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**Hipólito Ferreira Paulino Neto**



**História Natural e Interação Flores-  
Besouros em espécies de Cerrado**

**SÃO PAULO-SP**

**2009**

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**Hipólito Ferreira Paulino Neto**

**História Natural e Interação Flores-Besouros  
em espécies de Cerrado**

Tese apresentada ao Instituto de  
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Orientadora: Profa. Dra. Silvana Buzato

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**Prof(a). Dra. Silvana Buzato**

**Orientadora**

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**Aos meus pais Hipólito Filho (*in memoriam*) e  
Maria Sueli pelo amor e apoio incondicional.**

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"Compreendi, então, que a vida não é uma sonata que, para realizar a sua beleza, tem que ser tocada até o fim. Dei-me conta, ao contrário, que a vida é um álbum de mini-sonatas. Cada momento de beleza vivido e amado, por efêmero que seja, é uma experiência completa que está destinada à eternidade. Um único momento de beleza e amor justifica a vida inteira." **Rubem Alves**

“Toda nossa ciência comparada a nossa realidade é primitiva e infantil e, no entanto, é a coisa mais preciosa que temos”

**Albert Einstein**

"A persistência é o caminho do êxito." **Charles Chaplin**

"Foi, é claro, uma mentira o que você leu sobre minhas convicções religiosas, uma mentira que está sendo sistematicamente repetida. Eu não acredito em um Deus pessoal e eu nunca neguei isso, mas expressei claramente. Se existe algo em mim que pode ser chamado de religioso, esse algo é a admiração ilimitada pela estrutura do mundo tão longínqua quanto a nossa ciência pode revelar."

**Albert Einstein**

# Dedicatória

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Antes de mais nada, acredito que tudo que fazemos na vida e mesmo quem somos é consequência direta e indireta dos fatos que cada um de nós vive e de todas pessoas que conhecemos, principalmente aquelas que fizeram ou fazem parte de nossas vidas de forma mais assídua e intensa. Por isso, quero compartilhar essa minha imensurável alegria de estar concluindo mais essa etapa de minha vida, justamente com as pessoas que mais fizeram ou fazem parte dela. Como sou grato por ter encontrado tantas boas pessoas na minha vida, posso neste momento de relativo estresse de final de redação de tese, me esquecer de alguns que realmente foram importantes para mim e que de forma alguma deixarão de ser só por não terem sido incluídos na lista de agradecimentos abaixo. Assim, obrigado a todos colegas de profissão, amigos e familiares por fazer parte de minha vida e me fazer tão feliz!!

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## RESUMO

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Interações planta-animal, tais como polinização, são a chave de processos ecológicos in muitas comunidades terrestres. O estudo de quem interage com quem é uma importante ferramenta para se entender os processos ecológicos e evolucionários. Em algumas comunidades tropicais, mais de um quarto de todas as espécies de planta pode ser polinizado por besouros. Eles são um grupo de inseto muito antigo e diversificado e eles interagem com angiospermas desde o período de suas origens e princípio da diversificação. Adicionalmente, a interação entre besouros e recursos florais provém singular oportunidade para se avaliar a complexidade de interações e a possibilidade de generalização como a tendência para plantas para usar uma enorme proporção da fauna de besouros visitantes como polinizadores, ou especialização com plantas usando uma proporção relativamente pequena da fauna disponível de visitantes como polinizadores. A distribuição espacial de espécies vegetais tem sido considerada um importante componente na determinação de interações planta-animal, sendo esperado que muitos padrões observados nestes sistemas resultem de variações na distribuição de recursos vegetais. A disponibilidade de recursos florais apresenta variações espaço-temporais que podem influenciar a eficiência dos polinizadores do ponto de vista quantitativo, pela frequência de ocorrência da interação, ou qualitativo pela contribuição dada ao sucesso reprodutivo das espécies. Os principais objetivos da presente tese foram: 1) descrever a história natural e interação flores – besouros em espécies de Cerrado em relação à heterogeneidade espaço-temporal da distribuição de recursos entre fitofisionomias localizadas na Estação Ecológica de Itirapina (EEI) e em área de Cerradão pertencente ao Instituto Arruda Botelho (22°12'- 22°10'S e 47°55'- 47°57'W, respectivamente) durante dois anos consecutivos; 2) verificar o padrão local de distribuição de flores e besouros em quatro fitofisionomias de cerrado estudadas; 3) caracterizar a nível de comunidade os padrões de interações observados na comunidade composta por besouros associados a flores; 4) finalmente, compreender detalhadamente um dos diversos sistemas de interação besouros-plantas registrados na área de estudo. Escolhemos o sistema de interação de *D. furfuracea*-besouros com o objetivo de descrever sua fenologia de floração e frutificação e seu sistema reprodutivo, verificando se há

limitação polínica e de recursos. Também foi avaliada a função e o efeito dos visitantes florais sobre o sistema reprodutivo. Uma alta proporção de espécies de plantas tem suas flores visitadas por besouros em todas quatro fitofisionomias de Cerrado estudadas (12-40%), indicando que os dados disponíveis até o momento subestimam a ocorrência de cantarofilia para áreas de Cerrado. Este consiste no primeiro estudo focando toda uma comunidade de besouros associados a flores. Não houve variação temporal entre anos tanto para as redes de visitantes-flores ou para redes de polinizadores-plantas. Redes de polinizadores-plantas tiveram espécies de besouros altamente especialistas. *Attalea geraensis*, *C. pubescens*, *D. furfuracea*, *D. hispida*, *K. coriacea*, *S. petrea*, *T. formosa* e *X. aromatica* consistem em espécies de planta envolvidas em muitas interações e foram consideradas espécies centrais. Tanto as redes de visitantes-flores, como a rede de polinizadores-plantas evidenciaram uma estrutura composta combinando estrutura de rede aninhada com compartimentada, mas com predomínio do padrão compartimentado. Estes compartimentos são resultantes tanto das muitas interações espécie-específica entre espécies de besouros e plantas, como daquelas espécies de planta que interagem com várias espécies especialistas de besouros. De modo geral, focando atenção nos besouros, ambas as redes, visitantes-flores e polinizadores-plantas foram definidas como altamente especializadas já que visitaram flores poucas espécies de planta tanto para comparações entre anos, como entre fitofisionomias. O presente estudo mostrou que o Cerrado apresenta sistemas de interação entre besouros e flores de espécies de planta com alta especificidade. Dentre as várias espécies de planta compreendidas nestas redes de interação de alta especificidade, merece desta *D. furfuracea* que apresenta uma fauna composta por várias espécies de besouros polinizadores altamente especialistas. *Duguetia furfuracea* é uma espécie auto-incompatível, cuja população estudada apresentou limitação polínica e de recursos no solo. Há duas guildas especializadas de besouros interagindo com esta espécie de planta. A primeira é composta por uma única espécie de curculionídeo (*Plasilia* sp.) que visitou suas flores em baixa abundância (média de 0,55 besouros por flor), mas com alta frequência de ocorrência (44,9% das flores) e suas larvas consomem as sementes dos frutos que se desenvolvem. A segunda guilda é composta por várias espécies de besouros nitidulídeos, principalmente *Colopterus* sp.3 que visitaram as flores em grande abundância (média de 99 besouros por flor) e também com alta ocorrência de visitaç o (92% das flores) e que efetivamente promoveram a polinizaç o. O sistema de polinizaç o de *D. furfuracea* consiste no primeiro caso de polinizaç o mutualística obrigatória envolvendo diferentes guildas



de visitantes florais. Este também é o primeiro sistema de polinização combinando polinizadores previsíveis e confiáveis, consumo de sementes, limitação polínica e limitação por recursos, o que resulta em um complexo e eficiente mecanismo para regulação da população do visitante floral consumidor de sementes e para otimizar o sucesso reprodutivo da planta.

***Palavras-chave:*** Cerrado, Coleoptera, heterogeneidade espacial, ecologia de comunidade, interação besouros- flores, redes de interações, *Duguetia furfuracea*, polinização, mutualismo, consumo de sementes.

## ABSTRACT

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### **Natural history and flower-beetle interactions in Cerrado species.**

Plant-animal interactions, such as pollination, are a key element in many terrestrial communities. The study of who interacts with whom is an important approach for understanding ecological and evolutionary processes. In some tropical communities, up to one quarter of all plant species may be pollinated by beetles. They are an ancient and much diversified insect group and they interact with angiosperms since the time of their origin and early diversification. Additionally, the interaction between beetle and floral resource provide unique opportunity to evaluate the complexity of interactions and the possibility of generalization as the tendency for plants to use a large proportion of the available beetle-visiting fauna as pollinators, or specialization with plants using a relatively small proportion of the available beetle visiting fauna as pollinators. The spatial distributions of plant species have been considered an important component in the determination of plant-animal interactions, and it is expected that many patterns observed in these systems resulting from variations in the resource distribution of plants. The availability of floral resources presents spatio-temporal variations that may affect the pollinator efficiency in its quantitative traits through frequency of occurrence of the interaction, or qualitative by its contribution to the fitness plant. Thus, the main objectives of this present thesis were: 1) describe the natural history and interactions of beetle with flowers in Cerrado species focusing the spatio-temporal heterogeneity of the resource distribution among phytophysiognomies located in the Itirapina Ecological Station and in the Cerradão area belongs to the Arruda Botelho Institute (22°12'-22°10'S and 47°55'-47°57'W, respectively) during two consecutive years.; 2) verify the local pattern of flowers and beetle distribution in the four cerrado phytophysiognomies studied; 3) characterize in the community level the interaction patterns observed in the community composed by beetles associated to flowers; 4) finally, comprehend deeply one of the several beetle-flower systems recorded to this studied area. We chose the *D. furfuracea*-beetle interaction

