de Brito
(Titular)

Prof. Dr. Roberth Fagundes
(Titular)

Uberlândia, MG
Maio de 2016

Essa tese é dedicada aos meus dois

anjinhos João Gabriel e Maria Clara

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Resumo

Vilela, A.A.S. 2016. Condicionalidade nas interações ecológicas: características morfológicas e variações fenológicas afetando o sistema Malpighiaceae-formiga-herbívoros. Tese de Doutorado em Ecologia e Conservação de Recursos Naturais. Universidade Federal de Uberlândia. Uberlândia-MG. 104p.Orientação: Prof. Dr. Kleber Del Claro.

As interações ecológicas são diversas e os resultados podem variar em resposta a fatores bióticos e abióticos. Plantas e formigas podem se associar por meio de secreções açucaradas produzidas por nectários extraflorais (NEFs) e as formigas também podem receber alimento de hemípteros produtores de honeydew. Autores demonstram os efeitos positivos de tais interações, porém a magnitude desses benefícios pode variar em função de características morfológicas, fisiológicas e comportamentais das formigas, assim como de variações nos eventos reprodutivos das plantas e na sobreposição entre as espécies interagentes. O Cerrado apresenta inúmeras espécies com NEFs que se associam com formigas e hemípteros produtores de honeydew e tem como caraterística a sazonalidade com possíveis variações fenológicas das espécies entre os anos. Tendo o sistema Malpighiaceae-formigas-herbívoros como modelo no Cerrado, os objetivos foram avaliar as variações nos resultados da interação formiga-planta-hemíptero considerando duas espécies de formigas com diferentes adaptações fisiológicas e ecológicas para a interação (*Camponotus crassus* (Formicinae) e *Ectatomma tuberculatum* (Ectatomminae). Analisar os efeitos de variações fenológicas nas taxas de sobreposição de espécies de formigas e herbívoros endofiticos (Curculionideos) e as consequências para a espécie *Banisteriopsis laevifolia,* além de verificar o padrão de floração (sincronizado ou sequencial) na comunidade formada pelas espécies *B. laevifolia, Banisteriopsis malifolia, Banisteriopsis campestris* e *Peixotoa tomentosa* e os efeitos de variações na sobreposição para as taxas de herbivoria e produção de frutos. Os resultados corroboraram a hipótese da competição intraespecífica das cochonilhas pela proteção das formigas, assim como os efeitos da biologia das espécies de formigas no resultado da interação. As variações fenológicas também foram comprovadas e associadas às mudanças nos fatores climáticos de temperatura e precipitação. As variações fenológicas ocorridas com a *B. laevifolia* promoveram uma assincronia com os curculionídeos e uma maior sobreposição com a espécie simpátrica, *P. tomentosa.* Essas alterações impactaram as interações formiga-planta-herbívoro e planta-planta e confirmam a hipótese que alterações nos eventos fenológicos são capazes de afetar o resultado de interações chaves e, consequentemente, o sucesso reprodutivo das plantas. Em relação aos padrões de floração nas comunidades, houve um efeito diferencial na herbivoria e na produção de frutos para as quatro espécies estudadas. Tais efeitos foram espécie-específicos e dependentes da íntima associação dessas espécies com seus agentes polinizadores e herbívoros associados. O modelo matemático desenvolvido mostrou-se confiável e capaz de estimar as respostas espécie-específicas em situações de contínuas variações fenológicas. Compreender como as plantas e animais interagem é a chave para entender traços importantes dos processos evolutivos. A integração entre as investigações de campo, as teorias ecológicas e os modelos matemáticos nos permite interpretar a condicionalidade de importantes interações ecológicas, descrever a evolução da história de vida dos organismos e os efeitos para o estabelecimento e manutenção das espécies ao longo do tempo. Assim sendo, o trabalho contribuiu para uma crescente área de pesquisa sobre a inerente diversidade de interações associadas às atuais variações ambientais, tornando o estudo relevante para uma maior compreensão sobre a ecologia das interações e a conservação das comunidades naturais.

Palavras-Chave: Nectários extraflorais, Fenologia, Bioma Cerrado.

Abstract

Vilela, A.A.S. 2016.Conditionality in ecological interactions: morphological and phenological variations affecting the Malpighiaceae-ant-herbivore system. Ph D. Thesis. Universidade Federal de Uberlândia. Uberlândia-MG. 104p. Adviser: Prof. Dr. Kleber Del Claro.

The ecological interactions are diverse and the results may vary in response to biotic and abiotic factors. Plants and ants can be associated through sugary secretions produced by extrafloral nectaries (EFNs) and the ants can also get honeydew produced by Hemiptera. Authors have demonstrated the positive effects of such interactions, however the magnitude of benefits can vary depending on morphology, physiology and behavior of ants, as well as on variations in plants reproductive events and the overlap degree between the interacting species. The Cerrado has numerous EFNs bearing plants associated with ants and honeydew producing Hemiptera and presents a seasonal phenology. Considering Malpighiaceae-ants-herbivores as a model system in Cerrado, the aims were to evaluate the outcomes of ant-plant-hemiptera interaction considering two species of ants with different physiological and ecological adaptations to interaction (*Camponotu crassus* (Formicinae) and *Ectatomma tuberculatum* (Ectatomminae). Analyze the effects of phenological changes in ants and endophytic endophytic herbivores (Curculionids) overlap and the consequences for *Banisteriospsis laevifolia* and to verify the flowering strategy (synchronized or sequential) in the community composed by *B. laevifolia, Banisteriospsis malifolia, Banisteriospsis campestris* and *Peixotoa tomentosa* and the effects of different degrees of overlapping to herbivory and fruit production. The results confirmed the hypothesis of intraspecific competition of mealybugs by ant's protection, as well as the effects of the biology of ant species in the results of interactions. The phenological changes were also proven, associated with changes in climatic factors of temperature and precipitation. The phenological variations of *B. laevifolia* promoted asynchrony with Curculionids and greater overlap with a sympatric species, *P. tomentosa*. These changes affected the ant-plant-herbivore and plant-plant interactions and confirmed the hypothesis that changes in phenology are able to affect the outcomes of key interactions and consequently the reproductive success of plants. Regarding the flowering patterns in communities, there was a differential effect on herbivory and fruit production for the four species studied. Such effects were species-specific and dependent on the closed association of these species with their pollinators and associated herbivores. The mathematical model was reliable and able to estimate the species-specific responses in situations of continuous phenological changes. Understanding how plants and animals interact is a key to understand important features of evolutionary processes. The integration of field investigations, ecological theories and mathematical models allows us to interpret the cross of important ecological interactions, describe the evolution of life history of organisms and the effects for the establishment and maintenance of the species over time. Thus, the present work contributed to a growing area of research about the inherent diversity of interactions associated with current environmental variations, making it a relevant study for a greater understanding of the ecology of the interactions and the conservation of natural communities.

Keywords: Extrafloral nectaries, Phenology, Cerrado Biome

Lista de Figuras / List of Figures

Capítulo 1: Hemipteran competition for ant services in a Neotropical Savanna: conditional outcomes mediated by ant morphological traits and aggressiveness.

Capítulo 2: Phenological variations and the effects for ecological plant interactions in a Brazilian Tropical Savanna

Capítulo 3. Phenology strategies affecting biotic interactions and plant reproductive success in a savanna community

Lista de Tabelas/ List of Tables

Capítulo 1: Hemipteran competition for ant services in a Neotropical Savanna: conditional outcomes mediated by ant morphological traits and aggressiveness.

Capítulo 2: Phenological variations and the effects for ecological plant interactions in a Brazilian Tropical Savanna

Capítulo 3. Phenology strategies affecting biotic interactions and plant reproductive success in a savanna community

Índice

Cap 1 - Hemipteran competition for ant services in a Neotropical Savanna: conditional outcomes mediated by ant morphological traits and aggressiveness.

Cap 2 - Phenological variations and the effects for ecological plant interactions in a Brazilian Tropical Savanna

Cap 3 - Phenology strategies affecting biotic interactions and plant reproductive success in a savanna community

INTRODUÇÃO GERAL

Introdução Geral

As interações ecológicas estão entre as principais forças estruturadoras das comunidades e a compreensão dos mecanismos que moldam essas forças tem sido um dos temas mais importantes na ecologia na atualidade (Rafferty *et al.* 2015, Ehrlén 2015). A variedade das interações possíveis entre os organismos é muito diversa e os custos e/ou benefícios para os parceiros podem variar entre interações mutualísticas e antagônicas (Del Claro e Torezan-Silingardi 2012). Além disso, a direção e a magnitude dos resultados para cada grupo podem variar entre contextos ecológicos em resposta a uma variedade de fatores bióticos e abióticos (Vilela *et al.* 2014, Del-Claro e Marquis 2015).

As plantas se envolvem em múltiplas interações que afetam o seu sucesso reprodutivo, como associações com espécies de formigas (Del Claro e Torezan-Silingardi 2012, Lange *et al.* 2013). Nessas relações, as plantas oferecem abrigo e/ou recursos alimentares, como o néctar extrafloral, e em troca recebem proteção contra a ação de herbívoros (Rico-Gray e Oliveira 2007, Byk e Del-Claro 2011, Del Claro *et al.* 2013). Muitos estudos demonstram o efeito positivo para as plantas em relação à diminuição da herbivoria e/ou aumento do sucesso reprodutivo (Fagundes *et al.* 2013, Zhang *et al.* 2015). No entanto, os benefícios da associação nem sempre são evidenciados (O'dowd e Catchpole 1983, Rashbrook *et al.* 1992) ou a magnitude desses benefícios variam em função de fatores ambientais influenciando os resultados das interações (Vilela *et al.* 2014, Alves-Silva e Del Claro 2015, Del Claro e Marquis 2015).

Algumas espécies de formigas podem participar simultaneamente do mutualismo com plantas que produzem néctar extrafloral e com hemípteros produtores de honeydew (Del Claro e Oliveira 2000, Vilela *et al.* 2014). Nessa relação, além do néctar extrafloral das plantas, as formigas recebem o honeydew dos hemípteros como um alimento nutritivo e em troca os protegem contra inimigos naturais (Moreira e Del-Claro 2005, Fagundes *et al.* 2013). No entanto, a presença dos hemípteros pode representar um balanço entre o benefício indireto pelo aumento das formigas na planta e redução na herbivoria e/ou aumento da produtividade (Moreira e Del Claro 2005) e um custo pela herbivoria provocada pelos próprios hemípteros atendidos (Lach 2003). Além disso, estudos mostram que a intensidade e a ocorrência de proteção podem ser fortemente dependentes da espécie de formiga interagente (Del-Claro e Oliveira 2000). Características morfológicas, fisiológicas e comportamentais das formigas podem tornar as interações nulas para todos ou um dos interagentes, fortalecendo a condicionalidade dessas interações (Stadler e Dixon 2005, Del Claro e Oliveira 2000).

Além dos caracteres específicos das espécies de formigas interagentes, a fenologia das plantas tem um efeito potencial sobre as interações ecológicas (Forrest e Thomson 2011, Ehrlén 2015). A sincronização fenológica das plantas com os herbívoros determina a abundância desses animais e, consequentemente, a taxa de recrutamento e proteção das formigas (Palmer *et al.* 2010, Elzinga *et al.* 2007). Portanto, mudanças na fenologia e na sobreposição temporal entre herbívoros e plantas são capazes de afetar o mutualismo formiga-planta e influenciar a produção de flores e frutos (Singer e Parmesan 2010, Yang e Rudolf 2010). Muitas espécies têm apresentado variações nos seus eventos reprodutivos capazes de afetar as interações ecológicas (e.g. Forrest *et al.* 2010, Rafferty e Ives 2011), porém poucos estudos abordam quais as consequências para as interações e para o fitness (e.g. produção de frutos e sementes, sobrevivência) das plantas (Liu *et al.* 2011).

Variações nos padrões reprodutivos também são capazes de aumentar ou diminuir a sincronização temporal entre as espécies de plantas dentro das comunidades (Van Schaik *et al.* 1993, Forrest *et al.* 2010, Vilela *et al.* 2014). Espécies simpátricas, espécies que evoluíram a partir de um único ancestral na mesma região geográfica, podem apresentar sobreposição na floração capaz de promover a diluição da herbivoria (Hipótese da Saciedade dos Herbívoros - Beattie *et al.* 1973) ou apresentarem uma floração sequencial para evitar a competição por polinizadores (Hipótese de Competição por Polinizadores - Robertson 1895). Assim, a estratégia de floração das espécies se baseia em uma maior ou menor sobreposição temporal

(Boulter *et al.* 2006), afetada por mudanças fenológicas correlacionadas, por exemplo, com fatores abióticos de temperatura e precipitação (Franks *et al.* 2013, CaraDonna *et al.* 2014). Nesse sentido, modelos matemáticos podem ser desenvolvidos e gerar predições testáveis que abordam mudanças fenológicas de diferentes magnitudes e direções e suas consequências para o sucesso reprodutivo das espécies interagentes (Memmott *et al.* 2007, Diez *et al.* 2012, Fabina *et al.* 2010).

O Cerrado é um bioma com grande variação sazonal e cerca de 25,5% de espécies apresentando nectários extraflorais (NEFs) (Rico-Gray e Oliveira 2007). Portanto, variações sazonais e fenológicas nas interações seriam muito comuns nesse ecossistema. A família Malpighiaceae apresenta uma grande representatividade no Cerrado (Souto 2007) e espécies com NEFs associados a formigas e hemípteros produtores de honeydew (Vilela *et al.* 2014). Tendo o sistema Malpighiaceae-formigas-herbívoros como modelo no Cerrado, os objetivos do trabalho incluíram avaliar as possíveis variações nos resultados da interação formiga-planta-hemíptero considerando duas espécies de formigas com diferentes adaptações fisiológicas e ecológicas para a interação (*Camponotus crassus* (Formicinae) e *Ectatomma tuberculatum* (Ectatomminae)). Analisar os efeitos de variações fenológicas nas taxas de sobreposição de espécies de formigas e herbívoros (Curculionideos) e as consequências para a espécie *Banisteriopsis laevifolia.* Além de verificar o padrão de floração (sincronizado ou sequencial) na comunidade formada pelas espécies *B. laevifolia, Banisteriopsis malifolia, Banisteriopsis campestris* e *Peixotoa tomentosa* e os efeitos de variações na sobreposição para as taxas de herbivoria e produção de frutos.

Objetivos específicos:

Esta tese foi dividida em três capítulos apresentados na forma de artigos científicos:

Cap 1 - *Hemipteran competition for ant services in a Neotropical Savanna: conditional outcomes mediated by ant morphological traits and aggressiveness.*

Verificar se hemípteros produtores de honeydew (Cochonilhas da família Ortheziidae) competem intraespecíficamente pela proteção das formigas (Hipótese da Competição por mutualismo - Addicott 1978b; Cushman e Addicott 1989). Além disso, analisar se a espécie *C. crassus,* que apresenta proventrículo ("estômago social"), difere da espécie *E. tuberculatum*, que não apresenta essa estrutura, em relação aos benefícios oferecidos às cochonilhas (sobrevivência) e também à espécie *B. campestris* (produtividade de frutos). Inicialmente, espera-se a competição intraespecífica das cochonilhas pela proteção das formigas corroborando a hipótese da competição por mutualismo postulada por Addicott (1978b) e Cushman e Addicott (1989). Além disso, acredita-se que a espécie com proventrículo ("estômago social"), *C. crassus*, apresente uma relação de proteção mais estreita com as cochonilhas por apresentar as mandíbulas livres para o ataque contra os inimigos naturais. Ao mesmo tempo, essa maior proteção às cochonilhas pode representar um dano mais acentuado às estruturas reprodutivas da espécie *B. campestris*.

Cap 2 - *Phenological variations and the effects for ecological plant interactions in a Brazilian Tropical Savanna*

Verificar mudanças no padrão fenológico da espécie *B. laevifolia* ao longo de 4 anos e na sobreposição da floração com a ontogenia de um importante herbívoro endofitico (coleópteros da família Curculionidae) e com espécies simpátricas (*B. malifolia, B. campestris* e *P. tomentosa*). Para relacionar as variações fenológicas com as interações ecológicas da planta, foram considerados os resultados da associação formiga-planta-herbívoro e as interações planta-planta para o sucesso reprodutivo da espécie *B. laevifolia*. Além disso, um modelo matemático foi desenvolvido para elucidar como possíveis variações fenológicas podem afetar as taxas de produção de flores e frutos da *B. laevifolia* em um futuro próximo (cinco anos).

Baseado nas observações de campo, acredita-se que as datas de início dos eventos reprodutivos da espécie estejam variando ao longo do tempo alterando a sincronia entre a floração e o período de desenvolvimento dos curculionídeos, além da sobreposição com espécies simpátricas. Espera-se que tais variações sejam capazes de influenciar os resultados das interações formiga-planta-herbívoro e planta-planta em relação às taxas de produção de flores e frutos da *B. laevifolia*. Além disso, acredita-se que o modelo matemático desenvolvido seja capaz de estimar os efeitos de variações contínuas na fenologia da *B. laevifolia* com um alto grau de confiabilidade.