system with aim to describe its flowering and fruit phenology and its reproductive biology verifying if there are pollen and resource limitation. Also was evaluated the role and effect of floral visitors in the fitness plant. A high number of flowering species was visited by beetles (12-40%) indicating that the data available up to now underestimate the representation of this interaction in Cerrado areas. This work represents the first study focusing in the entire beetle community associated to flowers. Was found no temporal variation in the interaction between beetles and flowers. Pollinator-plant webs had beetle species highly specialist. *A. geraensis*, *C. pubescens*, *D. furfuracea*, *D. hispida*, *K. coriacea*, *S. petrea*, *T. formosa* and *X. aromatica*, involved in many interactions were considered as core species. Both the visitor-flower and the pollinator-plant webs showed a compound structures mixing nested and compartmented networks structure, but predominating the compartmented pattern. These compartments are resultant from both of the many species-specific interactions between beetle and plant species and of the plant species that interact with several specialist beetle species. Both visitor-flower and pollinator-plant webs may be denominated as highly specialist from the beetle perspective whereas that these beetle fauna visited flowers of very few plant taxa over time and space. The present study showed that the Cerrado presents interaction systems between beetles and plants species with high specificity. Among the several plant species comprised in these interaction webs with high specificity, *D. furfuracea* presents a very interesting pollinator system, presenting a fauna composed by several pollinator beetle species highly specialized. *D. furfuracea* is a self-incompatible species, which studied population presented pollen and resource limitation. There are two specialized beetle guilds interacting with this plant species. The first guild is composed just by one curculionid species (*Plasilia* sp.) that visited flowers with low abundance (median of 0.55 beetles per flower), but presenting high frequency of occurrence (44.9% of the flowers) and their larvae consumed seed of that fruits that developed. The second guild is composed by several nitidulid beetles, principally *Colopterus* sp.3 that visited flowers in large abundance (median of 99 beetles per flower) and also presented high visitation occurrence (92% of flowers) and that effectively promoted the pollination. The pollination system of *D. furfuracea* consists is the first case of obligate pollination mutualism case involving different guilds of floral visitors. This also constitutes the first pollination system combining predictable and reliable pollinators, seed consumption, pollen and resource limitation resulting in a complex and efficient mechanism to regulate the seed consuming by floral visitor's population and to optimize the plant fitness.

**Keywords:** Cerrado, Coleoptera, spatial heterogeneity, community ecology, beetle-flower interaction, network interactions, *Duguetia furfuracea*, pollination, mutualism, seed consumption.

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# **APRESENTAÇÃO**

Coleópteros constituem um dos grupos mais antigos dentre os insetos, possuindo a maior riqueza dentre os invertebrados (Price 2002). Este fato pode ser explicado por sua longa história evolutiva e inúmeras interações interespecíficas com plantas e animais, sendo algumas de alta especificidade (Gottsberger 1989, Webber 1996; Paulino Neto *et al.* 2006; Hemborg & Bond 2005; Andresen & Feer 2005; Valente 2005). As interações entre besouros e angiospermas datam da segunda metade do Cretáceo (90 - 100 milhões de anos, Friis & Crepet 1987), e apresentam variações nos resultados decorrentes das relações de custo-benefício da interação. Estes resultados possibilitam a classificação das interações como positivas (mutualismo) ou negativas (predação) e muitas interações mutualísticas podem apresentar conflitos, sendo p.e. comum no processo de polinização ocorrer também florivoria (García-Robledo *et al.* 2004, Paulino Neto & Teixeira 2006). Estes conflitos são comuns em outros sistemas de alta especificidade como *Yucca* (Althoff *et al.* 2005), o cactus *Lophocereus schottii* e mariposas *Upiga virescens* (Holland & Fleming 1999, Holland *et al.* 2004) e Araceae (García-Robledo *et al.* 2004).

A distribuição espacial de espécies vegetais tem sido considerada um importante componente na determinação de interações planta-animal, sendo esperado que muitos padrões observados nestes sistemas resultem de variações na distribuição de recursos vegetais (Hunter & Price 1992). A abundância local de espécies que pode ser representada tanto pela distância entre indivíduos (densidade), caracterizando a estrutura espacial, bem como pelo número de indivíduos (tamanho populacional) tem efeitos sobre as interações biológicas (Kunin 1997, Mustajärvi *et al.* 2001). A atividade de polinizadores apresenta padrões dependentes de densidade, tanto no que se refere às frequências de visita como à constância floral (Rathcke 1983, Waser 1986, Kunin & Iwasa 1996). Polinizadores forrageiam de modo não aleatório o recurso disponível (Gill & Wolf 1977, Waser 1982, Jones 2001), sendo que sua resposta à abundância local de espécies sofre influência não somente de densidades florais, mas da distribuição de recursos florais (Zimmerman 1988, Rathcke 1992).

A disponibilidade de recursos florais apresenta variações espaço-temporais e que podem ser avaliadas em diversas escalas (Real & Rathcke 1988, Kotliar & Wiens 1990, Bronstein 1995). Tais variações podem influenciar a eficiência dos polinizadores do ponto de vista quantitativo, pela frequência de ocorrência da interação, ou qualitativo pela contribuição dada ao sucesso reprodutivo das espécies (Herrera 1987). Aumento na densidade de flores da mesma espécie ou de espécies diversas tem efeitos no sucesso reprodutivo dos indivíduos via facilitação,

aumentando o sucesso na polinização ou promovendo competição, resultando em limitação polínica (Vandermeer *et al.* 1985, Feinsinger 1987, Rathcke 1988). Como as plantas são imóveis, dependem de vetores abióticos e/ou bióticos para o transporte de pólen para a reprodução sexual, inadequada quantidade ou qualidade de pólen pode reduzir o sucesso reprodutivo da planta, afetando a quantidade ou a qualidade das sementes. Em termos gerais, este fenômeno é denominado de “limitação polínica” (Ashman *et al.* 2004). Assim, no caso de plantas polinizadas por animais, a quantidade de pólen pode ser reduzida em consequência de poucas visitas de polinizadores ou diminuição na deposição de pólen por visitas, e a qualidade do pólen pode ser reduzida em casos em que pólen incompatível seja depositado nos estigmas (Ashman *et al.* 2004). Segundo Turnbull *et al.* (2000), o contexto ecológico, a história natural da planta, seu sistema reprodutivo, bem como a história filogenética podem influenciar ou estarem associados com a intensidade de limitação polínica.

Atualmente, ainda não se chegou a um consenso quanto aos efeitos de densidade nos sistemas planta-polinizador, indicando a complexidade dos mecanismos pelos quais os parâmetros populacionais influenciam a interação e o sucesso reprodutivo das plantas (Aizen 1997, Bosh & Waser 2001). Alguns estudos relatam que plantas com maior número de flores, ou situadas em manchas mais ricas em recursos florais podem ser mais visitadas e obter maior sucesso reprodutivo, via produção de frutos e sementes (Feinsinger *et al.* 1991, Kunin 1993, Roll *et al.* 1997, Thompson 2001). Porém, há casos em que a presença de maior número de flores provoca redução na produção de frutos e sementes. Tal efeito pode ser resultado da redução na frequência de visitas ou alterações comportamentais no polinizador (Murawski 1987) que acarretam menor quantidade de pólen coespecífico no estigma das flores, reduzindo o sucesso reprodutivo dos indivíduos (Kunin 1993). Espécies com mecanismos de incompatibilidade ou que apresentem depressão por endocruzamento podem, além de experimentar redução na produção de frutos e sementes, ter a germinação de sementes ou vigor das plântulas reduzido, caso recebam pólen geneticamente semelhante (Schemske & Pautler 1984). Portanto, baseando-se nas predições de que a distribuição espaço-temporal dos recursos afeta a força das interações planta-polinizador e que o conhecimento acerca dos sistemas de polinização pode auxiliar na compreensão dos mecanismos que regem a promoção e manutenção da diversidade nos diferentes ecossistemas, foi estabelecida a seguinte hipótese para este projeto:

1. A interação flores-besouros em fitofisionomias de Cerrado apresentam variações locais relacionadas à riqueza e à distribuição espacial de espécies vegetais nestas áreas. Supomos que quanto maior a abundância de uma dada espécie vegetal utilizada por besouros, maior será a densidade de besouros/planta.
2. Dada a alta especificidade da interação flores-besouros, a diversidade de besouros deve aumentar linearmente com o crescimento de espécies visitadas por besouros, sendo que maior diversidade de plantas não reduz a abundância ou diversidade de besouros por planta.
3. Baseado na antiga história evolucionária entre besouros e flores, bem como em sua alta especialização esperamos que a rede de interações flores-visitantes apresente um padrão muito compartimentado no qual um ou poucas espécies de besouros ajam como um único visitante floral de uma dada espécie de planta.
4. Esperamos que em interações cantarófilas de alta especificidade todas ou pelo menos, a maioria das fases do ciclo de vida do besouro polinizador ocorra na própria espécie de planta em que é o polinizador específico resultando em uma relação de custo-benefício.

Desta forma o objetivo geral da presente tese é descrever a história natural e interação flores – besouros em espécies de Cerrado em relação à heterogeneidade espaço-temporal da distribuição de recursos entre fitofisionomias. O **capítulo 1** descreve o padrão local de distribuição de flores e besouros em quatro fitofisionomias de Cerrado da Estação Ecológica de Itirapina (EEI) e Instituto Arruda Botelho durante dois anos (Figure 1). Neste estudo caracterizamos a intensidade da interação entre flores e besouros nas comunidades em função de variações na riqueza e abundância de espécies, o que nos proporcionou uma melhor compreensão da relação entre abundância de flores e a abundância de besouros, e nos forneceu informações ecológicas imprescindíveis para os capítulos subsequentes. No **capítulo 2** caracterizamos, em nível de comunidade, os padrões de interações observados na comunidade composta por besouros associados a flores na área de cerrado estudada. Em adição, também classificamos as espécies de besouros dentre as diferentes funções de acordo com a intensidade de interação com flores. Os resultados oriundos destes dois capítulos caracterizam a interação entre flores e besouros perante a variação de recursos nas quatro diferentes fisionomias e os níveis de especificidade nesta



interação. Portanto, os dois capítulos iniciais nos ajudam a compreender, de forma geral, como a rede de interação cantarófilas está estruturada nas áreas estudadas. No **capítulo 3**, descrevemos a fenologia de floração e frutificação, bem como o sistema reprodutivo de *Duguetia furfuracea* (Annonaceae). Adicionalmente, foi feito experimento combinando suplementação de pólen e flores controle sob condições naturais e exclusão de *Plasilia* sp. (Curculionidae) para verificar se há limitação polínica ou de recursos no solo. Também foi avaliada a função deste besouro curculionídeo sobre a produção de frutos e sementes de *D. furfuracea*. Finalmente, avaliamos o custo-benefício para a interação entre *D. furfuracea* e besouros que a polinizam (porcentagem de sementes produzidas pela polinização/pela porcentagem de sementes consumidas pelas larvas dos polinizadores). Finalizando, o **capítulo 4** apresenta as considerações finais oriundos do estudo como um todo.