Cap 3 - *Phenology strategies affecting biotic interactions and plant reproductive success in a savanna community*

Verificar variações nos padrões climáticos de temperatura e precipitação ao longo de dez anos (2005-2014) e possíveis correlações desses parâmetros com o início da floração de quatro espécies de Malpighiaceae (*B. laevifolia, B. malifolia, B. campestris* e *P. tomentosa*) durante três períodos reprodutivos (2008/2009, 2011/2012, 2013/2014). Determinar mudanças no grau de sobreposição fenológica entre as espécies e os efeitos para as taxas de herbivoria e produção de frutos. Além disso, um modelo matemático foi desenvolvido para elucidar como variações fenológicas podem afetar as taxas de produção de flores e frutos das quatro espécies de plantas em um futuro próximo (cinco anos).

Diante das pesquisas atuais sobre mudanças climáticas, espera-se variações nos padrões de temperatura e precipitação ao longo dos anos e correlações com o início das datas de floração das espécies estudadas. Além disso, variações nos graus de sobreposição temporal entre as espécies de Malpighiaceae (sincronizada ou sequencial) teria o potencial de afetar as taxas de herbivoria e frutificação de maneira espécie-específica.

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CAPÍTULO 1

HEMIPTERAN COMPETITION FOR ANT SERVICES IN A NEOTROPICAL SAVANNA: CONDITIONAL OUTCOMES MEDIATED BY ANT MORPHOLOGICAL TRAITS AND AGGRESSIVENESS.

Hemipteran competition for ant services in a Neotropical Savanna: conditional outcomes mediated by ant morphological traits and aggressiveness.

Andréa Andrade Vilela[1] and Kleber Del-Claro[1*]

[1] Laboratório de Ecologia Comportamental e Interações, Instituto de Biologia, Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, CEP 38400-920, Brasil.
[*]Corresponding author: delclaro@ufu.br

Abstract

Ants commonly participate simultaneously in mutualisms with honeydew-producing hemipterans and extrafloral nectar bearing host plants, protecting plants against herbivores and hemipterans against their natural enemies. However, the benefit may vary according to the species of ant interacting and the outcomes for the plant productivity will depend on the results of plant-ant-hemipteran relationship. We studied a plant-mealybug association with *Camponotus crassus* Mayr (Formicinae) and *Ectatomma tuberculatum* Olivier (Ectatomminae) in a Brazilian Tropical Savanna. Our main hypothesis was that mealybugs compete for ant's attendance indirectly affecting the host plant productivity. Results showed that only *C. crassus* affected positively the survivorship of mealybugs and, consequently, decreased the host plant productivity. Thus, we provided further evidence that ant-plant mutualism can be highly conditional in relation to ant species, reinforcing the hypothesis that ants can differ greatly in their deterrent abilities and the importance of their physiological and morphological traits conditioning the outcomes of these relationships.

Keywords: Conditional mutualism, Mealybugs, Cerrado Biome.

Introduction

At community level, organisms commonly participate in associations with different partners simultaneously and the outcomes from multiple interactions cannot be predicted by pairwise interactions alone (Savage and Rudges 2013; Thompson 2013; Stefani *et al.* 2015). Interaction networks consist of partner species acting directly or indirectly on each other to regulate the net outcomes of each interaction (Grinath *et al.* 2012; Lange and Del-Claro 2014; Datillo *et al.* 2015). In this sense, the relationships among ants, hemipterans and plants bearing extrafloral nectar (EFN) provide valuable models for disentangling the complex dynamics of mutualistic interactions exploring the range of outcomes for involved species (Del Claro and Oliveira 2000; Zhang *et al.* 2012; Fagundes *et al.* 2013).

Ants commonly participate simultaneously in mutualisms with honeydew-producing hemipterans and with EFN bearing host plants (Blüthgen *et al.* 2000; Del-Claro 2004). In ant-plants interactions, ants often protect plants against herbivores and plants provide ants with nesting sites and/or food resources (Rico-Gray and Oliveira 2007; Byk and Del-Claro 2011). In addition, ants may also be attracted by hemipterans producing honeydew, an exudate rich in sugars, amino acids, minerals and secondary compounds from the host plant (Blüthgen *et al.* 2004), and, in turn, can protect the hemipterans against enemies, which may result in increased survivorship, fecundity and developmental rate (Del Claro and Oliveira 2000; Moreira and Del-Claro 2005; Fagundes *et al.* 2013). As a consequence, distinct hemipteran aggregations can compete intra and interspecifically for ant protection (Competition for mutualism hypothesis - Addicott 1978b; Cushman and Addicott 1989). However, it is likely that the direction and magnitude of competition effects will vary among ecological contexts in response to the identity, abundance and behavior of the involved species, which can lead to different outcomes (Bronstein 1994, 1998, Stadler and Dixon 2005, Billick and Tonkel 2003).

The ant-hemipteran interactions have a variety of ecological indirect effects on the host plants (Styrsky and Eubanks 2007, Zhang *et al.* 2012, 2015). Hemipterans are sapsucking herbivores and can inject harmful substance and pathogens in the plant, affecting the plant growth and development (Delabie 2001, Oliveira and Isaias 2010). Thus, the consequences of these interactions on plant fitness may represent a trade-off between the indirect benefit by ant reducing the non-honeydew-producing herbivores and the direct costs of herbivory by the ant-tended hemipterans (Lach 2003, Trager *et al.* 2010). Many studies have confirmed that the ant-hemipteran mutualisms are beneficial to the host plant from the standpoint of herbivory reduction and increased fitness (e.g. Del Claro 2004, Moreira and Del Claro 2005, Strysky and Eubanks 2007, Zhang *et al.* 2012, 2015). However, some studies were unable to detect these positive effects (e.g Buckley 1983, Rashbrook *et al.* 1992, Rico Gray and Thien 1989, Itino *et al.* 2001a).

Ant-plant-hemipteran relationships are particularly pervasive in Cerrado (the Brazilian Tropical Savanna) due to the high incidence of exudates of insect and plant on foliage, which promotes intense ant activity on the vegetation (e.g. Del-Claro *et al.* 2013, Vilela *et al.* 2014). In this study, we sought to understand the effect of two ant species, *Ectatomma tuberculatum* (Ectatomminae) and *Camponotus crassus* (Formicinae), in an interaction with mealybugs of Ortheziidae family present on a typical EFN bearing plant of Cerrado, *Banisteriopsis campestris* (A. Juss.) Little (Malpighiaceae). Therefore, we intended to answer whether: a) both species of ants would interact in a mutualistic way with the mealybugs leading to an intraspecific competition by ant's protection, b) both species of ants are able to ensure a positive effect on the mealybug's survivorship and c) the effects of ant-hemipteran interaction for the plant productivity and herbivore deterrence would also vary according to the identity and strength of the interaction between the hemipteran and ant species. At first, we hypothesized that mealybug aggregations compete intra–specifically for the ant protectors (Competition for mutualism hypothesis - Addicott 1978b, Cushman and Addicott 1989). Second, different species of ants can produce different consequences for plant production.

Materials and Methods

Site and studied groups

Fieldwork was carried out from November 2013 to March 2014 at the *Reserva Ecológica do Clube Caça e Pesca Itororó de Uberlândia* (CCPIU) (18°59'S, 48°18'W), Uberlândia, Minas Gerais State, Brazil. We used a 640 ha Cerrado site consisting of a dense scrub of shrubs and trees, known as Cerrado *sensu stricto* (Oliveira-Filho and Ratter 2002). The climate is markedly seasonal, with a dry winter (April to September) and a rainy summer (October to March) (for additional details, see Réu and Del-Claro 2005, Ferreira and Torezan-Silingardi 2013).

The Malpighiaceae species selected for the study, *Banisteriopsis campestris* (A. Juss.) Little, was chosen because it is a very common small shrub in the Cerrado with paired EFNs on the leaf base (Anderson 1990, Gates 1982, Torezan-Silingardi 2011). This plant species has close relationships with protective ants and experiences intensive interactions with ant-honeydew-producing hemipterans, especially mealybugs of Ortheziidae family (Torezan-Silingardi 2007, Vilela *et al.* 2014).

The Ortheziidae is a morphologically well-defined family of scale insects (Vea *et al.* 2012) being known for their adornment with thick wax patches or plates that cover most of their body and a thick waxy ovisac that is attached to the body of the adult female (Miller and Kozár 2002). Females are apterous with little movement in their host plants, while males are winged and rarely seen (Kozár 2004). The life history of these scale insects is not well described but, in general, they show four female and most likely five male instars. They complete their life cycle in 30 days with offspring being deposited over 24 days and 80-102 nymphs being produced per female (Shivakumar and Lakshmikantha 2001). The taxonomy of Brazilian species in this group is poorly known and several new species are expected.

Control and treatment groups

In November of 2013, a total of 42 individuals of *B. campestris* of similar phenological state (without leaves), almost same size and architecture (0,5-1 m tall with 2-5 stems) and at least 5 m apart from each other were tagged. These plants were randomly divided into four groups: in the "control" group, plants were not manipulated in any way and ants and mealybugs had free access to all plant parts (7 plants with *C. crassus* and mealybugs; 7 plants with *E. tuberculatum* and mealybugs); In the second group, called "ants", the mealybugs were removed and only ants had access to the plants (7 plants with *C. crassus*; 7 plants with *E. tuberculatum*); In the third group, named "mealybugs", ants were removed maintaining only the mealybugs (n=7 plants) and in the fourth group, called "exclusion", all ants and mealybugs were excluded from plants (n=7 plants). Ants were excluded from plants manually and subsequently we applied an adhesive paper strap with a layer of Tanglefoot® resin on the main plant stem. All plants of groups without mealybugs (“exclusion” and “ant”) were treated with tobacco diluted in water and sprayed on the plant surface at two week intervals to prevent any mealybugs on plants (Renault *et al.* 2005).

Intraspecific mealybugs competition experiments

We performed two experiments from January until April of 2014 with only the “control” group: plants with *C. crassus* and mealybugs (n=7) and plants with *E. tuberculatum* and mealybugs (n=7). We tested whether neighboring hemipteran aggregations (two branches side by side from the same individual plant) would be able to reduce the number of ants that each aggregation attracts and whether the presence of ants would positively affect hemipteran survivorship. An intraspecific competition for protection by ants (Competition for mutualism hypothesis - Addicott 1978b, Cushman and Addicott 1989) can only be assumed if both assumptions are met.

We describe the experiments at a generic way, but the experiment was done for the plants with *C. crassus* (n=7) and *E. tuberculatum* (n=7) separately.

Interference from a neighboring aggregation – We randomly selected two branches side by side from the same individual plant (n=7) and defined them as control or treatment to assess whether the presence of one mealybug aggregation would be able to affect the frequency of ant visitation (represented by ant's touch using their antennas- Del-Claro and Oliveira 1996) on another neighbor aggregation. During all experiment the abundance of mealybugs was kept unchanged and equal for all branches (n=10). The individuals were accompanied during nine days and the experiment was divided in three periods. In a pre-manipulation period (days 1, 2 and 3), we counted the frequency of ants tending mealybug populations during 10 minutes for both control and treatment group of each plant. At the end of day 3, we excluded tending ants from group treatment by placing an adhesive paper strap with a layer of Tanglefoot® around the base of the branches. During the manipulation period (days 4, 5 and 6), we counted the frequency of ants tending mealybugs in the control group during 10 minutes. At the end of day 6, we removed the ant barriers from treatment branches and monitored subsequent ant's visitation frequency during 10 minutes in the days 7, 8 and 9 in both group (postmanipulation period).

At the same time, we did a neutral treatment using other plants with mealybugs and *C. crassus* (n=7) and mealybugs and *E. tuberculatum* (n=7) and also separated two branches side by side from the same individual plant and defined them as control or treatment, in the same way. Those individuals were also accompanied during the same nine days to record the frequency of visitation by ants but they only received an adhesive paper strap without a layer of Tanglefoot®. We intended to verify the real frequency of ant visitation without possible local environmental variations and validate our experimental results. A Repeated-Measure Analysis of Variance (One-way ANOVA) were performed using the frequency of ant's visitation on the group with ant access all time (control group) considering the premanipulation (days 1, 2 and 3) and manipulation periods (days 4, 5 and 6) to verify whether the frequency of ant attendance on control aggregation increased when prevented to visit the neighboring aggregation on treatment branch. The data were log-transformed.

Throughout the manipulative experiment (9 days), we also registered the frequency of visitation on EFNs of branches with ant access all time (control) to compare with the frequency of visitation on mealybugs. We performed Repeated-Measure Analysis of Variance (Two-way ANOVA) using the frequency visitation in each source over time for *C. crassus* and *E. tuberculatum*. The data were log-transformed.

Ant's effect on survivorship of mealybugs - After the end of the "*Interference from a neighboring aggregation experiment*" (9 days), we used the same plants to verify whether the mealybug's survivorship would depend on the presence of ants (plants with *C. crassus* and mealybugs (n=7) and plants with *E. tuberculatum* and mealybugs (n=7)). The treatment branches received an adhesive paper strap with a layer of Tanglefoot® around their base again in 01/23/2014 and the abundance of mealybugs (nymphs and adults) was monitored every three weeks (02/05/2014; 02/26/2014; 03/19/2014 and 04/09/2014). We considered their life cycle around 30 days and offspring being deposited over 24 days (sensu Shivakumar and Lakshmikantha 2001). We analyzed the effects of ant's visitation on the abundance of mealybugs throughout the time of monitoring. We performed a Friedman test with the abundance of nymphs and adults of mealybugs throughout the time for plants with *C. crassus* and *E. tuberculatum.* The Friedman test is a nonparametric counterpart of repeated-measures ANOVA for paired groups (Zimmerman and Zumbo 2003, e.g. Gonzaga and Leiner 2013).

Factorial design experiment for plant productivity test

The factorial designs use the combined effects of multispecies interactions to reveal whether the effects of species together differ from those we would expect on the basis of their separate ones (Savage and Rudgers 2013). All groups of *B. campestris* marked in November were used for the factorial experiment (n=7 per group for each species of ants): "control”, "ants", "mealybugs" and "exclusion" group. Each plant was monitored every two weeks during its four-month reproductive period (December to March, Vilela *et al.* 2014). The plants were always inspected by the same observer and the number of floral buds and fruits produced were counted. The plant productivity was expressed as the proportion of fruits formed per buds produced (e.g. Del-Claro *et al.* 1996, Vilela *et al.* 2014). The productivities were compared using Two-way Analysis of Variance (ANOVA) with the presence/absence of ants and mealybugs treated as a separate factor. A significant interaction would indicate the occurrence of an interactive effect of ants and mealybugs on plant productivity (see Nahas *et al.* 2012). Data of plant productivity were arcsine square root transformed.

We also quantified the abundance of ants, mealybugs and herbivores that not produce honeydew (non-trophobiont). We compared the abundance of mealybugs between plants with ant-hemipteran association and plants with only mealybugs using Test t for each species of ants (Zar 1996). The abundances of non-trophobiont were also compared between plants with hemipteran-ant association and plant with only ants using non-parametric Mann Whitney U tests for each species of ants. This test was used because the data were not normally distributed even after transformation (Zar 1996).

Results

Intraspecific mealybug’s competition experiments

Mealybugs competed intraspecifically for *C. crassus* protection. The frequency of *C. crassus* visitation increased in control aggregation when prevented from tending the treatment aggregation, considering the premanipulation (days 1, 2 and 3) and manipulation period (days 4, 5 and 6) (F=3.53; df=6; p=0.012 Fig.1A). On the other hand, the frequency of *E. tuberculatum* visitation did not increase when prevented from tending a neighboring aggregation (F= 1.51; df=6; p=0.220 Fig.1B). For the neutral treatment, i.e. with only adhesive paper strap without Tanglefoot®, the mean number of *C. crassus* and *E. tuberculatum* visitation remained the same during all experiment (F=1.53; df=6; p=0.213 for *C. crassus* and F=2.107, df=6; p=0.098 for *E. tuberculatum*), which validated our experimental results, ruling out any possibility of influence of environmental variations on results.