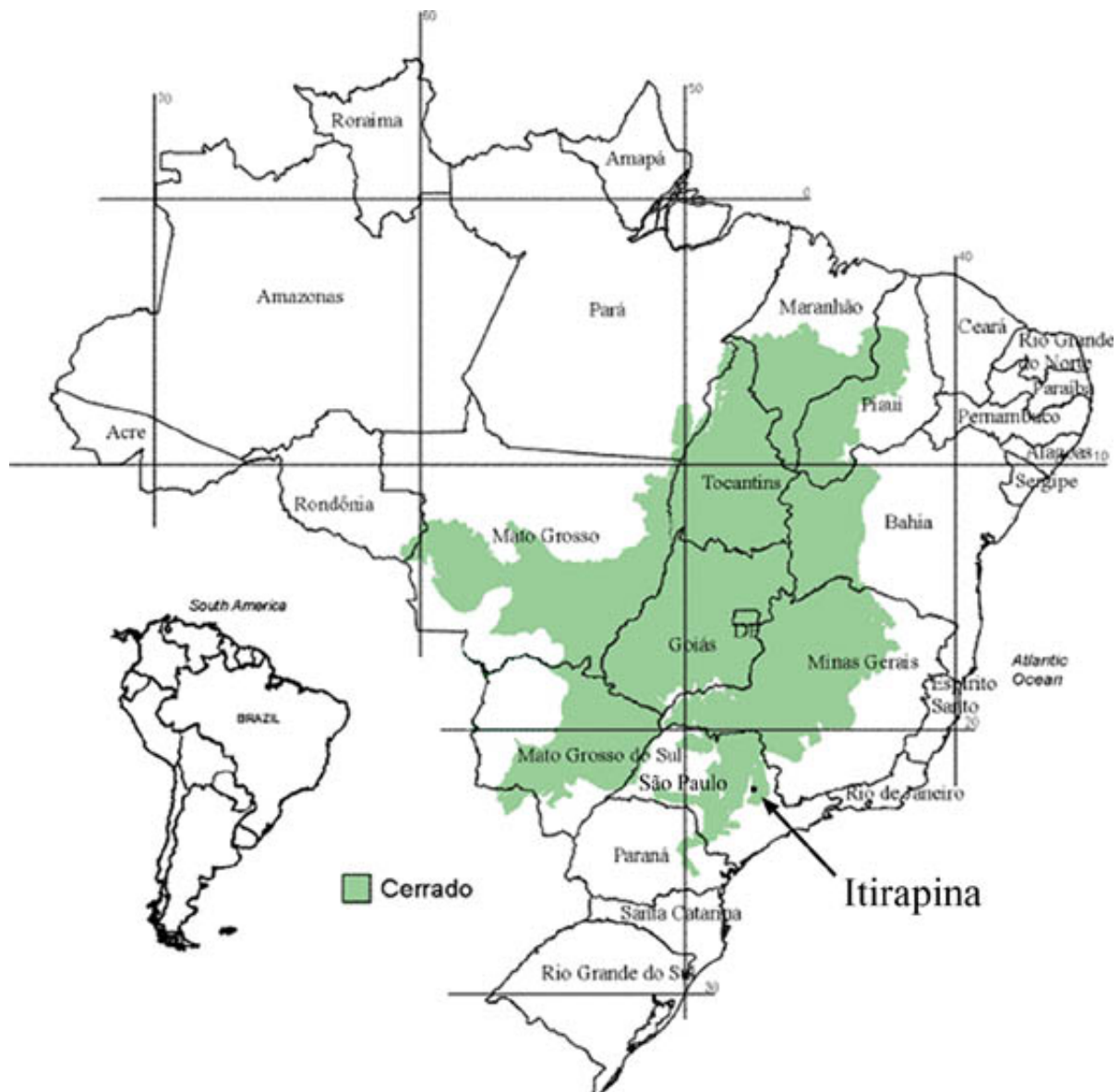


Figura 1 – Localização das áreas de estudo.

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# **CAPÍTULO 1**

**Distribution of Floral Resources and Beetles: a Small Scale Analysis  
of the Flower-Beetle Interaction within Cerrado Communities**

## ABSTRACT

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Flower visitors may respond differently to the diversity and abundance of floral resources. Beetles are the most diverse group of insects and they have an array of interaction with flowers in the tropics. This paper reports the small-scale pattern on the distribution of beetles and their floral resource in four phytophysiognomies of Cerrado during two years. Our data indicated that high number of flowering species was visited by beetles (12-40%), and most beetle species was found in one or very few plant species. As a result, a linear function describes the relationship between the diversity of plant species and beetles in Cerrado. Considering the variation within population, there is no simple relationship between flower abundance and beetles. However, there is a trend for generalist beetle species visiting flowers respond positively to variation in floral abundance while specialist beetles keep their abundance independent of their flower resource.

**Keywords:** abundance; Coleoptera; floral resources; floral visitors; spatial heterogeneity; richness; Neotropical savanna.



## RESUMO

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### **Distribuição de Recursos Florais e Besouros: uma Análise em Pequena Escala da Interação Flores-Besouros dentro de Comunidades de Cerrado**

Guildas de visitantes florais podem responder diferentemente à diversidade e abundância de recursos florais, e pouco ainda se sabe sobre a resposta de grupos de polinizadores a estas variáveis. Entre visitantes florais, a guilda composta por besouros é responsável por muitas relações com flores nos trópicos. Este artigo relata o padrão em pequena escala na distribuição de besouros que visitam flores e recursos florais disponíveis em quatro fitofisionomias de Cerrado durante dois anos. Nossos dados indicam que alto número de espécies em flor foi visitado por besouros (12-40%), e provavelmente os dados disponíveis até o momento subestimam a representação desta interação em áreas de Cerrado. No entanto, mesmo em pequenas escalas não há uma relação simples entre abundância de flores e de besouros. Dentre todas as espécies de besouros registradas, a maioria foi relacionada a uma única espécie de planta. É possível que a ocorrência de espécies generalistas seja diretamente relacionada com a abundância de flores, ao passo que besouros especialistas não apresentam relação dependente de densidade já que são capazes de localizar suas plantas hospedeiras até mesmo quando ocorrem em baixas densidades, entretanto, mais estudos são necessários para verificar esta hipótese.

***Palavras-chave:*** abundância; Coleoptera; recursos florais; visitantes florais; heterogeneidade espacial; riqueza; Cerrado.

## INTRODUCTION

Food resources are patchily distributed for most animals, regulating their feeding behavior, population dynamics, and ultimately their evolution (Bronstein 1995, Schöps 2002, Fonseca *et al.* 2005). Although plant phenological variation exists at every spatial and temporal scale, we rarely know the degree of phenological difference among patches. At present, we still know remarkably little about how floral visitors respond to spatial and temporal variation in their floral resources at local and regional scales (Bronstein 1995, Hegland & Boeke 2006, Lopes & Buzato 2007), and there is no consensus in relation to the density effects in the plant-pollinator systems, indicating complex patterns of density-dependence acting on this interaction (Rathcke, 1983, Aizen 1997, Bosh & Waser 2001). Some studies have discussed that plants with high number of flowers or located in rich floral resource patches can be more visited than the ones in low floral resource availability conditions (Feinsinger *et al.* 1991, Kunin 1993, Roll *et al.* 1997, Thompson 2001). However, there are situations in which frequency of visits are independent from density of floral resources (Bosh & Waser 2001).

Guilds of flower visitors may respond differently to diversity and abundance of floral resources, and little is still known about the general response of pollinator groups to these variables, especially at a local scale (Steffan-Dewenter & Tschardt 1999, Potts *et al.* 2003, Hegland & Boeke 2006). Among flower visitors, the guild composed by beetles is responsible for many flower-pollinator relationships in the tropics as well in temperate regions (Gottsberger 1989, Bernhardt 2000). In some tropical communities, up to one quarter of all plant species may be pollinated by beetles (Bawa 1990). They are an ancient and much diversified insect group and they interact with angiosperms since the time of their origin and early diversification (Proctor *et al.* 1996). Additionally, the interaction between beetle and floral resource provide unique opportunity to evaluate the complexity of interaction and the possibility of generalization as the tendency for plants to use a large proportion of the available beetle-visiting fauna as pollinators, or specialization as plants using a relatively small proportion of the available beetle visiting fauna as pollinators (Armbruster 2006).

Natural environments presenting high spatial heterogeneity are considered appropriate areas to study the effects of the resource variation on the occurrence of animal species (Kolasa & Rollo 1991). In this context, the Neotropical savanna – Cerrado – can be viewed as an adequate

scenario because the high spatial variation in resources and environmental conditions among its phytophysiognomies related mainly to soil fertility and ground water regimes (Oliveira-Filho & Ratter 2002, Fonseca *et al.* 2005). Additionally, in this Biome several plant species provide food and shelter for beetles (Oliveira & Gibbs 2002, Gottsberger & Silberbauer-Gottsberger 2006a).

This paper reports the small-scale pattern on the distribution of beetles that visit flowers and floral resources availability across four phytophysiognomies of Cerrado during two years. By supposing that plant richness and abundance can affect the abundance and richness of beetles through the bottom-up control (Fonseca *et al.* 2005), we expected to find great richness and abundance of beetles in areas of great floral resource availability. Additionally, as beetles have an ancient history with wood plants, we also expect to find high occurrence of beetle in the Cerrado areas, because of its high abundance of woody species.

## **MATERIAL AND METHODS**

**STUDY SYSTEM** — Beetles visited several plant species in the Cerrado. They can be found in generalist flowers or in very specialized flowers named cantharophylous very common in Annonaceae, Araceae and Arecaceae (Oliveira & Gibbs 2002, Gottsberger & Silberbauer-Gottsberger 2006a). The cantharophylous species usually show a floral chamber formed for its petals, thermogenic respiration and a precise attraction of their specific pollinator by strong odour emission (Gottsberger 1989, Endress 1994). Ancient groups of small beetles in the families Nitidulidae, Mordellidae, Chrysomelidae or Curculionidae visit several species with small flowers. On the other hand, species with large flowers are usually visited by beetles in the Scarabaeidae (Dynastinae: *Cyclocephala* spp., Gottsberger 1986, 1994). Dynastinae beetles were registered as pollinator in some species of Cyclanthaceae, Lecythidaceae, Nymphaeaceae and constitute the main pollinator in several tropical species of Araceae and Arecaceae (Gottsberger 1986). However, in Arecaceae, the most important pollinators are small nitidulids, in special, several species of *Mystryps* and curculionids (Oliveira *et al.* 2003).

THE CERRADO AND THE STUDY AREA —The Cerrado Biome is constituted of diverse vegetation physiognomies, termed cerrado *sensu lato* (Oliveira-Filho & Ratter 2002). These diverse vegetation physiognomies are associated mainly with factors such as soil fertility and drainage, seasonal precipitation and fire regime that create a mosaic represented by open grasslands (campo limpo) to dense woodlands (Cerradão) classified traditionally by a continuum of recognizable stages areas (Eiten 1972, Gottsberger & Silberbauer Gottsberger 2006b). In this study, we census four Cerrado phytophysiognomies: campo sujo, campo cerrado, cerrado *sensu stricto*, cerradão. These areas are distributed in the Itirapina Ecological Station, one of the southern reserves of Brazilian tropical savannas with 2,300 ha and a Cerradão fragment with 266 ha belongs to the Arruda Botelho Institute (22°12' - 22°10'S and 47°55' - 47°57'W, respectively, Durigan *et al.* 2002, Delgado *et al.* 2004, Figure 1). These areas are 730 a.s.l and the climate in this region is mesothermic with a marked dry season from April to September and a wet season between October and March (Figure 2) Average monthly temperatures ranges from 16 °C to 20 °C in the wet season (Figure 2).

# Itirapina Ecological Station

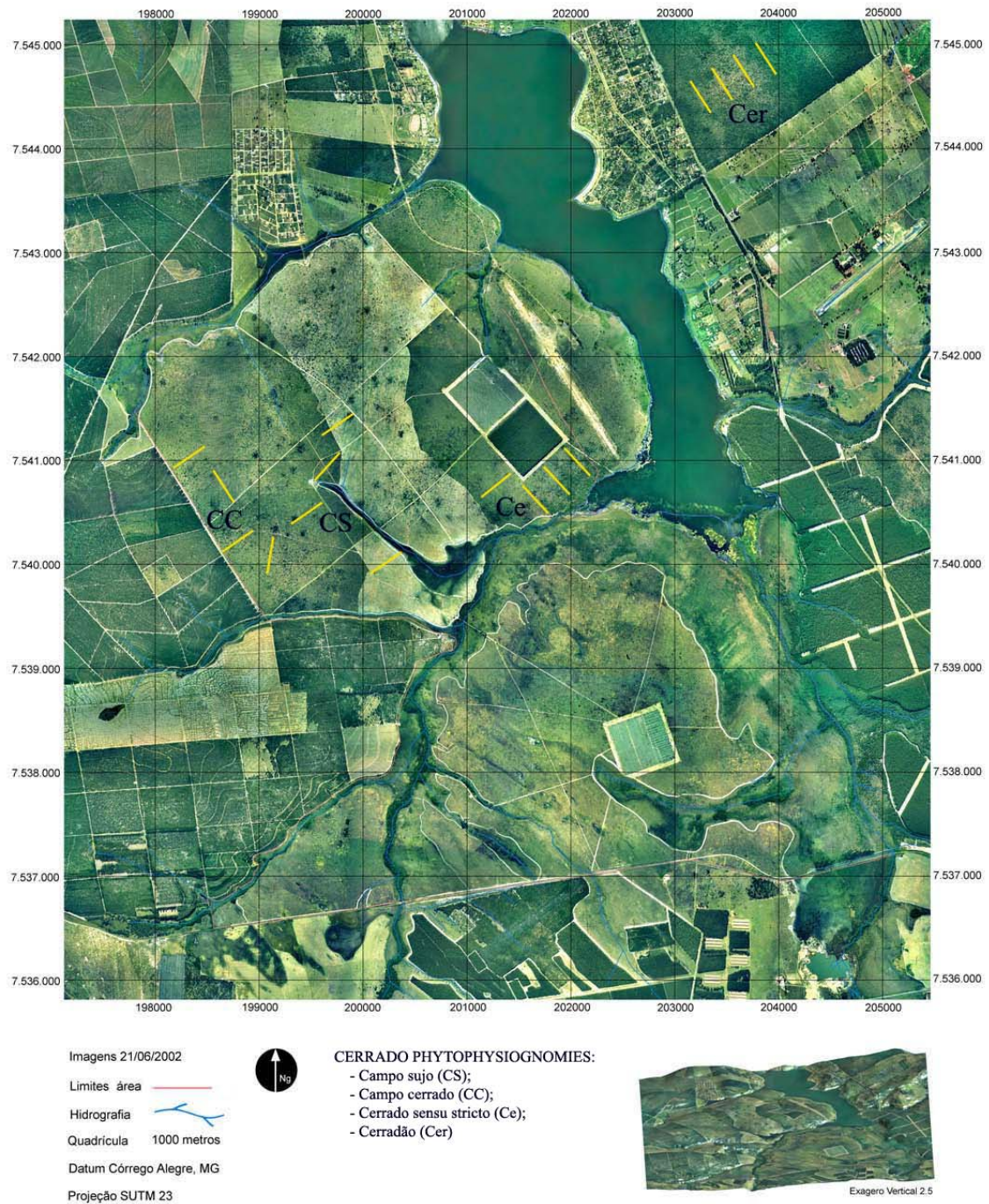


Figure 1 – Study areas: and trais of each Cerrado phytophysionomy.

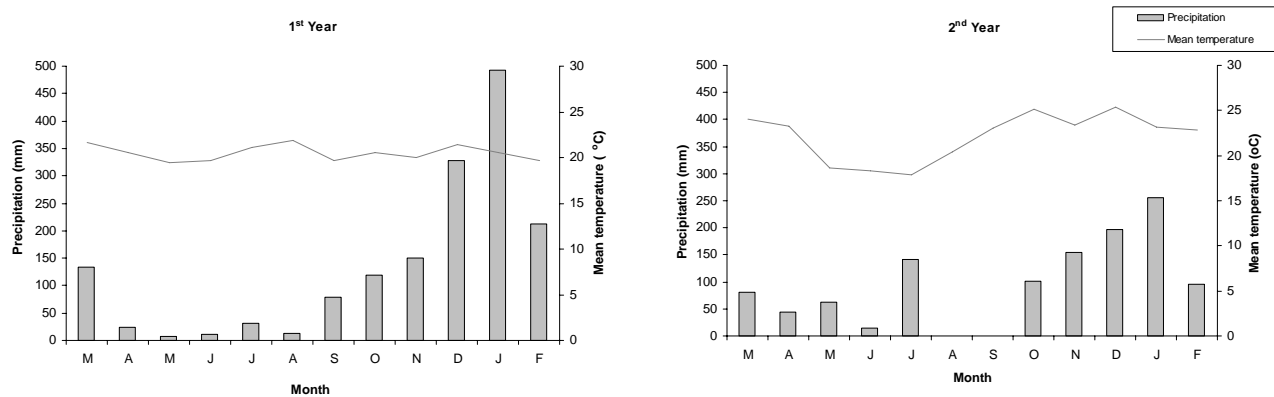


Figure 2 - Precipitation and mean temperature in the Itirapina municipality for the studied period. First year: from March 2006 to February 2007; Second year: from March 2007 to February 2008.

**SAMPLING METHODS** — To assess the relative abundance of the flowering species, we walked along four trails of 70 m, which were 8 m wide, at each phytophysionomy. Each trail was censused at intervals of two months from April 2006 to February 2008 (Figure1). In each moment, the number of flowering species and abundance of flowers per plant were registered. The occurrence of beetles on flowers was surveyed in three flowers haphazardly selected in plants within trails, by counting the number of beetles. Beetles were collected for further identification between 0700am to 0100pm. During this morning periods is possible to census beetle species also arrived during the night before.

**DATA ANALYSIS** — Curves of rank-abundance, ordering species by its relative frequency of floral resource abundance were used to analyze variation in composition and floral resource availability in Cerrado (Feinsinger 2001). The same analysis was performed for beetle abundance per species, and beetle species were classified in specialist if it was registered visiting flowers in only one plant species. To examine the relation between number of beetles and flower abundance per trails linear regression was applied (Sokal & Rohlf 1995). We compared floral resource availability and beetle abundance between years and phytophysionomies using a two-way ANOVA for ranked data, named the Scheirer-Ray-Hare extension of the Kruskal-Wallis test (Sokal & Rohlf, 1995). Phytophysionomies and year were random effects in the models and trails were the sampling units.

## RESULTS

A high proportion of plant species had their flowers visited by beetles in all Cerrado phytophysiognomies (Table 1). In areas with more woody plants, we recorded high proportion of plant species visited by beetles in both years (Cerrado *s. s.* and Cerradão, Table 1 and Figure 3). The number of beetle species varied among phytophysiognomies, and the high number of species occurred in areas of Campo cerrado and Cerrado *s.s.* (Table 1, Figure 4).

Table 1 - Proportion of plant species visited by beetles, richness and abundance of plant and beetle in four Cerrado phytophysiognomies, Campo sujo (CS), Campo cerrado (CC), Cerrado *sensu stricto* (Ce), Cerradão (Cer), at Itirapina from April 2006 to February 2007 (first year) and from April 2007 to February 2008 (second year).

	First year				Second year			
	CS	CC	Ce	Cer	CS	CC	Ce	Cer
Plant species visited (%)	29.6	25.0	33.3	40.0	12.5	18.2	23.7	26.5
Plant richness	27	32	27	25	40	44	38	34
Flower abundance	79098	9553	6925	18982	27244	8501	44656	7190
Beetle richness	21	36	24	11	5	22	25	8
Beetle abundance	2800	6535	2708	49	79	2985	891	11

There was low evenness in floral abundance among plant species visited by beetle in all phytophysiognomies (Figure 3). For example, the flower abundance of *Myrcia lingua* was higher than *Annona coriacea* in cerradão areas (Figure 3, Appendix 1a, b). Except for cerradão, the same pattern emerged for beetle abundance in the first year (Figure 4, Appendix 2 a, b). There was difference among phytophysiognomies in flower abundance ( $H_{3,268} = 8.48$ ,  $P < 0.05$ , Figure 5A), but we did not find difference in flower abundance between years ( $H_{1,268} = 1.99$ ,  $P > 0.05$ ). There was difference among phytophysiognomies in beetle abundance ( $H_{3,166} = 22.71$ ,  $P < 0.05$ , Figure 5B), but we did not find difference in beetle abundance between years ( $H_{1,166} = 0.59$ ,  $P > 0.05$ ).