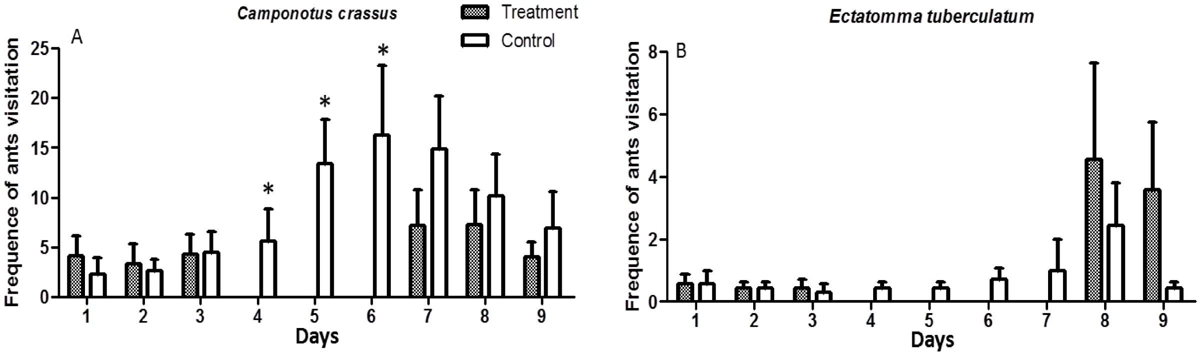

Figure 1. Frequency of visitation on mealybug aggregations during manipulative experiment (nine days) by *Camponotus crassus* (A) and *Ectatomma tuberculatum* (B) showing the treatment individuals (gray bars – with ant exclusion during manipulation period - days 4, 5 and 6) and control individuals (white bars – with ant access all time). The frequency of *C. crassus* visitation increased when prevented from tending the treatment aggregation (Repeated-Measures One-Way ANOVA, (*) means statistical difference; $p < 0.05$, Means ± 1SE are presented).

Camponotus crassus visited more often the mealybugs than EFNs (F= 4.50; df=1, p=0.036; Fig.2A), while there was no significant preference between honeydew and EFNs for *E. tuberculatum* (F= 0.005; df=1, p=0.945, Fig.2B).

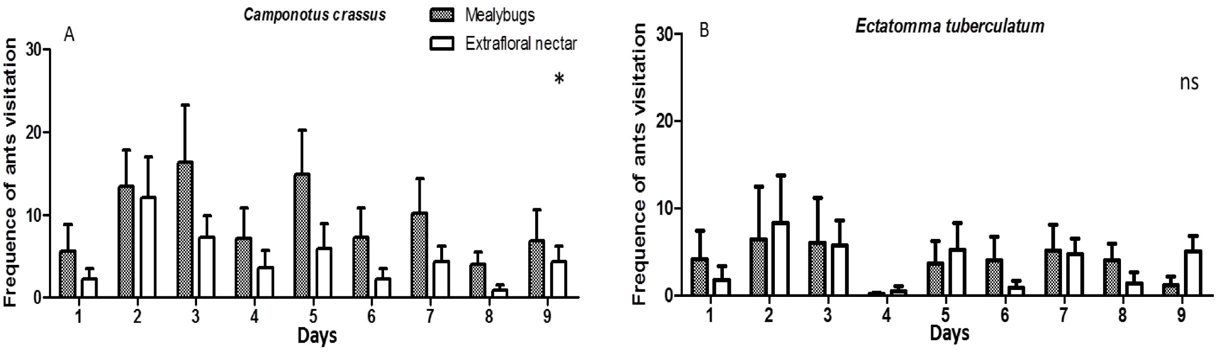

Figure 2. Frequency of visitation on mealybug aggregations and extrafloral nectaries by *Camponotus crassus* (A) and *Ectatomma tuberculatum* (B) on plants with ant access all time (gray bars: mealybugs; white bars: extrafloral nectaries). *C. crassus* visited the mealybugs more frequently with no difference for *E. tuberculatum* (Repeated-Measures Two-Way ANOVA, (*) means statistical difference; p < 0.05, Means ± 1SE are presented).

Furthermore, the survivorship of nymphs, but not of adults, of mealybugs was influenced by the presence of *C. crassus* (Friedman ANOVA Chi= 4, df=1 p<0.05 for nymphs; Chi=1, df=1, p>0.05 for adults; Fig.3A). The presence of *E. tuberculatum* did not affect the survivorship of neither nymphal nor adult mealybugs (Friedman ANOVA Chi= 1, df=1 p<0.05 for nymphs and adults Fig.3B).

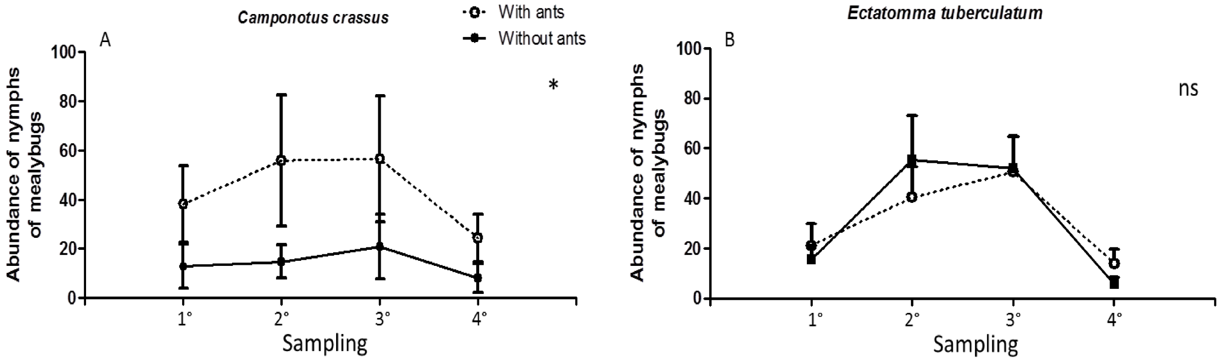

Figure 3. Comparative analysis of abundance of nymphs attended by *Camponotus crassus* (A) and *Ectatomma tuberculatum* (B) in individuals of *Banisteriopsis campestris* with and without ants over 76 days of monitoring (Sampling: 1º- 02/05/2014; 2º - 02/26/2014; 3°- 03/19/2014; 4°- 04/09/2014). Statistical difference was observed for nymphs attended by *C. crassus* (Friedman Test, (*) means statistical difference; p < 0.05, Means ± 1SE are presented).

Factorial design experiment for plant productivity test

The factorial experiment showed a significant effect of the *C. crassus*-mealybug interaction on plants with a greater negative effect on productivity than the groups with the presence of this ant and mealybugs considered separately (F=6.752; df=1; p=0.019; Fig.4; table 1). The result was not significant for plants with *E. tuberculatum*-mealybug interaction (F=3.403; df=1; p=0.08 table 1).

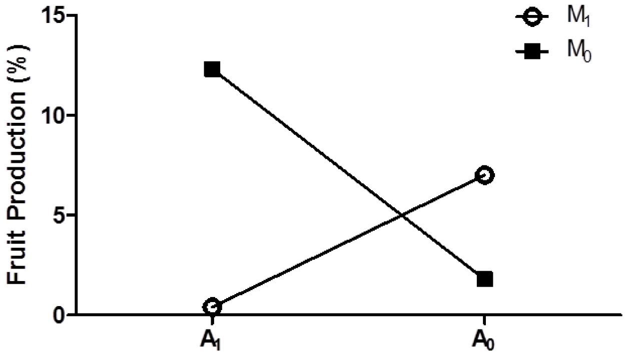

Figure 4. Productivity (fruit production) of plants with *Camponotus crassus* and mealybugs (A1M1 = with ants and mealybugs; A1M0 = only ants; A0M1 = only mealybugs; A0M0 = without ants and mealybugs). (Two-way ANOVA with the presence/absence of ants and mealybugs treated as a separate factor $p<0.05$ for A1M1; see Table 1).

Table 1. Two-way ANOVA results for the effects of the interaction between mealybugs and *Camponotus crassus* and *Ectatomma tuberculatum* on productivity (fruit production) of *Banisteriopsis campestris* in a Brazilian Tropical Savanna. (*) means statistical difference.

Source	df	SS	F	P
C. crassus				
Ants	1	0.004	0.161	0.069
Mealybugs	1	0.018	0.643	0.434
Ants x Mealybugs	1	0.187	6.752	0.019 *
Error	17	0.470		
E. tuberculatum				
Ants	1	0.057	1.423	0.247
Mealybugs	1	0.004	0.099	0.757
Ants x Mealybugs	1	0.137	3.403	0.080
Error	20	0.808		

The abundance of mealybugs on plants with *C. crassus* increased by 40.8% (t=2.5; df=12; p=0.024; Fig. 5A) while the abundance of mealybugs on plants with *E. tuberculatum* increased only by 9.6%, with no significant difference between plants with ants and mealybugs and plants with only mealybugs (t=1.65; df=11; p=0.126; Fig. 5B). The abundance of non-trophobiont herbivores (herbivores that not produce honeydew) was greater in plants with *C. crassus* and mealybug associations in relation to plants with only ants (58.6%) (U= 9.000; df=1; p=0.038; Fig. 6A). In plants with *E. tuberculatum* there was no difference in the abundance of non-trophobiont herbivores in plants with and without this ant-mealybug interaction (U=22.000, df=1; p=0.876; Fig. 6B)

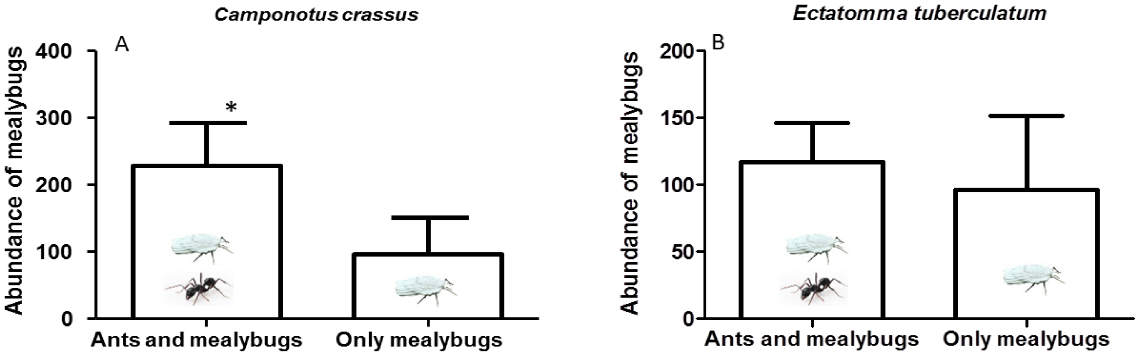

Figure 5. Comparative analysis of abundance of mealybugs on plants with and without *Camponotus crassus* (A) and *Ectatomma tuberculatum* (B) on shrubs of *Banisteriopsis campestris* in a Brazilian Tropical Savanna. (Test T, (*) means statistical difference; $p < 0.05$, Means ± 1SE are presented).

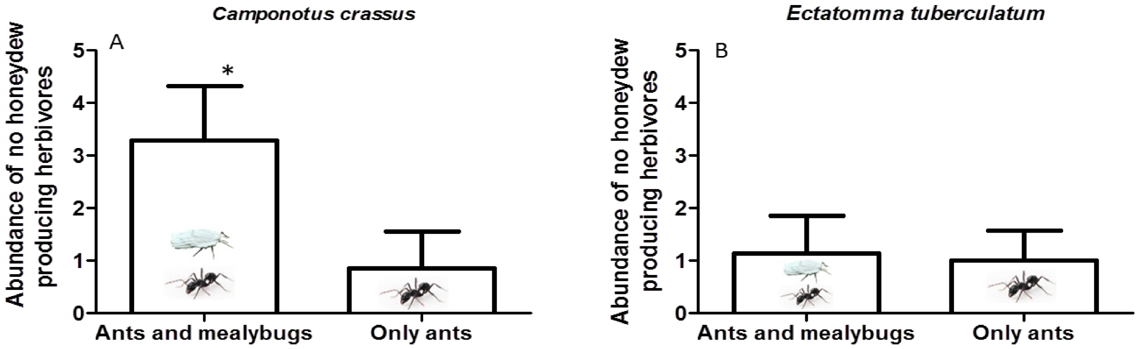

Figure 6. Comparative analysis of abundance of non-honeydew producing herbivores in plants with *Camponotus crassus* (A) and *Ectatomma tuberculatum* interaction (B) on shrubs of *Banisteriopsis campestris* in a Brazilian Tropical Savanna. (Mann Whitney U-test, (*) means statistical difference in plants with *C. crassus* attending mealybugs; $p < 0.05$, Means ± 1SE are presented)

Discussion

Our results showed that mealybugs compete intraspecifically for ant protection and that the outcomes of these interactions are ant-species dependent, with clear benefits provided to the hemipteran only by *C. crassus*. So, our first hypothesis was confirmed: mealybug aggregations compete for ant attendance (Competition for mutualism hypothesis - Addicott 1978b, Cushman and Addicott 1989) with difference in the abilities of ants to protect the hemipterans. We also demonstrated an indirect negative effect on fruit production in the presence of *C. crassus* and mealybugs association, confirming our second hypothesis that different species of ants can produce different consequences for plant production.

We reinforced the facultative feature of ant-mealybug mutualism and also the conditionality since two different species of ant revealed different outcomes in the benefits provided to the mealybugs. While *C. crassus* was able to positively interact with mealybugs promoting an intraspecific mealybugs competition for ant's visitation and a consequent positive effect for nymphs survival, *E. tuberculatum* did not have any significant effect. Some studies also demonstrated the positive effects of ants on hemipteran's fitness and the factors that influenced these results, highlighting the physiological and ecological features of ants (see Morales 2000, Renault *et al.* 2005, Herbert and Horn 2008).

Del-Claro and Oliveira (2000) was the first study to simultaneously demonstrate the conditionality in ant-derived benefits to hemipteran. The authors showed that by having their mandibles loaded with honeydew, ant species from lineages derived more primitively cannot store liquid in the stomach, such as *Ectatomma edentatum* Roger (Ectatomminae), thus may have become less prompt in chasing the hemipteran's enemies than the more agile *Camponotus rufipes* Fabricius (Formicinae) whose mandibles are ever free for aggression due to its capabilities to store liquid in its stomach ("social stomach", sensu Hölldobler and Wilson 1990). Therewith, ant partners can differ greatly in their direct protective effectiveness to hemipterans (see Buckley and Gullan 1991, Itioka and Inoue 1999) and also in their effects on host plant (see Messina 1981, Byk and Del Claro 2010, Del-Claro and Marquis 2015). Formicinae species are equipped with an advanced proventriculus and are able to carry a greater amount of sugary liquid in relation to Ectatomminae species. We suggest that the physiological and ecological features of *C. crassus* possessing a specialized proventriculus, a large crop capacities and mandibles free for defense, can explain their effectiveness in result a positive effects on nymphs survival.

Some genus of Formicinae, such as *Camponotus* sp., is intensively recorded as avid consumers of plant and insect exudates in tropical forests (Davidson *et al.* 2003) and also the most common genus in ant-hemipteran interactions in Cerrado vegetation (Sendoya *et al.* 2009, Nascimento and Del Claro 2010, Lange and Del-Claro 2014). In some studies that presented positive effects of tending ants on hemipterans and host plant (e.g. Moreira and Del-Claro 2005, Fagundes *et al.* 2013), there was a presence of several ant species, but more often species of *Camponotus* sp. reinforcing the importance of these species as worldwide attendance of trophobiont insects. In these studies, by becoming more aggressive while protecting the associated hemipterans, the ants attacked and drive away hemipteran's natural enemies and even the herbivores of the host plant. In our study, the *C. crassus* defended the nymphs of mealybugs, based on the positive effects on nymph’s survival, however, the ants did not protect the plants against herbivores.

The outcomes of ant-hemipteran interactions for host plants represent a trade-off between the indirect benefit of increased ant suppression of non-trophobiont herbivores and the direct cost of herbivory by ant-tended hemipterans (Lach 2003). In our system, the abundance of trophobiont mealybugs was higher in plants visited by *C. crassus* (40.8%) as well as the abundance of other herbivores (58.6%). In plants with mealybugs and *E. tuberculatum* differences were not detected. The factorial experiment also demonstrated a negative effect of *C. crassus* and mealybug interaction on plant productivity. Thus, *C. crassus* increased the number of mealybugs and also did not attack and drive away other herbivores which can have resulted in the lower productivity rate. Some studies that demonstrated negative effects on plant fitness also detected the outbreaks of hemipterans promoted by ant protection (Holway *et al.* 2002), which can impose tremendous costs on plant fitness via consumption of plant sap and transmission of plant pathogens (Cooper 2005, Delabie 2001). Renault *et al.* (2005), for example, reported that the aphid *Aphis coreopsidis* (Thomas) (Hemiptera, Aphididae) was 34% more abundant in the presence of tending *Camponotus* ants than in their absence and plants produced fewer viable seeds than did plants without the interaction.

Moreover, trophobiont hemipterans can attract the ants away from EFNs and decrease the efficacy of ant's defense to the interacting host plant (e.g. Buckley 1983, Rico-Gray and Thien 1989). In our study, the frequency of *C. crassus* visitation on mealybugs was statistically higher than the visitation rate on EFNs. We suggest that the total amount of honeydew offered could be higher than nectar secretion and such high-rewarding resources were more profitably defended than comparably low-rewarding nectaries (see Blüthgen *et al.* 2000). Besides, the sporadically temporal pattern of nectar secretion (Heil *et al.* 2001) compared to more constant production of honeydew (Yao and Akimoto 2002) could decrease the probability of nectar resources being defended. Lastly, the honeydew is considered relatively richer in complex amino acids (Maltais and Auclair 1952) and it might be a much more valuable diet than most extrafloral nectar sources. In this sense, we suggest that in the presence of a stronger relationship with mealybugs, the ant *C. crassus* protected the honeydew source more than they did with the EFN-bearing host plants. But, this aspect is still an issue of controversy (e.g. Chanam *et al.* 2014).