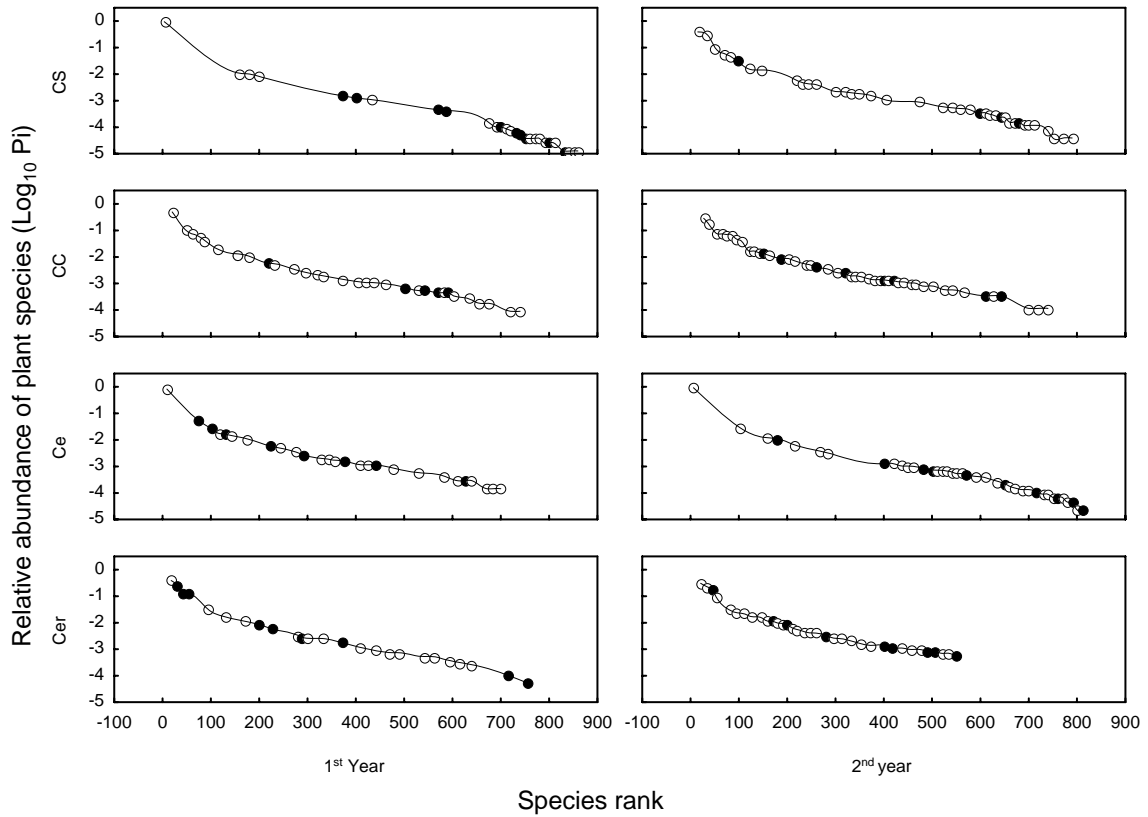


Figure 3 - Plant ranked by their relative abundance of flowers ( $\text{Log}_{10} \text{Pi}$ ) for the first and second years. Black ( $\bullet$ ) circles represent plant species visited by beetles. Plant species identification as indicated by sequence of relative abundance in Appendix 1a, b.



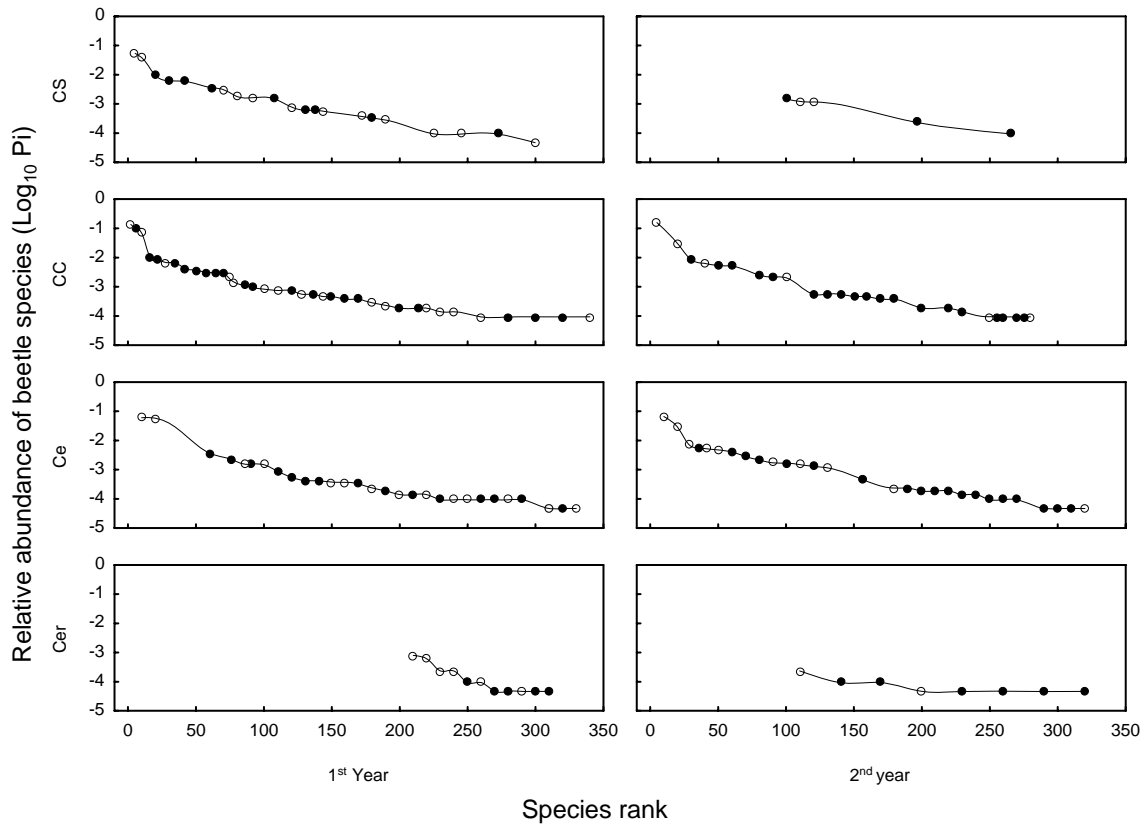


Figure 4 - Floral visitors ranked by their relative abundance on flower ( $\text{Log}_{10} \text{Pi}$ ) for the first and second years. Black circles represent beetle species that visit only one plant species. Beetle species identification as indicated by sequence of relative abundance in Appendix 2a, b.

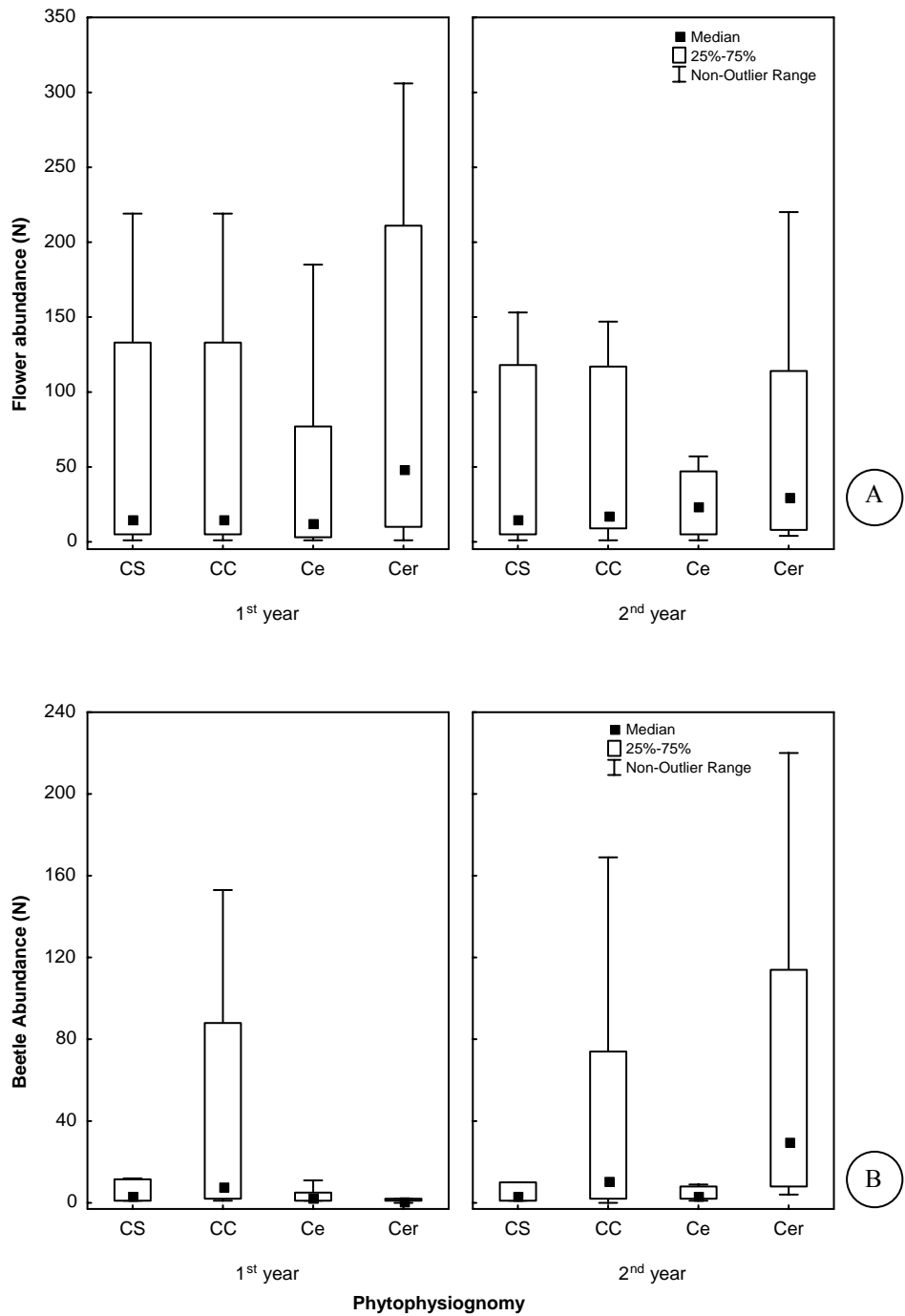


Figure 5 - Floral resource and beetle abundance in four Cerrado phytophysiognomies, Campo sujo (CS), Campo cerrado (CC), Cerrado *sensu stricto* (Ce), Cerradão (Cer), at Itirapina from April 2006 to February 2007 (first year) and from April 2007 to February 2008 (second year).