Thus, we provided the importance of the evolution of physiological and morphological traits of ants to the conditionality of ant- plant- hemipteran interaction, important to understand better how plants and animals interact and evolve in a natural community (Thompson 2013).

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CAPÍTULO 2

PHENOLOGICAL VARIATIONS AND THE EFFECTS FOR ECOLOGICAL PLANT INTERACTIONS IN A BRAZILIAN TROPICAL SAVANNA

Phenological variations and the effects for ecological plant interactions in a Brazilian Tropical Savanna

Andréa Andrade Vilela[1], Vergilio Torezan Silingardi Del Claro[2], Helena Maura Torezan-Silingardi[1] and Kleber Del-Claro1*

1 Laboratório de Ecologia Comportamental e Interações, Instituto de Biologia, Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, CEP 38400-920, Brasil.

2 Laboratório de Mecânica de Estruturas, Faculdade de Engenharia Mecânica, Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, CEP 38400-920, Brasil.

*Corresponding author: delclaro@ufu.br

Abstract

Phenology is a critical life history trait in angiosperms and many plant species have exhibited variation on phenological synchrony with other plants and animals species able to influence their ecological interactions. We studied the reproductive pattern of *Banisteriopsis laevifolia* (Malpighiaceae) in a Brazilian Tropical Savanna for four consecutive years looking for variations on plant phenological traits. We studied the influence of *B. laevifolia* phenological changes on the synchrony with an important endophytic herbivore (Curculionids beetles) and with sympatric plant species. To relate phenological variations with plant ecological interactions, we considered the outcomes of synchrony variation on ant-plant-herbivore and plant-plant interactions and the effects on reproductive success of *B. laevifolia* (flower and fruit productivities). Shifts on reproductive timing resulted in a phenological asynchrony between *B. laevifolia* and curculionids and increased the protection of flowers by ants. Besides, a greater flowering synchronization with other Malpighiaceae, *Peixotoa tomentosa* (Malpighiaceae), decreased fruit productivity suggesting an increase in the competition by pollinators and an inefficient ant's protection for fruits. We developed a mathematical modelling able to present a tendency on flower and fruit production of *B. laevifolia* for the next five years in a scenario with continued phenological changes. Our results suggest that there are a wide range of potential consequences to species relationships depending on phenological shifts. Thus we focused on the importance of phenology to the outcomes of plant interactions in natural communities.

Keywords: Ant-plant interaction, Conditional mutualism, Sympatric species, Cerrado Biome

Introduction

Phenology represents the temporal dimension of natural history of plant species and has potential influence on key ecological interactions (e.g. Torres and Galetto 2011, Vilela *et al.*2014, Ehrlén 2015). Many interactions depend on temporal overlap of interacting species and phenological variations, especially in response to climate changes (e.g. Forrest *et al.*2010, Forrest and Thomson 2011, Rafferty and Ives 2011, Diez *et al.*2012, Liuth *et al.*2013), may alter the synchrony and strength of interspecific relationships affecting plant productivity and survival (Memmott *et al.*2007, Encinas-Viso *et al.*2012, Rafferty and Ives 2012).

The timing of flowering includes the seasonal ontogeny of buds, flowers and fruit production resulting in a direct interaction with herbivore insects (Marquis *et al.* 2012, Thompson 2013). At the same time, some plant species may also produce extrafloral nectar secreted by extrafloral nectaries (EFNs) and promote a protective indirect interaction with ants in periods of high vulnerability to herbivores (Rico-Gray and Oliveira 2007, Del-Claro *et al.*2013, Nahas *et al.*2012). A close phenological synchronization between specialist herbivores and their host plants frequently determine the herbivory pressure on plants (Yukawa 2000, Silva and Oliveira 2010), and then, the necessity of ant recruitment for effective protection (Del-Claro and Oliveira 2000, Palmer *et al.*2010, Elzinga *et al.*2007). Therefore, shifts on phenology and disruption between herbivores and host plants synchrony are able to affect ant-plant mutualism and the final outcomes to plant fitness (Memmott *et al.* 2007, Both *et al.* 2009, Singer and Parmesan 2010, Yang and Rudolf 2010).

Variations on reproduction time may also alter the phenological synchrony among plant species (Elzinga *et al.*2007). For sympatric species, a greater overlap flowering could promote an increase in competition by pollinators according to the Pollinator Competition Hypothesis (see Robertson 1895). This hypothesis assumes pollinators as a limiting resource and, during periods of overlap, different plant species may attract the same pollinators which can lead them to compete for these cross-pollination vectors (e.g. Tanakura *et al.*2009, Muchhala and Thomson 2012). Some studies demonstrated variation on plant phenologies resulting in a higher overlap among plant species with consequential share of pollinators (e.g. Forrest *et al.*2010) and decreased fruit and seed productivity (e.g. Rafferty and Ives 2011, 2012).

Recent studies indicated effects of phenological change on mutualistic (e.g. Rafferty *et al.* 2015, Revilla *et al.* 2015) and competitive interaction (Cleland *et al.* 2015), demonstrating indirect effects for population dynamics (Nakazawa and Doi 2012, Revilla *et al.*2014) and plant-pollinator communities (Memmott *et al.*2007, Burkle *et al.*2013, Revilla *et al.*2015). Nonetheless, the most ecological implications of changing phenology for plant fitness remain to a large extent unknown. Johansson *et al.* (2015) pointed out the need to shed light on the interplay between phenology and ecological interactions from different perspectives. In this sense, there is an urgency to also translate a basic understanding of phenology into forecasts about continued phenological changes to predict the ecological consequences of them (see Diez *et al.*2012, Vilela *et al.* 2014). Using mathematical models is possible to create a model based on real field data and explore some specific aspects of species life-histories (see also Fabina *et al.*2010, Diez *et al.*2012) to address how phenological shifts can affect plant interaction and their consequent reproductive success (Memmott *et al.* 2007, Fabina *et al.*2010, Gilman *et al.*2012).

Cerrado is the Brazilian Tropical Savanna with great seasonal variation and communities possessing around 25.5% of EFN bearing plants closely associated with protective ants (Rico-Gray and Oliveira 2007, Del-Claro and Torezan-Silingardi 2009, 2012). *Banisteriopsis laevifolia* (A. Juss) B. Gates is an abundant species of Malpighiaceae (Anderson 1990, Gates 1982) that presents EFNs visited by ants and a main herbivore in the studied area, weevils of the genus *Anthonomus* (Anthonominae: Curculionidae) (Torezan-Silingardi 2007, 2011, Vilela *et al.*2014). This plant species has presenting phenological variation on the onset of reproductive period in the studied area (Vilela *et al.*in prep, Claro *et al.*, in prep), which can alter the degree of synchronization with this threatening herbivore and with sympatric plant species.

In this sense, we intended to (a) monitor the phenology of *B. laevifolia* over four reproductive periods to verify the variation on the onset of buds, flowers and fruits production and (b) verify the phenological synchronization with curculionids and sympatric plants that also possess EFNs. We also intended to (c) verify the outcomes of ant-plant-herbivore and plant-plant synchrony on reproductive success of plant (flower and fruit productivities). Here we used a mathematical model we developed (Programa CSVM® - INPI - BR 51 2015 000482-4 Claro *et al.*in prep.) to (d) elucidate how continuous changes on *B. laevifolia* phenology can affect plant productivity rate in a nearby future. We hypothesized that the phenology of *B. laevifolia* has been fluctuating over time changing the degree of synchronizations with curculionids and sympatric species able to influence the outcomes of interactions in relation to plant productivity. We also expect that our software will be able to estimate the flower and fruit production of *B. laevifolia* for the next five years in a scenario with continued phenological changes.

Materials and Methods

Study site

Fieldwork was carried out from March 2008 to November of 2013, reaching four reproductive season of *B. laevifolia* (2008; 2011; 2012; 2013) at the *Reserva Ecológica do Clube Caça e Pesca Itororó de Uberlândia* (CCPIU) (18°59'S, 48°18'W), Uberlândia, Minas Gerais State, Brazil. We used a 640-ha Cerrado site consisting of a dense scrub of shrubs and trees, known as Cerrado *sensu stricto* (Réu and Del-Claro 2005, Ferreira and Torezan-Silingardi 2013)

Studied species

In the Cerrado of central Brazil, shrubs of the family Malpighiaceae are diverse and abundant (Anderson, 1990; Gates, 1982). *B. laevifolia* was chosen because it possesses paired EFNs on the leaf base and have close relationships with protective ants (Torezan-Silingardi, 2007, 2011, Alves-Silva *et al.*2013, Vilela *et al.*2014).

Torezan-Silingardi (2007) and Vilela *et al.* (2014) have already described the importance of weevils of the genus *Anthonomus* (Anthonominae: Curculionidae) as one of the main herbivore of *B. laevifolia* in the studied area due to its intense breeding season overlap with *B. laevifolia* flowering period and its endophytic feeding behavior. Weevil's larvae infest the buds and blossoms eating the plant embryonic tissue, which make them protected against sun and drying winds and also parasites and predators, such as ants (Clark and Martin 1987, Torezan-Silingardi 2011, Vilela *et al.*2014).

Phenology variation

In each year, we tagged 30 resprouting individuals of *B. laevifolia* (without leaves and with the presence of vegetative gems), of similar aspect (height and number of stems), and separated 3m least. Each plant was monitored every two weeks during its four-month reproductive period. Plant phenology (intensity and number of buds, flowers and fruits) was recorded according to Torezan-Silingardi and Oliveira (2004) and Vilela *et al.* (2014)

Circular statistical analyses were performed to verify variations on the onset of buds, flowers and fruits among the years. To calculate the circular statistic parameters, months were converted to angles from 0= January to 345= December at intervals of 15 because the measurements were made every two weeks (e.g. Cardoso *et al.*2012, Vilela *et al.*2014). We considered the frequency of individuals at the onset of each phenophase to calculate the follow parameters: the mean vector (μ), length of mean vector (r), median, circular standard deviation, rayleigh test (z). The mean date for each phenophase is determined by converting the mean angular directions to corresponding mean dates (see Morellato *et al.*2000, 2010, Staggemeier *et al.*2010). When the mean angle was significant, we performed the two-sample Watson-Williams tests (F) to determine the difference among the mean data of the onset parameter over time (Zar 1996).

Ant-plant-herbivore interaction

We selected plants occupied by ant species recognized for their close protection mutualism with EFNs-bearing plants, such as *Camponotus* sp and *Ectatomma* sp (e.g. Del Claro and Marquis 2015, Stefani *et al.*2015, Calixto *et al.*2015). The individuals tagged (N=30) were randomly divided in two groups of 15 individuals at the beginning of the breeding season for each year and by the flip of a coin were named control or treatment. In control groups, plants did not receive any manipulation and ants had free access to all plant parts. In treatment groups, ants were all manually removed from the plants and the trunk was covered with a 5 cm large adhesive paper strap and a layer of sticky resin was applied over it (Tanglefoot ®; The Tanglefoot Company, Rapids, Michigan, USA) to avoid ants climbing. All structures of other plants that could be used by ants as bridges to get access to these experimental plants were removed. To be sure that the sticky resin did not interfere in the results in control plants, we also covered the trunk with a paper strap, but we applied the resin only on one side of the trunk.

Each plant was monitored every two weeks during the reproductive period (4 months) (Vilela *et al.*2014) and always inspected by the same observer (AVV). We recorded the abundance of herbivores, including curculionids and other herbivores considered secondary ones. The variation on the abundance of herbivores in the presence or absence of ants along the years was analyzed using Two-Way ANOVA after log transformation.

Considering the greater damage of curculionds (endophytic herbivores) on flower buds, we obtained the flower production as the proportion of flower formed per bud produced (e.g. Vilela *et al.*2014). The variation on flower productivity in the presence and absence of ants over time was obtained from Two-Way ANOVA after arcsine of the square root transformation to represent the conditionally of ant-plant interaction.

Plant-plant interaction

The phenologies of sympatric plants were also monitored, including *Peixotoa tomentosa* A. Juss., *Banisteriopsis campestris* (A. Juss.) Little and *Banisteriopsis malifolia* (Ness and Mart.) (see Vilela *et al.*2014). We tagged 30 individuals of each species and phenological traits (intensity of flowers) were recorded according to Vilela *et al.*2014 every two weeks during their four-month reproductive period in 2008, 2012 and 2013. Linear graphics using flowering intensity were performed to compare with *B. laevifolia* phenology over time.

Based on Pollinator Competition Hypothesis (Robertson 1895) and considering that plant species studied are bee-pollinated and self-compatible (see Torezan-Siligardi 2007) with distinct taxa of natural and self-pollination (Torezan-Silingardi *et al.*in preparation), we analyzed the fruit production intending to figure out the effect of phenological synchrony for *B.laevifolia* fruit set. We used the proportion of fruit formed per bud produced and the variations on fruit productivities along the years with the presence or absence of ants were obtained from Two-Way ANOVA after arcsine of the square root transformation.

Mathematical Modelling

We developed a model for the group of four sympatric Malpighiaceae species living in the studied area *(B. laevifolia, B. campestris, B. malifolia* and *P.tomentosa*) (*Programa CSVM*® - INPI - BR 51 2015 000482-4; Claro *et al.*, in prep. Vilela *et al.*in prep). The model uses field parameters measure (at least four previous seasons) based on generic herbivores and pollinators. Considering that temporal and spatial variations on the abundance of specific partners could represent local variation on the results of flower and fruit production, the program intends to ensure a qualitative validity demonstrating a tendency in different scenarios. We adjusted and validated the model parameters by the use of Differential Evolution optimization methods (Lobato and Steffen, 2008, Vanderplaats, 2005). This procedure is made by estimating the interval in which the variables considered may vary, normalizing the variables and then applying an optimization procedure to achieve the best approximation of the real parameter/variable in a specific scenario (Lobato and Steffen, 2007). Initially, we considered the field data obtained by Torezan-Silingardi (2007) in 2005/2006 aiming to ensure a greater robustness for the model. In order to standardize the sample method, we employed the same methodology applied to Torezan-Silingardi (2007).

The inputs considered were rainfall, leaf sprouting and production of floral buds, flowers and fruits. Other parameters estimated by the researchers and adjusted by the software, were the weekly rates of herbivory, pollination and self-fecundation. The preliminary data as input parameters were: area of 2500m², with 100x100 square elements of 0.5x0.5 meters each, simulating for 5 years of 48 weeks time steps each (1 time step equals 1.0863 week). Containing 50 *B. malifolia* individuals (3x3 elements in size), 50 *P. tomentosa* individuals (2x1 elements in size), 30 *B. laevifolia* individuals (2x2 elements in size) and 40 *B. campestris* individuals (1x1 elements in size) based on their real size and presence measured on the field. The herbivory and pollination were supposed to occur in 90% of the elements per iteration. The randomness of herbivory considered was: base ± random of 3%. The randomness of insect pollination and autonomous self-pollination considered was: base ± random of 1% and we considered 13% of randomness for the baselines (rainfall, leaves sprouting, and flowering). All variation parameters were based on real field data.

We simulated three phenological scenarios with advancement of *B. laevifolia* phenology in 2, 4 and 6 weeks to investigate the consequences of phenological changes of flower and fruit productivities for the next five years.

Results

Phenology variation

Analyses of the reproductivity period over years showed an advance of the onset of flower buds production in *B. laevifolia*. In 2008 and 2011 the bloom was concentrated from August to November (Rayleigh test Z= 28.934, p<0.001 and Z=29.502, p<0.001, respectively), with the average date of bud's production in 08/13/2008 and 08/02/2011. In 2012 the bloom was concentrated from July to October (Z= 24.075 p<0.001), with the average date of bud's production in 07/09/2012. In 2013, the bloom begun in June until September (Z= 19.217, p<0.001), with the average date of bud's production in 07/04/2013. Thus, comparisons of the mean data of bud flowering, flowering and fruit among the years showed that the onset of *B. laevifolia* varied over time for bud flowers and flowers for all year (see table 1), showing an advance over last years. There were no significant different only for fruiting between 2008 and 2011 and 2012 and 2013 (see table 1).

We compared the phenology of flowers bud, flowers and fruits with the abundance of weevils and observed the asynchrony between this herbivore group and host plant phenology over time. Linear graphics were performed (Fig. 1)

Table 1. Circular statistical analyzes testing for seasonality of phenological patterns of *Banisteriopsis laevifolia* (2008, 2011, 2012 and 2013). Watson-Williams tests (F) were performed to test the difference among the mean date of onset of flowers buds, flowers and fruits over time.

	Year	N	a +-DP	Mean Data	r	Z (p)	Watson- Willian (F)		P
Flower bud	2008	29	225.512° ± 2.731°	**13/08/2008**	0.999	**28.934 (<0.001)**	2008 & 2011	44.618	**<0.001**
	2011	30	213.457° ± 7.417°	**02/08/2011**	0.992	**29.502 (<0.001)**	2008 & 2012	88.298	**<0.001**
	2012	26	190.053° ± 15.891°	**09/07/2012**	0.962	**24.075 (<0.001)**	2008 & 2013	56.304	**<0.001**
	2013	23	184.854° ± 24.289°	**04/07/2013**	0.914	**19.217 (<0.001)**	2011 & 2012	76.732	**<0.001**
							2011 & 2013	59.833	**<0.001**
							2012 & 2013	4.459	**0.038**
Flower									
	2008	30	233.995° ± 8.313°	**22/08/2008**	0.99	**29.375 (<0.001)**	2008 & 2011	12.273	**0.002**
	2011	27	240.55° ± 2.826°	**29/08/2011**	0.99	**26.934 (<0.001)**	2008 & 2012	31.832	**<0.001**
	2012	20	221.943° ± 13.97 °	**10/08/2012**	0.971	**18.846 (<0.001)**	2008 & 2013	80.928	**<0.001**
	2013	16	207.574° ± 19.615°	**26/07/2013**	0.943	**14.23 (<0.001)**	2011 & 2012	37.936	**<0.001**
							2011 & 2013	57.089	**<0.001**
							2012 & 2013	28.566	**<0.001**
Fruit									
	2008	22	252.963° ± 6.848°	**10/09/2008**	0.993	**21.688 (<0.001)**	2008 & 2011	0.392	**0.537**
	2011	28	253.402° ± 6.125°	**11/09/2011**	0.994	**27.682 (<0.001)**	2008 & 2012	34.027	**<0.001**
	2012	17	239.104° ± 13.136°	**27/08/2012**	0.974	**16.13 (<0.001)**	2008 & 2013	39.175	**<0.001**
	2013	5	242.983° ± 5.999°	**31/08/2013**	0.995	**4.945 (=0.001)**	2011 & 2012	35.966	**<0.001**
							2011 & 2013	51.822	**<0.001**
							2012 & 2013	1.654	**0.206**

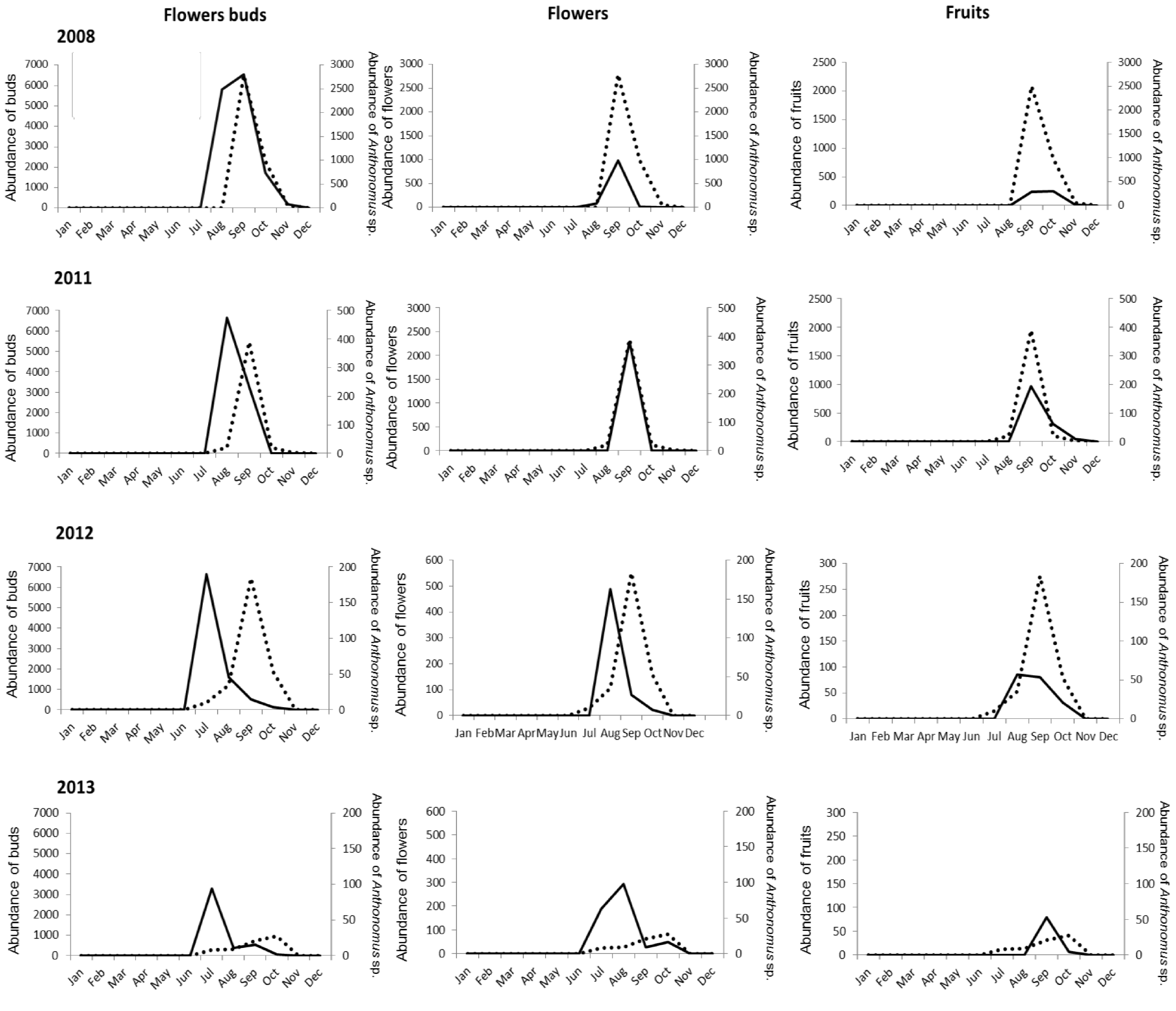

Figure 1. Linear graphics demonstrating the asynchrony between the phenological traits of *Banisteriopsis laevifolia* (bud flowers, flowers and fruits) with Curculionds abundance during 2008, 2011, 2012 and 2013 in a Brazilian Tropical Savanna (Solid line means the type of resource – flowers buds, flowers and fruits – Dotted line means abundance of Curculionids).

Ant-plant-herbivore interaction

Curculionidae was the most abundant herbivore over the four reproductive season (2008 (n=3906); 2011 (n=431); 2012 (n=282) and 2013 (n=134). Among the secondary herbivores (less abundant) we recorded thysanopterans, hemipterans, orthopterans and other coleopterans accounting in 2008 (n= 220), 2011 (n= 209), 2012 (n=380) and 2013 (n=338).

The abundance of Curculionids decreased over time (F=37.07, p<0.001, df=1 Fig. 2A) and these herbivores infested as much control as ant-excluded plants with no variation along the years (F=0.089, p=0.966, df=3, Fig. 2A). However, the other herbivores were significantly more abundant in ant-excluded plants all years studied (F= 3.983, p=0.048, df=1 Fig.2B). Thus ants were effective in the removal of exophitic herbivores in these plants, but not endophitic curculionds for all year studied.

The mutualism between ants and plants varied over time in relation to flower protection (F=3.059, p=0.032, df=3 Fig. 3).

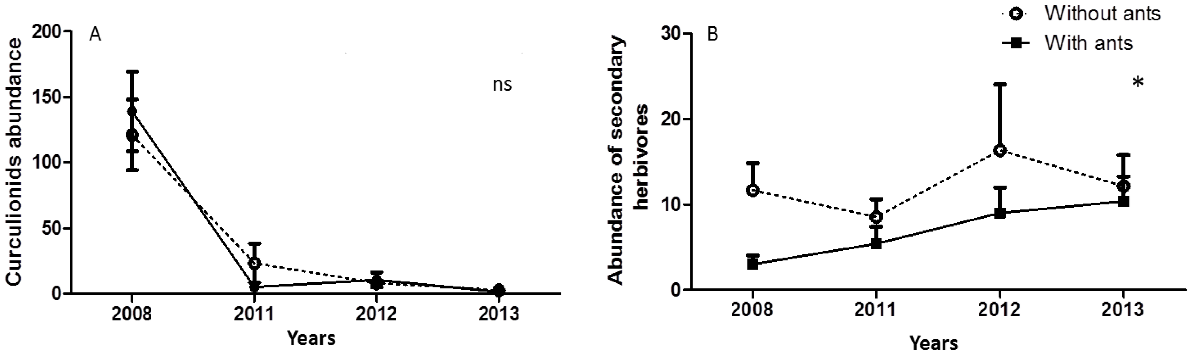

Figure 2. Abundance of curculionids (A) and secondary herbivores (B) from *Banisteropsis laevifolia* during 2008, 2011, 2012 and 2013 in the presence and absence of ants in a Brazilian Tropical Savanna. (Two way ANOVA, (*) means statistical difference; p < 0.05, Means ± 1SE are presented).

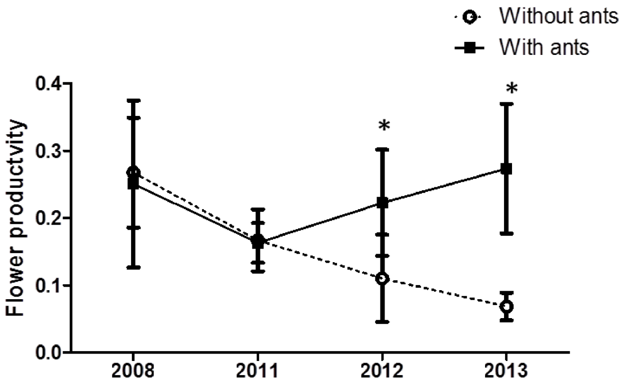

Figure 3. Flower productivity of *Banisteropsis laevifolia* during 2008, 2011, 2012 and 2013 in the presence and absence of ants in a Brazilian Tropical Savanna. (Two way ANOVA, (*) means statistical difference; p < 0.05, Means ± 1SE are presented).

Plant-Plant interaction

We compared the phenology of sympatric plants with the phenology of *B. laevifolia* along the years and detected a greater synchronization with the phenology of *P.tomentosa.* Linear graphics were performed (Fig. 4).

The results of fruit productivity demonstrated a decrease on fruit production (F=6.221, p=0.001, df=3 Fig. 5) with no variation with or without ants over time (F=1.360, p=0.259, df=3 Fig. 5), suggesting a decrease in production due to a competition by pollinators between *B. laevifolia* and *P. tomentosa* and an inefficient ant's protection to few fruits produced.

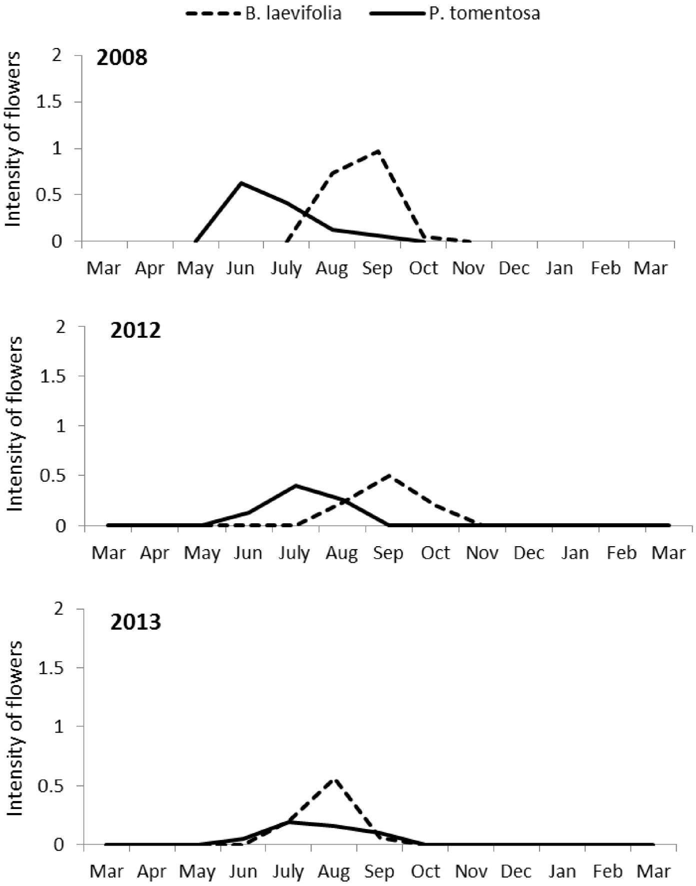

Figure 4. Linear graphics with the intensity of flowers of *Peixotoa tomentosa* and *Banisteriopsis laevifolia* during 2008, 2012 and 2013 in a Brazilian Tropical Savanna (axis y means intensity of flowering phenology being 1=25% and 2=75%; n=30).

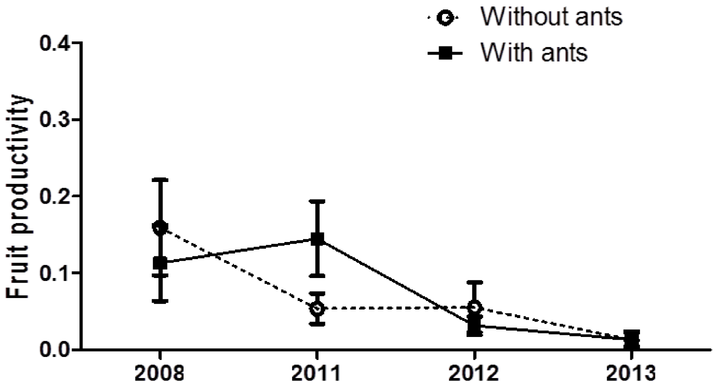

Figure 5. Fruit productivity of *Banisteropsis laevifolia* during 2008, 2011, 2012 and 2013 in the presence and absence of ants in a Brazilian Tropical Savanna. (Two way ANOVA, (*) means statistical difference; $p < 0.05$, Means ± 1SE are presented).

Mathematical Modelling

The model parameters were adjusted and validated by the use of Differential Evolution optimization methods (Lobato and Steffen, 2008, Vanderplaats, 2005, also see Claro *et al.*in prep. Vilela *et al.*in prep). The model reached a mean squared error of 3.87% when compared to the mean field data, considered a great approach between the model and the real-case scenario.

The results of advance in *B. laevifolia* phenology demonstrated a tendency in increasing the production of flowers, however with a proportionally lower production of fruits (Fig. 6). It is also important to consider that the model was based on generic herbivores and pollinators and temporal and spatial variations on the abundance of specific partners could represent local variation on productivity results.

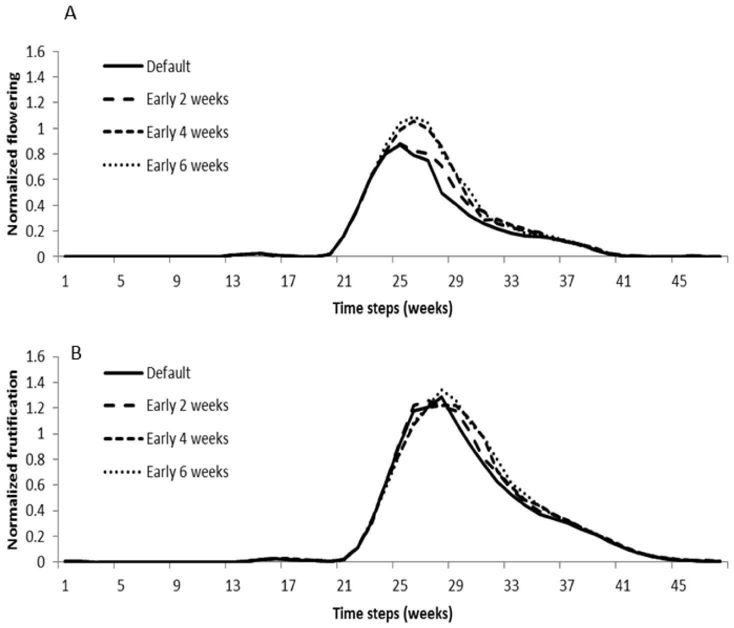

Figure 6. Results of flower (A) and fruit (B) productivities of *Banisteriopsis laevifolia* in four different phenological simulations, considering the phenology advance in 2, 4 and 6 weeks. Default means the standard setting.