Except for the campo sujo in second year and cerradão in first year, there was no relation between beetle and flower abundance for studied phytophysiognomies (Campo sujo first year:  $r = -0.32$ ,  $p = 0.40$ , second year:  $r = -0.89$ ,  $p = 0.006$ ; Campo cerrado first year:  $r = 0.26$ ,  $p = 0.34$ , second year:  $r = 0.22$ ,  $p = 0.42$ ; Cerrado *s.s* first year:  $r = 0.25$ ,  $p = 0.59$ , second year:  $r = 0.09$ ,  $p = 0.78$ ; Cerradão first year:  $r = 0.74$ ,  $p = 0.03$ , second year:  $r = 0.38$ ,  $p = 0.30$ ; Figure 6).

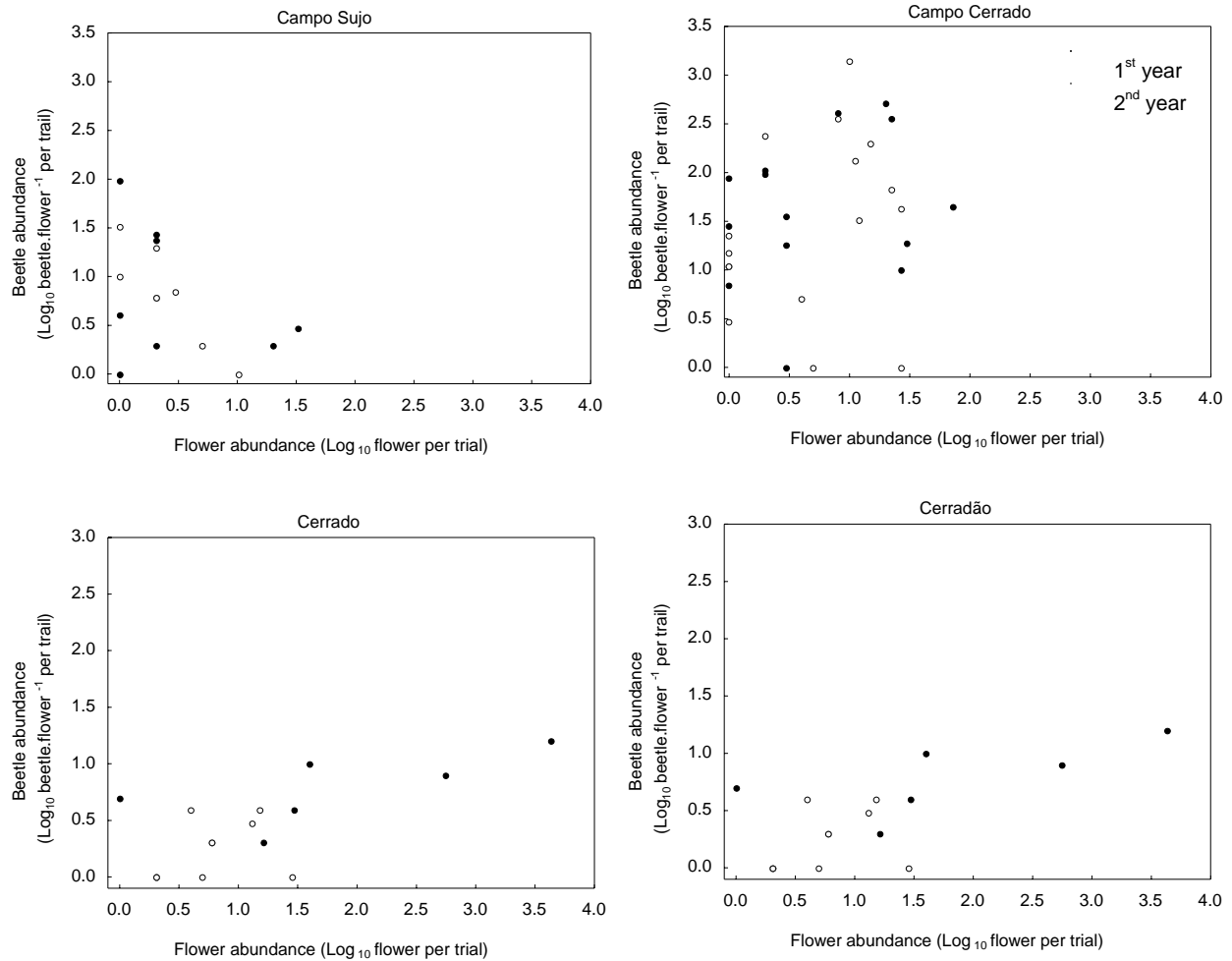


Figure 6 - Relation between beetle and flower abundance in four Cerrado phytophysiognomies, Campo sujo (CS), Campo cerrado (CC), Cerrado *sensu stricto* (Ce), Cerradão (Cer), at Itirapina from April 2006 to February 2007 (first year- black dots) and from April 2007 to February 2008 (second year- white dots).

## DISCUSSION

Our data indicated that beetles visited high number of flowering species, and probably the data available up to now underestimate the representation of this interaction in Cerrado areas. For example, Oliveira and Gibbs (2002) indicated that beetle-flower interaction occurred only in 6% of woody plants in Cerrado. Probably, the underestimation of this interaction might be related to the register of conspicuous interaction such as that occurred between beetle and species in the Annonaceae (Gottsberger 1986, Gottsberger & Silberbauer Gottsberger 2006a, Paulino Neto 1999). This work represents the first study focusing in the entire beetle community associated to flowers.

As described in other papers (Bosh & Waser 2001), even in a small scale there is no simple relationship between flower abundance and beetles. Although beetles can respond to changes in “landscapes” (Wiens & Milne 1989). Additionally, the absence of some beetle species even in the presence of high abundance of floral resources indicated that other factors than floral abundance is determining the occurrence of beetles in the studied areas.

Except for the Cerrado, all phytophysiognomies had a deep reduction in flower abundance, and in the second year, the number of species visited by beetles decreased. Although statistical analysis did not indicated differences between years, we considered that precipitation might be affected the flowering and beetle abundance. Although the open phytophysiognomies as campo sujo and campo cerrado present the higher abundance of flowers and plant richness in the study period, the biggest proportion of visits by beetles were recorded in the cerrado and cerradão in the both years. Apparently, this result is related to high abundance of woody species in these areas (Oliveira-Filho & Ratter 2002). Up to now, almost all flower-beetle interaction was registered for woody species related to ancient families such as that found in the Magnoliidae (Gottsberger 1988, 1989).

From all beetle species recorded, most of them were related to only one plant species. This result indicated that other pattern than that mentioned as generalization for the interaction between flowers and floral visitor can emerge in tropical areas (Waser et al 1996). The occurrence of generalist beetle species visiting flowers might be directly related with abundance of floral resources, but specialist beetles might find their host plants even in the presence of few floral resources. More studies are necessary to verify this hypothesis.

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Appendix 1a - Floristic composition, variation on flower abundance and life habit of plant species in four phytophysiognomies of cerrado in Itirapina-SP during 04.2006 to 02-2007.

Areas	Nº	Plant species	Habit
Campo Sujo	1	<i>Lippia corymbosa</i>	herb
	2	<i>Jacaranda decurrens</i>	wood
	3	<i>Pouteria torta</i>	wood
	4	<i>Piptocarpha rotundifolia</i>	herb
	5	<i>Byrsonima coccolobifolia</i>	wood
	6	<i>Banisteriopsis stellaris</i>	wood
	7	<i>Byrsonima intermedia</i>	wood
	8	<i>Banisteriopsis campestris</i>	wood
	9	<i>Diospyros hispida</i>	wood
	10	<i>Senna rugosa</i>	wood
	11	<i>Lippia lupulina</i>	herb
	12	<i>Syagrus petrea</i>	wood
	13	<i>Eugenia pyriformis</i>	wood
	14	<i>Byrsonima verbascifolia</i>	wood
	15	<i>Duguetia furfuracea</i>	wood
	16	<i>Annona dioica</i>	wood
	17	<i>Attalea geraensis</i>	wood
	18	<i>Leandra lacunosa</i>	wood
	19	<i>Memora axillaris</i>	herb
	20	<i>Ouratea spectabilis</i>	wood
	21	<i>Davilla rugosa</i>	wood
	22	<i>Eriotheca gracilipes</i>	wood
	23	<i>Jacaranda caroba</i>	wood
	24	<i>Annona crassiflora</i>	wood
	25	<i>Caryocar brasiliensis</i>	wood
	26	<i>Couepia grandiflora</i>	wood
	27	<i>Evolvulus sp.</i>	herb
Campo Cerrado	1	<i>Pouteria torta</i>	wood
	2	<i>Aegiphila lhotzchiana</i>	wood
	3	<i>Banisteriopsis campestris</i>	wood
	4	<i>Styrax ferrugineus</i>	wood
	5	<i>Jacaranda decurrens</i>	wood
	6	<i>Banisteriopsis stellaris</i>	wood
	7	<i>Diospyros hispida</i>	wood
	8	<i>Byrsonima coccolobifolia</i>	wood
	9	<i>Duguetia furfuracea</i>	wood
	10	<i>Kielmeyera coriacea</i>	wood
	11	<i>Byrsonima intermedia</i>	wood
	12	<i>Bidens brasiliensis</i>	herb
	13	<i>Annona crassiflora</i>	wood
	14	<i>Caryocar brasiliensis</i>	wood
	15	<i>Alibertia myrciifolia</i>	wood
	16	<i>Ouratea spectabilis</i>	wood
	17	<i>Lippia lupulina</i>	herb
	18	<i>Chamaecrista desvauxii</i>	herb
	19	<i>Serjania sp.</i>	herb
	20	<i>Palicourea rigida</i>	wood
	21	<i>Campomanesia pubescens</i>	wood
	22	<i>Byrsonima verbascifolia</i>	wood
	23	<i>Syagrus petrea</i>	wood
	24	<i>Attalea geraensis</i>	wood
	25	<i>Hypenia paulina</i>	herb
	26	<i>Memora axillaris</i>	herb
	27	<i>Byrsonima pachyphilla</i>	wood