Discussion

Plant reproductive phenology plays a key role in the dynamics of communities in many ways (e.g. Vilela *et al.*2014, Lange *et al.*2013, Datillo *et al.*2014a, 2015). Changes in *B. laevifolia* phenological traits among years were followed by a phenological asynchrony between the onset of plant reproduction and the breeding stage of curculionids and also resulted in a greater synchronization with the sympatric plant, *P. tomentosa*. These phenological variations directly impacted ant-plant-herbivore and plant-plant interactions and corroborate our hypothesis that changes in the timing of life-history events for plants is able to affect the outcome of key ecological interaction (e.g. Durant *et al.*2005, Visser and Both 2005) and consequently the plant fitness (e.g. English-Loeb and karban 1992, Thomson 2010, Lange and Del-Claro 2014).

Phenological changes on *B. laevifolia* promoted an asynchrony with its main herbivores and resulted in a protection of plants by ants in 2012 and 2013, which had not occurred in the early years of study. Ant can vary greatly in quality of defense they provide depending on the herbivore specie and its abundance (Bronstein 1998, Del Claro and Oliveira 2000) making the outcomes notoriously variable from year to year (Del-Claro and Oliveira 2000, Chamberlain and Holland 2008, Trager *et al.*2010). Vilela et al. (2014) had already demonstrated an extensive damage to the buds of *B. laevifolia* promoted by a high abundance of curculionids and the failure of ants in drive away them. These herbivores feed and complete their development inside the flower buds ensuring a steady food supply, protection against sun and drying winds and shelter against parasites and predators, such as ants (Clark and Martin, 1987; Torezan-Silingardi, 2011). Thus, we suggested that the phenological variation and desynchronization with curculionids resulted in a lower abundance of them during the reproductive period of 2012 and 2013, which was crucial for the effective protection by the ants. Other authors also related plant phenology with the presence and abundance of ants and herbivores (e.g. Heil *et al.*2000, Holland *et al.*2010, Aranda Rickert *et al.*2014).

The plant species received a great benefit due to desynchronization, however, the curculionids may have been harmed. In some circumstance, changes on plant-herbivore synchronization have the potential to be extremely detrimental to the consumer (e.g. Both *et al.*2006, Donnelly *et al.*2011). Singer and Parmesan (2010) explored the Edith's checkerspot butterfly (*Euphydryas editha*) and the winter moth (*Operophtera brumata*) and demonstrated an asynchrony between the insect and its host plant with conspicuous mortality rate of herbivores. We also noted a considerable decrease in curculionids abundance along the years which may be a result of a mismatch with plant phenology. This vulnerability can likely contribute to the decline of this herbivore population, as observed for herbivores studied by Singer and Parmesan (2010). Relatively few studies have quantified the demographic effects and is still large uncertainty their long-term consequences for species and ecosystems (Miller-Rushing *et al.* 2010, Bennett *et al.* 2015). Thus, phenological variation could have relevant consequences for both interacting species making studies about mismatches worthy to analyze the evolution and maintenance of natural populations (Forkner *et al.*2008, Amsellem and Mckey 2006, Muniz *et al.*2012).

When que compared the phenology of sympatric plants with the phenology of *B. laevifolia* we detected a greater synchronization with the phenology of *P.tomentosa* along the years. Change on plant phenology can result in minimization or maximization of phenological overlap among plant species (Van Schaik *et al.* 1993) and, in this cases, some studies have demonstrated the importance of pollination relationship influencing the outcomes to plants reproductivity (e.g Forrest *et al.*2010, Rafferty and Ives 2011, 2012). Observations of annual variation on plant phenologies in subalpine meadows in Colorado, USA, demonstrated a higher overlap among plant species with a sizeable share of pollinators by some species (Forrest *et al.*2010) and other authors simulated changes on phenology of two perennial wildflowers and demonstrated that the seed set can vary as a result of changes in pollination rate (Rafferty and Ives 2011). Therewith, flowering phenology is commonly thought to affect plant reproduction through the influence of pollinators, as well as the degree of plant dependence from this interaction (see Johnson *et al.*2011).

Plants range from complete dependence of pollinators to those able to ensure sexual reproduction via autonomous self-pollination (Vogler and Kalisz 2001). In general, self-compatible plants can be considered facultatively autonomous whereas they can require animal pollinators to outcross pollen or reproduce via spontaneous autogamy. Conversely, self-incompatible plants present high dependence of pollinators because they can use only outcross pollen to produce seeds (Richards 1997). In our system, both *B. laevifolia* and *P. tomentosa* are bee-pollinated and self-compatible, however, with different rate of self-pollination. The spontaneous self-pollination produces about 2% of fruits in *B. laevifolia* and 10 to 43% in *P. tomentosa* (Torezan-Silingardi *et al.*in preparation). In this sense, it is possible that the greater dependence of *B. laevifolia* in relation to pollinators and the increase of phenological synchronization with *P. tomentosa* resulted in a competition by pollinators with a negative effect for *B. laevifolia* fruit production. Bishop *et al.* (1998) working with a self-pollinated species also suggested that this reproductive advantage decreased the influence of pollinators on fruit ripening, and Moller (2006) discuss that autonomous self-pollination confers reproductive assurance by elevating fruit and seed production being an important drivers of plant mating system evolution.

Our model demonstrated a considerable approach between the model and the real-case scenario with a mean squared error of only 3.87% when compared to the mean field data. These results ensure the model as robust enough to surely indicate a tendency of plant productivity (flowers and fruit) in a scenario with progress changes on *B. laevifolia* phenology. The advance simulation on *B. laevifolia* phenology demonstrated a tendency in increasing the production of flowers, however a proportionally lower production of fruits. We suggest the flowering production rate as a result of asynchrony between curculionids and onset of buds associated with effectiveness ant protection. However, the increase on overlapping and sharing of pollinators with *P. tomentosa* and the greater dependence on theses vectors resulted in a tendency of lower production of fruits (e.g. Forrest and Thomson 2011).

Thus, we were able to explore some specific aspects of species life-histories and demonstrated that when phenological change has a positive influence on some fitness components but a negative influence on others, the response to the shift will depend on the balance between these different effects (see Johansson et al.2015). In this sense, our work demonstrated the wide range of potential consequences on plant fitness from phenological shifts based on variation in different ecological interactions. We also contributed to a growing area of research about phenological changes and ecological interactions (also see Jonzén *et al.* 2007, McNamara *et al.* 2011, Visser *et al.* 2011, Lof *et al.* 2012, Nakazawa and Doi 2012, Johansson and Jonzén 2012a, Revilla *et al.* 2014) making our study relevant to a broader understanding about the ecology of interactions and the conservation of natural communities.

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CAPÍTULO 3

PHENOLOGY STRATEGIES AFFECTING BIOTIC INTERACTIONS AND PLANT REPRODUCTIVE SUCCESS IN A SAVANNA COMMUNITY

PHENOLOGY STRATEGIES AFFECTING BIOTIC INTERACTIONS AND PLANT REPRODUCTIVE SUCCESS IN A SAVANNA COMMUNITY

Andréa Andrade Vilela[1], Vergilio Torezan Silingardi Del Claro[2], Helena Maura Torezan-Silingardi[1] and Kleber Del-Claro[1*]

[1] Laboratório de Ecologia Comportamental e Interações, Instituto de Biologia, Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, CEP 38400-920, Brasil.
[2] Laboratório de Mecânica de Estruturas, Faculdade de Engenharia Mecânica, Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, CEP 38400-920, Brasil.
[*]Corresponding author: delclaro@ufu.br

Abstract

Shrubs of Malpighiaceae family in the Brazilian Tropical Savanna exhibit a sequential flowering phenology. Climatic parameters are able to influence the timing of life history events in a wide array of plant communities affecting the degree of phenological synchronization among species, their mutualistic and antagonistic interactions and reproductive success. In this work we verified variations on climatic factors (temperature and precipitation) over a period of ten years (2005 to 2014) and asked whether the onset of flowering of four Malpighiaceae species is correlated with these climatic parameters. Besides, we asked whether the phenological synchronization among species has changed over time, and thus affecting herbivory rate and fruit production. For this purpose, herbivory and fruit production were recorded during three reproductive seasons (2008/2009, 2011/2012, 2013/2014) and a mathematical model was developed to estimate the flower and fruit production in face of phenological shifts for the next 5 years. We detected variations on annual mean of temperature and total precipitation from 2005 to 2014. Circular statistics determined variations on the mean data of onset of flowering for all species and on the degree of overlapping among them. The onset of plants flowering were correlated with the environmental conditions and the effects for herbivory and fruits production were specie-specifics. The mathematical model was able to present a tendency on flower and fruit production for each plant species contributing to the predictions of the outcomes for species facing phenological changes. Our results endorsed the climate variations in recent years and the manifestation on phenology of different plant species and on their biotic interactions reinforcing the species-specific effects for the survival and maintenance of these plant species

Keywords: Synchrony, Floral Phenology, Abiotic Factors, Malpighiaceae, Savanna,

Introduction

Flowering phenology is a critical life history trait in angiosperms and has potential significance for plant ecology and evolution (e.g. Amasino 2010, Torres and Galetto 2011). Due to its great influences on a wide range of ecological processes, including plant interactions and their reproductive success (Pilson 2000, Kawagoe and Kudoh 2010), the pattern of flowering is one of the most investigated aspects of plant phenology (Hegland *et al.* 2009, Vilela *et al.* 2014), especially nowadays with the occurrence of noticeable climate changes (Franks *et al.*2013, CaraDonna *et al.*2014). Shifts in the timing of flowering have great consequences on plant developmental traits and interactions with mutualistic (pollinators) and antagonistic (herbivores) partners which may affect plant success, survival rates and maintenance of species (Herrera *et al.*2002, Memmott *et al.*2007, Torezan-Silingardi 2011, Encinas-Viso *et al.*2012).

The flowering pattern of individual species varies widely (Forrest *et al.*2010, Forrest and Thomson 2011). For example, in Cerrado, for some species it is staggered over a long period while others present a more pronounced peak with relatively few individuals occurring in the early and late parts of the flowering period (Ferreira and Torezan-Silingardi 2013). These variations are likely to be the result of a combination of abiotic, biotic and phylogenetic factors (Mitchell *et al.* 2009b, Rusquist and Staton 2013; Del-Claro and Marquis 2015) and the action of these factors may have led to either the minimization or the maximization of flowering within and among plants species, inducing synchronous and asynchronous phenologies at population and community level (Van Schaik *et al.*1993; Vilela *et al.* 2014).

Sympatric species can exhibit clumped flowering phenologies that overlap extensively, possibly reducing herbivory according to the Herbivore Satiation Hypothesis (Beattie *et al.* 1973), *i. e.*, a plant-plant association in which one species could decrease the likelihood of detection or vulnerability of other species by insect herbivores (see Barbosa *et* *al.*2009, Lamarre *et al.*2014). Besides, the desynchronization of herbivores in relation of plant flowering period (e.g. Forrest and Thomson 2011) with a mismatch in the timing of interacting organisms can also reduce the herbivore pressure. (Hoye and Forchhammer 2008a). Nevertheless, during periods of overlap, different plant species may attract the same pollinators which can lead to competition for these cross-pollination vectors (e.g. Tanakura *et al.*2009, Muchhala and Thomson 2012). The Pollinator Competition Hypothesis introduced by Robertson (1895) assumes that pollinators are a limiting resource and flowering events should be evenly spread through time (staggered phenology) to reduce the competition for pollinators and increase the effectiveness of pollination. Thus, a balance in plant life-history strategies based on a trade-off between a staggered phenology and a more overlapped flowering is expected (see Herrera *et al.*2002, Forrest *et al.* 2010).

Many plant species have presented phenological shifts in the timing of their life-history events (Rafferty and Ives 2011), affecting the strength of mutualists and antagonistic interactions (Burkle *et al.* 2013, Rafferty *et al.*2013, 2015) and consequently their fitness (see English-Loeb and karban 1992, Thomson 2010, Lange and Del-Claro 2014). Recent studies attempt to answer why and how the phenological overlap of interacting species has been changing. Many of them have been reinforcing the climate change as an important disturber on the timing of life history events in a wide array of communities (Forrest *et al.* 2010, Rafferty and Ive 2012, Diez *et al.*2012). Mainly due to its potential to desynchronize the phenologies of interdependent species (Rafferty *et al.* 2015) and vary the degree of overlapping among related species (Forrest *et al.*2010).

In a recent meta-analysis, Chambers *et al.* (2013) related phenological drivers and trends among Southern Hemisphere species and discussed how phenological information contribute to our knowledge of the adaptive capacity of species, their resilience and constraints. However, the authors concluded that our general understanding and capacity to predict phenological responses remains patchy and constrained. Furthermore, few studies have been able to directly address what are the consequences of phenological shifts for species interactions (but see van Asch *et al.* 2007; Fabina *et al.* 2010; Liu *et al.* 2011) and for plant fitness (but see Dominguez and Dirzo 1995, Miller-Rushing *et al.* 2010). Therefore, there is an urgency to translate a basic understanding of phenology into forecasts about continued phenological changes and to predict the ecological consequences of these changes (Diez *et al.*2012), especially for South American species (Staggemeier and Morellato 2011, Morellato *et al.* 2013). Integrating life-history theory and mathematical models into ecological studies should help in forecasting changes and in understanding what may be the consequences for plant interaction and reproductive success (Fabina *et al.* 2010). Mathematical models could be used to generate testable predictions that address how phenological shifts can be generated to investigate consequences for temporal overlap of interacting species (Memmott *et al.* 2007, Gilman *et al.* 2012) and how it could affect plant fitness.

Cerrado (the Brazilian Tropical Savanna) is the second largest South American ecosystem and presents great seasonal variation as a remarkable characteristic (Oliveira-Filho and Ratter 2002). Initially, we (a) tested whether the climatic parameters (temperature and precipitation) has changed over ten years (2005 to 2014) and then we used empirical data of four Malpighiaceae species of Cerrado, which present previously described sequential flowering (Torezan-Silingardi 2007; Vilela *et al.*, 2014), to (b) answer whether the onset of flowering is correlated with those climatic parameters and (c) whether the phenological synchronization among species has therefore changed over time. We also aimed to (d) detect the effects of a sequential flowering and a higher overlap phenology for herbivory and fruit set of species. We used a mathematical model we developed to (e) estimate the flower and fruit production of each plant species for the next five years in a scenario with continued phenological changes. We hypothesized that temperature and precipitation rates have presented variation over the years analyzed and the onsets of flowering have also changed affecting the degree of overlap among Malpighiaceae species. Furthermore, we expected that the degree of plant phenological overlap (staggered or synchronized) influence the herbivory and fruit set of species depending on the relationship that each species establishes with its mutualists (pollinators) and antagonists (herbivores) partners.

Materials and Methods

Study site

Fieldwork was carried out from May 2008 to March of 2014, reaching three reproductive seasons for each species studied (2008/2009; 2012/2013; 2013/2014), at the *Reserva Ecológica do Clube Caça e Pesca Itororó de Uberlândia* (CCPIU) (18°59’S, 48°18’W), Uberlândia, Minas Gerais State, Brazil. We used a 400-ha Cerrado site consisting of a dense scrub of shrubs and trees, known as Cerrado *sensu stricto* (Oliveira-Filho and Ratter 2002).

Plant species

In the Cerrado of central Brazil, shrubs of Malpighiaceae family are diverse and abundant (Anderson, 1990; Gates, 1982; Ferreira and Torezan-Silingardi 2013). Several species exhibit a staggered phenological development, in which individuals of different species resprout, bloom and set fruit sequentially over time (Munhoz and Felfili 2005, Torezan-Silingardi 2007, Mendes *et al.* 2011). Four Malpighiaceae species were selected for the study: *Peixotoa tomentosa* A. Juss., *Banisteriopsis laevifolia* (A. Juss) B. Gates, *Banisteriopsis campestris* (A. Juss.) Little and *Banisteriopsis malifolia* (Ness and Mart) B. Gates. These species were chosen because they present a sequential flowering phenology previously described by Torezan-Silingardi (2007) and Vilela *et al.* (2014). Also, they are deciduous, hermaphroditic, bee-pollinated, self-compatible and share both pollinators and herbivores (see Torezan-Siligardi 2007, Vilela *et al.*2014).

Climatic analysis

The climate is markedly seasonal, with dry/cold season from April to September (rainfall 22 ± 20 mm; temperature 19° ± 3° C) and a wet/warm season occurring from October to March (rainfall, 270 ± 50 mm; temperature, 23° ± 5° C) (additional details in Réu and Del-Claro 2005). The annual mean of temperature and total precipitation were provided by the Climate Station of the Geography Institute of Federal University of Uberlandia (Fig. 1). Initially, we performed the Repeated Measures ANOVA to compare the annual mean of temperature and total precipitation from 2005 to 2014. We considered climatic factors since 2005 based on the sequential phenological results provided by Torezan-Siligardi (2007) for the same Malpighiaceae species at same place during 2005/2006. Further, we conducted Spearman's correlations to examine the association between climatic factors (temperature and precipitation) and phenological responses (number of individuals which begin their flowering in each month) from our field data (e.g Morellato *et al.*2010, Staggemeier and Morellato 2011).

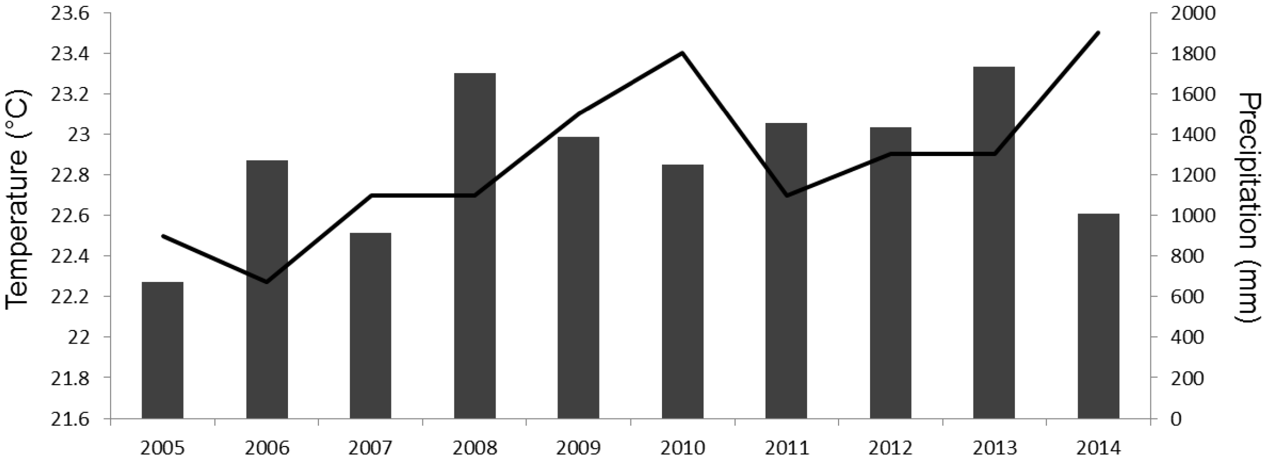

Figure 1. Annual mean temperature (°C, line) and precipitation (mm, bar) from 2005 to 2014 in Uberlândia, Minas Gerais State, Brazil.

Data Collection

Individual plants (N=30) of each plant species were monitored every two weeks during their reproductivity period (see Vilela *et al.* 2014). Plant phenology (intensity and number of buds, flowers and fruits) was recorded according to Torezan-Silingardi and Oliveira (2004) and Vilela *et al.* 2014 and fruit production was expressed as the proportion of fruit produced per buds (e.g. Del-Claro *et al.* 2013). At the end of the breeding season we also quantified herbivory (leaf area loss). We recorded data from nine leaves per plant (three from the most apical stem, three from a middle stem and three from the most basal stem) in order to determine the mean herbivory rate per species. This procedure was done without leaf removal. Measurements of herbivory rates on leaves were assessed by placing leaves on a transparent grid (divided into millimeters). An index of herbivory from each leaf was calculated as the proportion of points in the grid falling within damaged and undamaged areas of the leaf blade (see Moreira and Del-Claro 2005).

Statistical analysis

Species flowering variation

Circular statistical analyses were performed using the date of first flowering of each specie separately for each year studied. To calculate the circular statistic parameters, months were converted to angles from 0= January to 345= December at intervals of 15 because the measurements were made every two weeks (e.g. Cardoso *et al.* 2012). The frequency of individuals at the onset of flowering for each specie was considered for calculating the parameters: the mean vector (μ), length of mean vector (r), median, circular standard deviation, rayleigh test (z). The mean date for each phenophase is determined by converting the mean angular directions to corresponding mean dates (see Morellato *et al.*, 2000, 2010, Staggemeier *et al.*, 2010). When the mean angle was significant, we performed the two-sample Watson-Williams tests (F) to determine differences among the mean data of the onset flowering of each species over time (Zar 1996).

Community flowering variation

Circular statistical analyses were also performed considering all species studied to analyze the degree of overlap in each reproductive period to verify for possible community flowering variation. In this case, we used more phenological variables: date of first bud, first flowering and first fruiting, date of peak of bud, flowering and fruiting. The phenological data were analyzed with the statistical software Oriana 4.0.

Intending to confirm a segregated phenology, a null model was performed to indicate if the temporal overlap among species was less than expected by chance. Overlap was quantified via Pianka (Pianka 1973) and Czechanowski (Feinsinger *et al.* 1981) indices and the values were generated using a randomization algorithm (Rosario). This algorithm was designed specifically for use with interval data important (Castro-Arellano *et al.* 2010). Rosario maintains the shape of the empirical activity distributions for each species in the randomly generated matrices by shifting entire activity patterns a random number of intervals. For each analysis, overlap indices were calculated for 10,000 randomly generated matrices of temporal flowering phenology, creating a null distribution of overlap values. Then a one-tailed test was conducted and the p-value was calculated as the proportion of randomizations that resulted in overlap that is equal to or less than the empirical overlap value (observed) (e.g. Brito *et al.* 2012). Simulations were conducted with the TimeOverlap program (Castro-Arellano *et al.* 2010).

Herbivory and productivity analyses

We compared the rates of herbivory and fruit production between the periods with minimal (2008/2009) and higher overlap (2013/2014) using Test t after arcsine of the square root transformation of the percentage values. The data were analyzed using the software Systat 12.

Mathematical Modelling

The model we developed is part of a software (*Programa CSVM*® - INPI - BR 51 2015 000482-4) to present a tendency of plant production in face of phenological shifts associated with climate variability in agricultural systems (Claro *et al.*, in prep.). The model uses field parameters measure (at least four previous seasons) based on generic herbivores and pollinators. Considering that temporal and spatial variations on the abundance of specific partners could represent local variation on the results of flower and fruit production, the program intends to ensure a qualitative validity demonstrating a tendency in different scenarios. We adjusted and validated the model parameters by the use of *Differential Evolution optimization* methods (Lobato and Steffen 2008, Vanderplaats 2005). This procedure is made by estimating the interval in which the variables considered may vary, normalizing the variables and then applying an optimization procedure to achieve the best approximation of the real parameter/variable in a specific scenario (Lobato and Steffen, 2007). Initially, we considered the field data obtained by Torezan-Silingardi (2007) in 2005/2006 aiming to ensure a greater robustness for the model. In order to standardize the sample method, we employed the same methodology applied to Torezan-Silingardi (2007).

The inputs considered were rainfall, leaf sprouting and production of floral buds, flowers and fruits. Other parameters estimated by the researchers and adjusted by the software, were the weekly rates of herbivory, pollination and self-fecundation. The preliminary data as input parameters were: area of 2500m², with 100x100 square elements of

0.5x0.5 meters each, simulating for 5 years of 48 weeks time steps each (1 time step equals 1.0863 week). Containing 50 *B. malifolia* individuals (3x3 elements in size), 50 *P. tomentosa* individuals (2x1 elements in size), 30 *B. laevifolia* individuals (2x2 elements in size) and 40 *B. campestris* individuals (1x1 elements in size) based on their real size and presence measured on the field. The herbivory and pollination were supposed to occur in 90% of the elements per iteration. The randomness of herbivory considered was: base ± random of 3%. The randomness of insect pollination and autonomous self-pollination considered was: base ± random of 1% and we considered 13% of randomness for the baselines (rainfall, leaves sprouting, and flowering). All variation parameters were based on real field data.

Simulations were performed from an advance in the phenology of *B. laevifolia*, already observed in 2012 and 2013, to investigate the consequences of a continuous shift in its phenology for the degree of overlap among species and the outcomes for plant reproduction, *i.e.*, production of flowers and fruit set, for the next five years. We simulated three phenological scenarios with advancement of phenology in 2, 4 and 6 weeks on the *B. laevifolia* flowering, keeping all other parametes set as before.

Results

Climatic analysis

The annual mean of temperature and total precipitation varied from 2005 to 2014 (Repeated-Measure One-Way ANOVA F=3.785; df=9; p<0.001 for temperature and F=2.21; df=9; p=0.027 for precipitation) (Fig. 1). The Spearman's correlations between the onset of flowering of each species and the climatic variable (temperature and precipitation) during each year studied (2008/2009, 2012/2013, 2013/2014) showed that the species were differently correlated with climatic factors over the years with *P. tomentosa* presenting the highest correlation results and *B. malifolia* as the species suffering less interference from climatic variations (see table 1).

Table 1. Spearrman correlations between the onset of flowering of *P. tomentosa, B. laevolia, B. campestris* and *B. malifolia* with climatic parameters (temperature and precipitation) in a Brazilian Tropical Savanna of Uberlândia, Minas Gerais State, Brazil. (*) means significant correlation, p<0.05.

specie	Temperature			Precipitation		
	2008/2009	2012/2013	2013/2014	2008/2009	2012/2013	2013/2014
P.tomentosa	-0.57*	-0.66*	-0.71*	-0.57*	-0.67*	-0.47
B. laevifolia	0.27	0.10	-0.58*	-0.44	-0.59*	-0.71*
B. campestris	0.08	0.61*	0.59*	0.76*	0.58*	0.39
B. malifolia	-0.59*	0.06	-0.25	0.22	0.18	0.18

Species flowering variation

For all species, the mean angles of onset of flowering were significantly seasonal for whole analyzed period (Rayleigh test p<0.05) and the lengths of mean vectors (r) were nearly 1 (Table 2). The vector r varies from 0 (when phenological activity is distributed uniformly throughout the year) to 1 (when phenological activity is concentrated around one single date or mean angle) (Zar 1996). Comparisons of the mean data among the years showed that the onset of species flowering varied over time (F=59.176, p<0.001 for *P. tomentosa*; F=48.445, p<0.001 for *B. laevifolia*; F=33.087, p<0.001 for *B. campestris*; F=60.729, p<0.001 for *B. malifolia,* Table 2).

Table 2. Circular statistical analysis testing for seasonality on floral phenology of *P. tomentosa, B. laevolia, B. campestris* and *B. malifolia* in a Brazilian Tropical Savanna over three reproductive season (Years 1º -2008/2009; 2º - 2012/2013; 3º - 2013/2014). Watson-Williams tests (F) were performed to test the difference among the mean date of onset of flowering along the years.

Species	Year	N	a +-DP	Mean Data	r	Z (p)	Watson-Willian (F)	P
P. tomentosa	1°	19	154.649° ± 9.775°	03/06/2008	0.986	18.455 (<0.001)	59.176	<0.001
	2°	14	181.009° ± 11.97°	30/06/2012	0.978	13.402 (<0.001)		
	3°	7	173.858° ± 19.433°	22/06/2013	0.944	6.239 (<0.001)		
B. laevifolia	1°	30	233.995° ± 8.313°	20/08/2008	0.99	29.375 (<0.001)	48.445	<0.001
	2°	20	221.943° ± 13.97°	08/08/2012	0.971	18.846 (<0.001)		
	3°	16	207.574° ± 19.615°	25/07/2013	0.943	14.23 (<0.001)		
B. campestris	1°	26	349.866° ± 15.99°	15/12/2008	0.962	24.052 (<0.001)	33.087	<0.001
	2°	13	357.807° ± 14.234°	23/12/2012	0.97	12.222 (<0.001)		
	3°	5	11.932° ± 14.772°	12/01/2014	0.967	4.678 (0.003)		
B. malifolia	1°	22	110.053° ± 19.451°	20/04/2008	0.944	19.605 (<0.001)	60.729	<0.001
	2°	22	82.547° ± 9.86°	23/03/2012	0.985	21.358 (<0.001)		
	3°	17	111.088° ± 17.195°	21/04/2013	0.956	15.536 (<0.001)		

Community flowering variation

For the first reproductive season (2008/2009) the mean angles of onset and peak for all phenophases were not significantly seasonal (Rayleigh test p>0.05) and the lengths of mean vectors (r) were nearly 0 (Table 3). These results show that phenological activity of the Malpighiaceae was distributed uniformly throughout the year featuring a segregated phenology. The simulations of null model for this reproductive period indicated that the distributions overlap were significantly less than expected by chance, which confirms the sequential flowering (Pianka=0.202, P<0.001; Czechanowski=0.968, P<0.001). For the second reproductive season (2012/2013) the mean angle of peak for buds and flowers and onset of fruit were not significantly seasonal (Rayleigh test p>0.05) and the lengths of mean vectors (r) were nearly 0 (Table 3). These results show that phenological activity exhibited a degree of overlap among some phenophases. In the third year of study the mean angles were significantly seasonal (Rayleigh test p<0.05) and the lengths of mean vectors (r) were nearer 1 (Table 3). Linear graphics with the intensity of flowering of Malpighiaceae species showing the degree of overlap among the years were performed (Fig. 2).

Table 3. Circular statistical analyzes testing for seasonality on phenological pattern of community composed by *P. tomentosa, B. laevolia, B. campestris* and *B. malifolia* in a Brazilian tropical savanna during three reproductive seasons (2008/2009; 2012/2013; 2013/2014). Rayleigh test performed the significance of the mean angle and the mean data were omitted when it were not significant.

	Flower bud		Flower		Fruit	
2008/2009	Onset	Peak	Onset	Peak	Onset	Peak
Mean data						
Number of Observations	110	109	97	90	87	88
Mean Vector (µ)	197,599°	166,697°	183,012°	206,112°	198,797°	219,599°
Length of Mean Vector (r)	0.162	0.101	0.174	0.169	0.175	0.177
Median	225°	225°	225°	240°	240°	255°
Circular Standard Deviation	109,408°	122,594°	107,109°	108,082°	106,992°	106,622°
Rayleigh Test (Z)	2.87	1.12	2,945	2,563	2,661	2,758
Rayleigh Test (p)	**p>0.05**	**p>0.05**	**p>0.05**	**p>0.05**	**p>0.05**	**p>0.05**

	Flower bud		Flower		Fruit	
2012/2013	Onset	Peak	Onset	Peak	Onset	Peak
Mean data	03/05/2012		28/05/2012			01/07/2012
Number of Observations	93	93	69	69	64	64
Mean Vector (µ)	123.18°	134.554°	148.521°	160.316°	159.064°	182.271°
Length of Mean Vector (r)	0.197	0.13	0.216	0.191	0.188	0.251
Median	180°	180°	90°	210°	120°	225°
Circular Standard Deviation	103.299°	115.796°	100.261°	104.28°	104.702°	95.32°
Rayleigh Test (Z)	3.604	1.565	3.228	2.513	2.269	4.02
Rayleigh Test (p)	**0.027**	**p>0.05**	**0.04**	**p>0.05**	**p>0.05**	**0.018**

	Flower bud		Flower		Fruit	
2013/2014	Onset	Peak	Onset	Peak	Onset	Peak
Mean data	10/05/2013	16/05/2013	04/06/2013	17/06/2013	28/05/2013	15/06/2013
Number of Observations	73	74	45	45	32	32
Mean Vector (µ)	129.454°	135.948°	154.857°	167.452°	147.297°	165.694°

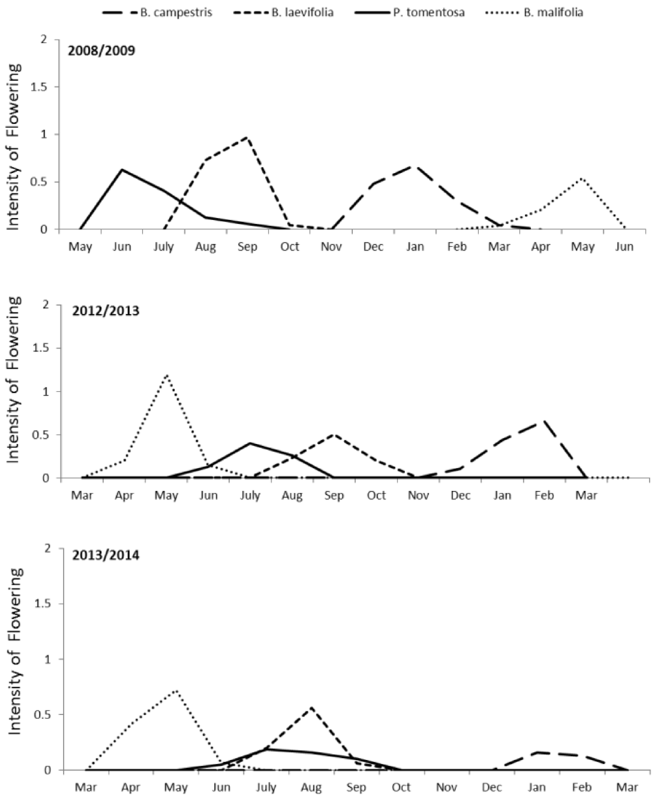

Figure 2. Linear graphics with the intensity of flowering of *Peixotoa tomentosa*, *Banisteriopsis laevifolia*, *Banisteriopsis campestris* and *Banisteriopsis malifolia* during 2008/2009, 2012/2013 and 2013/2014 in a Brazilian Tropical Savanna (axis y means intensity of flowering phenology being 1=25% and 2=75%; n=30).