	28	<i>Chromolaena pungens</i>	herb
	29	<i>Eugenia aurata</i>	wood
	30	<i>Peltaea polymorpha</i>	herb
	31	<i>Jacaranda caroba</i>	wood
	32	<i>Jacaranda rufa</i>	wood
Cerrado <i>sensu stricto</i>	1	<i>Pouteria torta</i>	wood
	2	<i>Myrcia lingua</i>	wood
	3	<i>Xylopia aromatica</i>	wood
	4	<i>Trichogonia salveaeifolia</i>	herb
	5	<i>Diospyros hispida</i>	wood
	6	<i>Styrax ferrugineus</i>	wood
	7	<i>Eriotheca gracilipes</i>	wood
	8	<i>Erythroxylum suberosum</i>	wood
	9	<i>Bromelia balansae</i>	herb
	10	<i>Ouratea spectabilis</i>	wood
	11	<i>Jacaranda caroba</i>	wood
	12	<i>Bauhinia rufa</i>	wood
	13	<i>Banisteriopsis campestris</i>	wood
	14	<i>Miconia albicans</i>	wood
	15	<i>Kielmeyera coriacea</i>	wood
	16	<i>Campomanesia pubescens</i>	wood
	17	<i>Hypenia paulina</i>	herb
	18	<i>Tocoyena brasiliensis</i>	wood
	19	<i>Cissus erosa</i>	herb
	20	<i>Byrsonima pachyphylla</i>	wood
	21	<i>Cybistax antisiphylla</i>	wood
	22	<i>Annona crassiflora</i>	wood
	23	<i>Attalea geraensis</i>	wood
	24	<i>Ipomea sp.</i>	herb
	25	<i>Centrosema virginianum</i>	herb
	26	<i>Memora axillaris</i>	herb
	27	<i>Temnadenia violacea</i>	wood
Cerradão	1	<i>Pouteria torta</i>	wood
	2	<i>Myrcia lingua</i>	wood
	3	<i>Miconia albicans</i>	wood
	4	<i>Anadenanthera falcata</i>	wood
	5	<i>Miconia stenostachia</i>	wood
	6	<i>Erythroxylum pelleterianum</i>	wood
	7	<i>Byrsonima pachyphylla</i>	wood
	8	<i>Tocoyena formosa</i>	wood
	9	<i>Byrsonima intermedia</i>	wood
	10	<i>Palicourea rigida</i>	wood
	11	<i>Xylopia aromatica</i>	wood
	12	<i>Erythroxylum suberosum</i>	wood
	13	<i>Acosmium dasycarpum</i>	wood
	14	<i>Diospyros hispida</i>	wood
	15	<i>Leandra lacunosa</i>	wood
	16	<i>Vochysia cinnamomea</i>	wood
	17	<i>Calea cuneifolia</i>	herb
	18	<i>Pyrostegia venusta</i>	herb
	19	<i>Eriotheca gracilipes</i>	wood
	20	<i>Galeandra sp.</i>	herb
	21	<i>Chamaecrista desvauxii</i>	wood
	22	<i>Jacaranda caroba</i>	wood
	23	<i>Memora axillaris</i>	herb
	24	<i>Temnadenia violacea</i>	herb
	25	<i>Annona coriacea</i>	wood

Appendix 1b - Floristic composition, variation on flower abundance and life habit of plant species in four phytophysiognomies of cerrado in Itirapina-SP during 04.2007 to 02-2008.

Areas	Nº	Plant species	Nº
Campo Sujo	1	<i>Piptocarpha rotundifolia</i>	1
	2	<i>Lippia corymbosa</i>	2
	3	Euphorbiaceae sp.	3
	4	<i>Piptocarpha rotundifolia</i>	4
	5	<i>Chromolaena pungens</i>	5
	6	<i>Byrsonima intermedia</i>	6
	7	<i>Banisteriopsis stellaris</i>	7
	8	<i>Byrsonima cocolobifolia</i>	8
	9	<i>Jacaranda decurrens</i>	9
	10	<i>Banisteriopsis campestris</i>	10
	11	<i>Campomonesia pubescens</i>	11
	12	<i>Aspidosperma tomentosum</i>	12
	13	<i>Ouratea spectabilis</i>	13
	14	<i>Pouteria torta</i>	14
	15	<i>Alibertia myrciifolia</i>	15
	16	<i>Centrosema virginianum</i>	16
	17	<i>Bauhinia rufa</i>	17
	18	<i>Hypenia paulina</i>	18
	19	<i>Bidens brasiliensis</i>	19
	20	<i>Chamaecrista desvauxii</i>	20
	21	<i>Lippia lupulina</i>	21
	22	<i>Diospyros hispida</i>	22
	23	<i>Memora axillaris</i>	23
	24	<i>Annona dioica</i>	24
	25	<i>Senna rugosa</i>	25
	26	<i>Caryocar brasilienses</i>	26
	27	<i>Jacaranda caroba</i>	27
	28	<i>Annona crassiflora</i>	28
	29	<i>Erythroxylum suberosum</i>	29
	30	<i>Byrsonima pachyphilla</i>	30
	31	<i>Jacaranda rufa</i>	31
	32	<i>Syagrus petrea</i>	32
	33	<i>Mandevilla ilustris</i>	33
	34	<i>Turnera hilaireana</i>	34
	35	<i>Solanum lycocarpum</i>	35
	36	<i>Palicourea rigida</i>	36
	37	<i>Byrsonima verbascifolia</i>	37
	38	<i>Gomphrena macrocephala</i>	38
Campo Cerrado	1	<i>Ipomoea</i> sp.	1
	2	<i>Styrax ferrugineus</i>	2
	3	<i>Serjania</i> sp.	3
	4	<i>Chromolaena pungens</i>	4
	5	<i>Piptocarpha rotundifolia</i>	5
	6	<i>Pouteria torta</i>	6
	7	<i>Mikania</i> sp.	7
	8	<i>Byrsonima cocolobifolia</i>	8
	9	<i>Byrsonima intermedia</i>	9
	10	<i>Gochnatia pulchra</i>	10
	11	<i>Peritassa campestris</i>	11
	12	<i>Baccharis</i> sp.	12
	13	<i>Senna rugosa</i>	13
	14	<i>Banisteriopsis campestris</i>	14
	15	<i>Diospyros hispida</i>	15
	16	<i>Jacaranda decurrens</i>	16
	17	<i>Talisia angustifolia</i>	17

	18	<i>Solanum lycocarpum</i>	18
	19	<i>Alibertia myrciifolia</i>	19
	20	<i>Duguetia furfuracea</i>	20
	21	<i>Lippia lupulina</i>	21
	22	<i>Erythroxylum suberosum</i>	22
	23	<i>Annona crassiflora</i>	23
	24	<i>Chamaecrista desvauxii</i>	24
	25	<i>Caryocar brasiliense</i>	25
	26	<i>Banisteriopsis stellaris</i>	26
	27	<i>Centrosema virginianum</i>	27
	28	<i>Miconia stenostachia</i>	28
	29	<i>Stryphnodendron obovatum</i>	29
	30	<i>Syagrus petrea</i>	30
	31	<i>Bauhinia rufa</i>	31
	32	<i>Memora axillaris</i>	32
	33	<i>Bidens brasiliensis</i>	33
	34	<i>Jacaranda caroba</i>	34
	35	<i>Mandevilla illustris</i>	35
	36	<i>Psidium cinereum</i>	36
	37	<i>Cissus sp.</i>	37
	38	<i>Eupatorium sp.</i>	38
	39	<i>Vernonia sp.</i>	39
	40	<i>Attalea geraensis</i>	40
	41	<i>Hypenia pauliana</i>	41
	42	<i>Psidium cinereum</i>	42
	43	<i>Anemopaegma arvense</i>	43
	44	<i>Ipomea sp.</i>	44
	45	<i>Gomphrena macrocephala</i>	45
Cerrado	1	<i>Rapanea ferruginea</i>	1
	2	<i>Campomanesia pubescens</i>	2
	3	<i>Pouteria torta</i>	3
	4	<i>Xylopia aromatica</i>	4
	5	<i>Chromolaena pungens</i>	5
	6	<i>Byrsonima intermedia</i>	6
	7	<i>Miconia albicans</i>	7
	8	<i>Tocoyena brasiliensis</i>	8
	9	<i>Trichogonia salveaefolia</i>	9
	10	<i>Eupatorium sp.</i>	10
	11	<i>Jacaranda caroba</i>	11
	12	<i>Eriotheca gracilipes</i>	12
	13	<i>Diospyros hispida</i>	13
	14	<i>Kielmeyera coriacea</i>	14
	15	<i>Bauhinia rufa</i>	15
	16	<i>Palicourea rigida</i>	16
	17	<i>Miconia stenostachia</i>	17
	18	<i>Miconia sp.</i>	18
	19	<i>Myrcia lingua</i>	19
	20	<i>Zeyhera montana</i>	20
	21	<i>Styrax ferrugineus</i>	21
	22	<i>Byrsonima pachyphylla</i>	22
	23	<i>Banisteriopsis campestris</i>	23
	24	<i>Senna rugosa</i>	24
	25	<i>Attalea geraensis</i>	25
	26	<i>Hypenia pauliana</i>	26
	27	<i>Lippia lupulina</i>	27
	28	<i>Annona crassiflora</i>	28
	29	<i>Byrsonima coccolobifolia</i>	29
	30	<i>Psidium cinereum</i>	30
	31	<i>Caryocar brasiliense</i>	31