Herbivory and productivity analyses

The periods with higher overlap of flowering accounted for lower rates of herbivory for *B. laevifolia* (t=2.05; df=161; p=0.041) and *P. tomentosa* (t=2.99; df=105; p=0.003). However, there were no significant difference for *B. campestris* (t=1.43; df=178; p=0.153) and *B. malifolia* (t=0.21; df=173; p =0.826) (Fig. 3a). In relation of fruit set, the higher overlap period accounted for lower rates of productivity only for *B. laevifolia* (t=2.35; df=58; p=0.022). There were no significant difference for *B. malifolia* (t=0.88; df=58; p=0.379) and *P. tomentosa* (t=0.121; df=60; p=0.900). *B. campestris* presented an increase in productivity during the period with higher overlap, however there was no significant difference (t=1.469; df=57; p=0.147) (Fig. 3b).

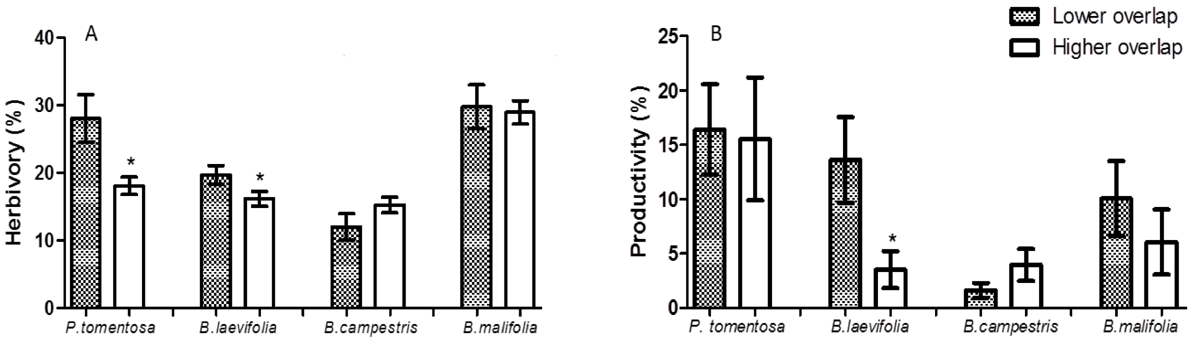

Figure 3. Comparative analysis of herbivory (leaf area loss) (A) and fruit production (B) of *Peixotoa tomentosa*, *Banisteriopsis laevifolia*, *Banisteriopsis campestris* and *Banisteriopsis malifolia* in a Brazilian Tropical Savanna between the period with lower (2008/2009) and greater overlap of flowering (2013/2014). (Test T, (*) means statistical difference; $p < 0.05$, Means ± 1SE are presented)

Mathematical Modelling

The program obtained a mean squared error of 3.87% when compared to the mean field data, achieving a considerable approach between the model and the real-case scenario. All mean percentage squared error achieved for each species after the optimization procedure are exhibited in table 4. The results of advance in the phenology of *B. laevifolia* demonstrated a tendency in increasing the production of flowers, however a proportionally lower production of fruits (Fig. 4a). In relation of *P. tomentosa,* the model indicated a tendency in increasing the amount of flowers and fruits, as well (Fig. 4b). There were no tendencies of variation for *B. malifolia* and *B. campestris* (Fig 4c and 4d, respectively).

Table 4 Total percentual errors given by the integral of the squared error between the real and simulated data, on a random simulation scenario

Total percentual squared error (%)	*B. malifolia*	*P. tomentosa*	*B. laevifolia*	*B. campestris*
F. Buds	1.22	4.11	2.46	1.10
Flowers	1.71	3.69	3.99	5.51
Fruits	1.65	9.03	3.16	9.45

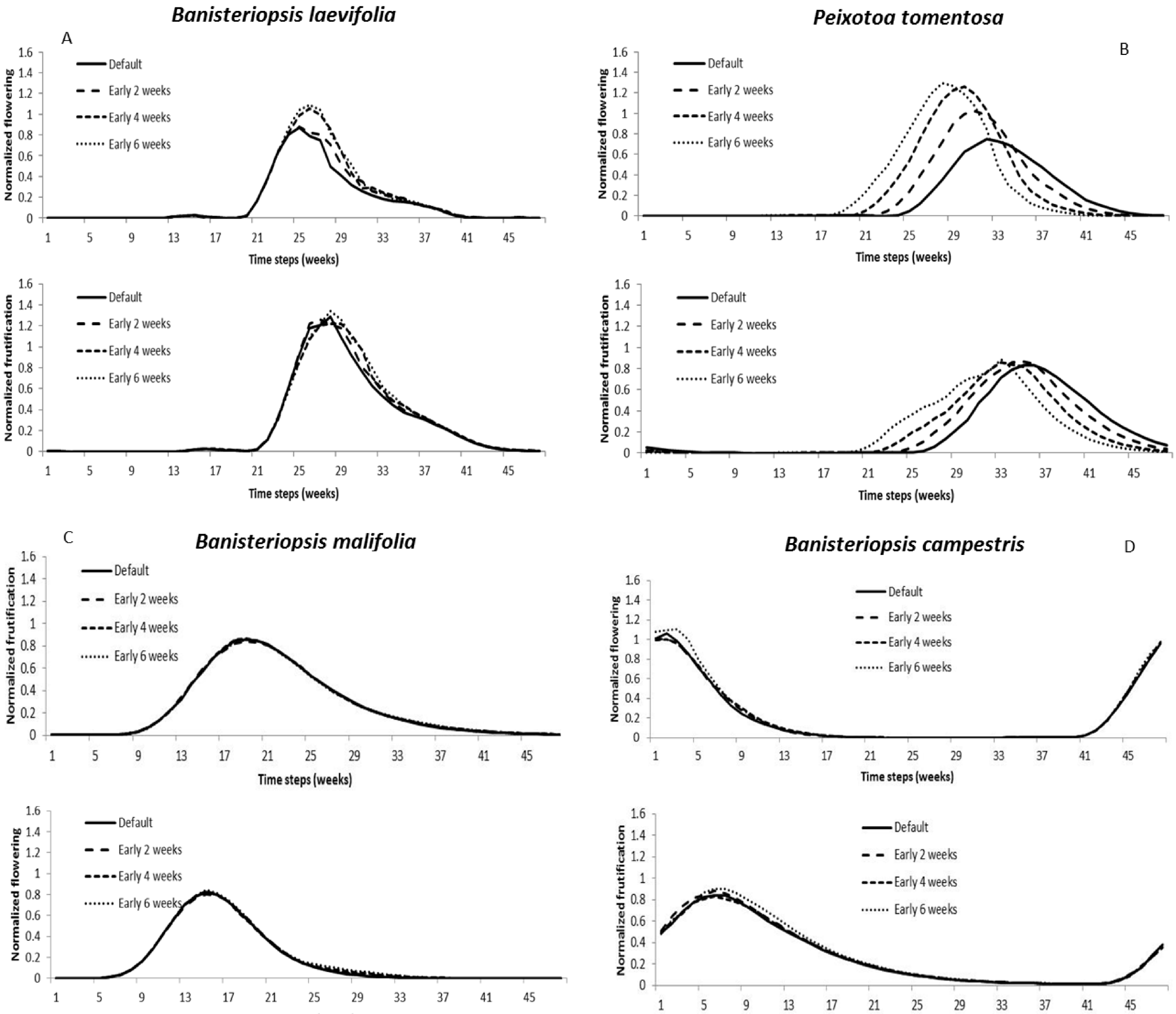

Figure 4. Results of flower and fruit production for *Banisteriopsis laevifolia* (A) *Peixotoa tomentosa* (B), *Banisteriopsis malifolia* (C) and *Banisteriopsis campestris* (D) in four different phenological simulations, considering an anticipated phenology of *B. laevifolia* in 2, 4 and 6 weeks. Default means the standard setting.

Discussion

Our results demonstrated a climatic variation on annual mean of temperature and total precipitation from 2005 and 2014 and detected correlations with the onset of plant flowering during the three reproductive seasons studied (2008/2009, 2011/2012, 2013/2014). Besides, shifts on the onset of flowering resulted in a greater overlap among Malpighiaceae species during the third reproductivity period analyzed, corroborating our first hypothesis. We also compared herbivory and fruit production during both phenological strategies (staggered or synchronized) and demonstrated that the outcomes varied according the interaction each species establishes with its mutualists (pollinators) and antagonists (herbivores) partners, corroborating our second hypothesis. The mathematical model indicated a tendency in flower and fruit production for each plant species contributing to a better understanding of how the phenology may vary on a continuing climate change.

The correlations between onset of plant flowering and temperature and precipitation suggests an influence of these climatic parameters on the phenology of plant species. Plants may advance or delay their reproductive period in a seasonal climate by avoiding the sprouting of new leaves before or during unfavorable periods (e.g water stress) or by producing new leaves to coincide with the onset of periods of favorable conditions (e.g. high radiation) (Van Shaik *et al.* 1993; Del-Claro and Torezan-Silingardi 2012). The impacts of climatic variation on phenological shifts are among the most documented process occurring during the last years, as demonstrated by several studies (e.g., Forrest *et al.*2010, Forrest and Thomson 2011, Rafferty and Ive 2012, Diez *et al.* 2012, Liuth *et al.*2013), especially the effects of rainfall and temperature in tropical climates (e.g Morellato 2013, Bock *et al.* 2014).

Changes in flowering patterns are likely to affect plant reproduction if competitive or facilitative interactions between plant species affect their associations with pollinators and herbivores partners (see Gross *et al.*2000, Lazaro *et al.* 2009, Forrest *et al.*2010). Annual variation on plant phenologies in subalpine meadows in Colorado, USA, demonstrated a higher overlap among plant species with a share of pollinators by some species (e.g. Forrest *et al.*2010) and decreased fruit set for other species due to their phenology desynchronization with pollinators (Thomson 2010). Rafferty and Ives (2011) showed that species experimentally induced to flower earlier in a wildflower community received more pollinator visits and Rafferty and Ives (2012) simulated changes on phenology of two perennial wildflowers and demonstrated that the seed set can vary as a result of changes in pollinator taxonomic composition and effectiveness. Therewith, flowering phenology is commonly thought to affect plant reproduction through the influence of pollinators, as well as the degree of plant dependence from this interaction (see Johnson *et al.*2011).

In our system, all species are self-compatible, they can require animal pollinators to outcross pollen or reproduce via spontaneous autogamy, however, the rate of autonomous self-pollination varies greatly among species (Torezan-Silingardi *et al.*in preparation). Fruit set via spontaneous self-pollination in *Banisteriopsis laevifolia* and *B. campestris* is about only 2%, while in *P. tomentosa* this value can range from 10 to 43%, and in *B. malifolia* accounts for about 30% of the fruit set. In this sense, the species of Malpighiaceae were affected differently by the greater overlap of flowering period with *B. laevifolia,* the only significantly affected in a negative way. Based on these results, we can suggest that *P. tomentosa* and *B.malifolia* presented a more robust response to a greater overlap of phenology due to their relative independence of pollinators. *Banisteriopsis campestris* can not be considered in this line of thought because its flowered separated from the core of other species. Therefore, the staggered strategy is more essential for *B. laevifolia* due to its greater dependence in relation to pollinators. Bishop *et al.* (1998) and Moeller (2006) have demonstrated and discussed the importance of self-compatibility by increasing fruit and seed production as an important driver of plant mating system evolution.

Our results also showed that the herbivory rate was significantly reduced for *P. tomentosa*, *B. laevifolia* and no significantly for *B. malifolia* and *B.campestris* in a scenario with greater overlap. Therefore, the effects of overlap of plants in reducing herbivory were corroborated and it is likely that the effect on *B. malifolia* and *B. campestris* was lower due to its less overlapping than *P. tomentosa* and *B .laevifolia.* In addition, these species flourishes in the rainy season, the period with the greatest abundance and activity of herbivore insects (e.g. Alves- Silva *et al.* 2013, 2014, Vilela *et al.*2014). Thus, we suggest that a greater synchronization among *P. tomentosa* and *B. laevifolia* may have allowed the occurrence of dilution of herbivory (Herbivore Satiation Hypothesis) (e.g. Clark and Clark 1991, English-Loeb and Karban 1992). A classic study of Aide (1991) investigated this effect for leaves growing synchronously within the population of the sub-canopy tree *Gustavia superba* (Lecythidaceae) and found out that individuals out of synchrony with their conspecifics suffered significantly more herbivore damage. It is also possible the occurrence of desynchronization of herbivores in relation of plant reproductive period with a mismatch in the timing of flowering resulting in a decreased herbivore rate. The importance of synchrony for effective herbivore consumption was already evidenced for larva of Lepidoptera feeding on leaf flush from experimental studies (e.g. Hunter and Elkinton 2000; Van Asch *et al.* 2007).

The model we developed was able to demonstrate with great precision (mean squared error < 3%) any given scenario with variations in the rainfall or herbivory for the species considered. In scenario extrapolations with input not directly linked to the species themselves (*i.e.* rainfall, herbivory, etc.) the model is able to make a very precise prediction, which has been optimized and validated. In the opposite way, when the variations are directly related to the species (*i.e.* flowering) the results should be interpreted as a tendency with unknown error, due to unavailability of real field data for comparisons and model adjustment. We considered variation in the onset of plant flowering and, therefore, we have to interpret the results as a valid tendency. The simulations showed a species specific response, also demonstrated by Diez *et al.* (2012) to three North American plant communities in response to climate change.

In our study, the most pollinator-dependent species*,* *B. laevifolia*, presented a tendency in increasing the production of flowers possibly as a result of the dilution in herbivore pressure (e.g. Bishop *et al.*1998) or a desynchronization in relation to plant flowering (e.g. Forrest and Thomson 2011). However, the greater synchronization with *P. tomentosa* and possibly share of pollinators add to its dependence on these vectors resulted in a tendency of lower production of fruits. On the other hand, plants possessing the combination of simultaneously autonomous self-pollination and escape from herbivores, as demonstrated by *P. tomentosa,* were able to keep a disproportionate fitness advantage over plants possessing any of the other possibilities (see Herrera *et al.*2002). Thus, within communities, variation in responses by species will determine community-level patterns of phenology and resilience of species (Memmott *et al.* 2007; Kaiser-Bunbury *et al.* 2010).

We endorsed variations on climatic parameters over the recent years and the manifestation of these changes on plant phenology and biotic interactions of different plant species from South Hemisphere. There is a concern that climate change is altering the timing of life history events in a wide array of species, especially for species from Northern Hemisphere. However, few papers have related the effects of climatic change on plant phenology and interactions of South American species (Morellato *et al.* 2013). Besides, we evidenced the significant variability in species responses related to their mutualistic and antagonistic partners. The plasticity traits have shown important for survival and maintenance of these plant species in a near future. Thus, integrating phenological investigations in the field with the existing theory and mathematical modeling allow us to interpret different phenological synchronization degree and enable us to describe the possible maintenance and evolution of life histories in response to climate changes.

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CONSIDERAÇÕES FINAIS

Os resultados corroboraram a hipótese da competição intraespecífica das cochonilhas pela proteção das formigas, assim como os efeitos da biologia das espécies de formigas no resultado da interação. As mudanças fenológicas ocorridas com a *B. laevifolia* promoveram uma assincronia com os curculionídeos e uma maior sobreposição com a espécie simpátrica, *P. tomentosa.* Essas variações impactaram diretamente as interações formiga-planta-herbívoro e planta-planta e confirmam a hipótese que alterações nos eventos fenológicos são capazes de afetar o resultado de interações chaves e, consequentemente, o sucesso reprodutivo das plantas. As variações nos fatores climáticos de temperatura e precipitação também foram comprovadas e houve correlação desses fatores abióticos com o início da floração das espécies nos três períodos reprodutivos estudados. Houve variação no grau de sobreposição do período reprodutivo entre as espécies estudadas (sincronizado ou sequencial), com um efeito diferencial na taxa de herbivoria e produção de frutos. Tais efeitos foram espécie-específicos e dependentes da intima associação dessas espécies com seus agentes polinizadores e herbívoros associados. O modelo matemático desenvolvido mostrou-se confiável, muito próximo aos dados de campo, e capaz de apresentar as tendências na produção de flores e frutos para cada espécie em situações de contínuas variações fenológicas para os próximos cinco anos.

Compreender como as plantas e animais interagem é a chave para entender traços importantes dos processos evolutivos. A integração entre as investigações de campo, as teorias ecológicas e os modelos matemáticos nos permite interpretar a condicionalidade de importantes interações ecológicas, descrever a evolução da história de vida dos organismos e os efeitos para o estabelecimento e manutenção das espécies ao longo do tempo**.** Assim sendo, o trabalho contribuiu para uma crescente área de pesquisa sobre a inerente diversidade de interações associadas às atuais variações ambientais, tornando o estudo relevante para uma maior compreensão sobre a ecologia das interações e a conservação das comunidades naturais.