	32	<i>Vernonia</i> sp.	32
	33	<i>Cybistax antisiphylla</i>	33
	34	<i>Duguetia furfuracea</i>	34
	35	<i>Memora axillaris</i>	35
	36	<i>Cochlospermum regium</i>	36
	37	<i>Psidium cinereum</i>	37
	38	<i>Annona coriacea</i>	38
	39	<i>Syagrus petrea</i>	39
Cerradão	1	<i>Myrcia lingua</i>	1
	2	<i>Qualea grandiflora</i>	2
	3	<i>Talisia angustifolia</i>	3
	4	<i>Miconia albicans</i>	4
	5	<i>Pouteria torta</i>	5
	6	<i>Chromolaena pungens</i>	6
	7	<i>Xylopia aromatica</i>	7
	8	<i>Byrsonima campestris</i>	8
	9	<i>Byrsonima intermedia</i>	9
	10	<i>Styrax ferrugineus</i>	10
	11	<i>Desmoscelis villosa</i>	11
	12	<i>Anadenanthera falcata</i>	12
	13	<i>Psychotria barbiflora</i>	13
	14	<i>Tocoyena formosa</i>	14
	15	<i>Palicourea rigida</i>	15
	16	<i>Jacaranda caroba</i>	16
	17	<i>Byrsonima pachyphylla</i>	17
	18	<i>Erythroxylum suberosum</i>	18
	19	<i>Pyrostegia venusta</i>	19
	20	<i>Memora axillaris</i>	20
	21	<i>Erythroxylum pelleterianum</i>	21
	22	<i>Ananas ananassoides</i>	22
	23	<i>Alibertia myrciifolia</i>	23
	24	<i>Lippia lupulina</i>	24
	25	<i>Baccharis</i> sp.	25
	26	<i>Campomanesia pubescens</i>	26
	27	<i>Byrsonima coccolobifolia</i>	27
	28	<i>Vernonia</i> sp.	28
	29	<i>Leandra lacunosa</i>	29
	30	<i>Tabebuia</i> sp.	30
	31	<i>Eugenia pyriformis</i>	31
	32	<i>Miconia stenostachia</i>	32
	33	<i>Calea cuneifolia</i>	33
	34	<i>Psidium cinereum</i>	34
	35	<i>Kielmeyera coriacea</i>	35

Appendix 2a –Beetle species composition, variation on beetle abundance and your ecological interaction with host plants in four phytophysiognomies of cerrado in Itirapina-SP during 04.2006 to 02-2007.

Areas	No	Beetle species
Campo Sujo	1	<i>Palmocentrinus</i> cf. <i>punctatus</i>
	2	<i>Bitoma palmarum</i>
	3	Coccinellidae sp.1
	4	<i>Telemus</i> sp.
	5	<i>Mystrops</i> sp.4
	6	<i>Imatidium</i> sp.1
	7	<i>Parisoschoenus plagiatus</i>
	8	<i>Dialomia polyphaga</i>
	9	<i>Anchylolorhynchus</i> cf. <i>camposi</i>
	10	Scaphidiinae sp.1
	11	Staphilinidae sp.1
	12	<i>Celestes</i> sp.1
	13	<i>Colopterus</i> sp.1
	14	Nitidulidae sp.2
	15	<i>Lobiopa</i> sp.1
	16	Nitidulidae sp.4
	17	<i>Celestes bipunctatus</i>
	18	<i>Celestes</i> sp.2
	19	<i>Celestes</i> sp.3
	20	<i>Mystrops</i> sp.3
	21	<i>Mystrops</i> sp.1
Campo Cerrado	1	Chrysomelidae sp.1
	2	Galerucinae sp.2
	3	Galerucinae sp.3
	4	Galerucinae sp.4
	5	Tenebrionidae sp.1
	6	<i>Mystrops</i> sp.4
	7	<i>Parisoschoenus plagiatus</i>
	8	<i>Macroductylus</i> sp.1
	9	<i>Mystrops</i> sp.6
	10	Nitidulidae sp.1
	11	<i>Palmocentrinus</i> cf. <i>punctatus</i>
	12	<i>Telemus</i> sp.
	13	<i>Bitoma palmarum</i>
	14	<i>Celestes</i> sp.1
	15	Scaphidiinae sp.1
	16	Scarabaeidae sp.1
	17	<i>Anchylolorhynchus</i> cf. <i>camposi</i>
	18	Scaphidiinae sp.2
	19	<i>Colopterus</i> sp.4
	20	<i>Dialomia polyphaga</i>
	21	<i>Colopterus</i> sp.1
	22	<i>Colopterus</i> sp.5
	23	Nitidulidae sp.6
	24	Staphilinidae sp.1
	25	Nitidulidae sp.5
	26	<i>Lobiopa</i> sp.2
	27	<i>Celestes</i> sp.3
	28	<i>Celestes bipunctatus</i>
	29	<i>Mystrops</i> sp.5
	30	<i>Plasilia</i> sp.
	31	<i>Celestes</i> sp.2

	32	Nitidulidae sp.2
	33	<i>Colopterus</i> sp.2
	34	Nitidulidae sp.4
	35	<i>Mystrops</i> sp.3
	36	<i>Colopterus</i> sp.3
	37	<i>Mystrops</i> sp.1
Cerrado <i>sensu stricto</i>	1	Chrysomelidae sp.1
	2	<i>Conotrachelus</i> sp.1
	3	Galerucinae sp.1
	4	<i>Parisoschoenus plagiatus</i>
	5	Baridini sp.1
	6	Chrysomelidae sp.4
	7	Galerucinae sp.3
	8	Galerucinae sp.5
	9	Galerucinae sp.7
	10	Nitidulidae sp.3
	11	<i>Revena rubiginosa</i>
	12	<i>Colopterus</i> sp.1
	13	Scaphidiinae sp.1
	14	Staphilinidae sp.1
	15	<i>Celestes</i> sp.1
	16	<i>Dialomia polyphaga</i>
	17	Galerucinae sp.2
	18	Nitidulidae sp.2
	19	Nitidulidae sp.5
	20	<i>Celestes</i> sp.3
	21	Chrysomelidae sp.6
	22	Nitidulidae sp.4
	23	Galerucinae sp.4
	24	Chrysomelidae sp.1
	25	<i>Cillaeus</i> sp.1
	26	Cucujoidae sp.1
	27	<i>Celestes bipunctatus</i>
	28	<i>Celestes</i> sp.2
	29	<i>Mystrops</i> sp.1
	30	<i>Mystrops</i> sp.3
Cerradão	1	Chrysomelidae sp.8
	2	<i>Cillaeus</i> sp.1
	3	Cucujoidae sp.1
	4	Galerucinae sp.6
	5	Scarabaeidae sp.2
	6	Chrysomelidae sp.4
	7	Chrysomelidae sp.5
	8	<i>Lobiopa</i> sp.1
	9	Nitidulidae sp.5
	10	Galerucinae sp.7
	11	Nitidulidae sp.3

Note: Were considered as generalists those beetle species that use a large proportion of the available plant species, and as specialists the beetle species that visited a relatively small proportion of the available plants.

Appendix 2b –Beetle species composition, variation on beetle abundance and your ecological interaction with host plants in four phytophysiognomies of cerrado in Itirapina-SP during 04.2007 to 02-2008.

Areas	No	Beetle species
Campo Sujo	1	<i>Palmocentrinus cf. punctatus</i>
	2	<i>Bitoma palmarum</i>
	3	Coccinellidae sp.1
	4	<i>Telemus sp.</i>
	5	<i>Mystrops sp.4</i>
	6	<i>Imatidium sp.1</i>
	7	<i>Parisoschoenus plagiatus</i>
	8	<i>Dialomia polyphaga</i>
	9	<i>Anchylolorhynchus cf. camposi</i>
	10	Scaphidiinae sp.1
	11	Staphilinidae sp.1
	12	<i>Celestes sp.1</i>
	13	<i>Colopterus sp.1</i>
	14	Nitidulidae sp.2
	15	<i>Lobiopa sp.1</i>
	16	Nitidulidae sp.4
	17	<i>Celestes bipunctatus</i>
	18	<i>Celestes sp.2</i>
	19	<i>Celestes sp.3</i>
	20	<i>Mystrops sp.3</i>
	21	<i>Mystrops sp.1</i>
Campo Cerrado	1	Chrysomelidae sp.1
	2	Galerucinae sp.2
	3	Galerucinae sp.3
	4	Galerucinae sp.4
	5	Tenebrionidae sp.1
	6	<i>Mystrops sp.4</i>
	7	<i>Parisoschoenus plagiatus</i>
	8	<i>Macroductylus sp.1</i>
	9	<i>Mystrops sp.6</i>
	10	Nitidulidae sp.1
	11	<i>Palmocentrinus cf. punctatus</i>
	12	<i>Telemus sp.</i>
	13	<i>Bitoma palmarum</i>
	14	<i>Celestes sp.1</i>
	15	Scaphidiinae sp.1
	16	Scarabaeidae sp.1
	17	<i>Anchylolorhynchus cf. camposi</i>
	18	Scaphidiinae sp.2
	19	<i>Colopterus sp.4</i>
	20	<i>Dialomia polyphaga</i>
	21	<i>Colopterus sp.1</i>
	22	<i>Colopterus sp.5</i>
	23	Nitidulidae sp.6
	24	Staphilinidae sp.1
	25	Nitidulidae sp.5
	26	<i>Lobiopa sp.2</i>
	27	<i>Celestes sp.3</i>
	28	<i>Celestes bipunctatus</i>
	29	<i>Mystrops sp.5</i>
	30	<i>Plasilia sp.</i>
	31	<i>Celestes sp.2</i>
	32	Nitidulidae sp.2

	33	<i>Colopterus</i> sp.2
	34	Nitidulidae sp.4
	35	<i>Mystrops</i> sp.3
	36	<i>Colopterus</i> sp.3
	37	<i>Mystrops</i> sp.1
<b>Cerrado sensu stricto</b>	1	Chrysomelidae sp.1
	2	<i>Conotrachelus</i> sp.1
	3	Galerucinae sp.1
	4	<i>Parisoschoenus plagiatus</i>
	5	<i>Baridini</i> sp.1
	6	Chrysomelidae sp.4
	7	Galerucinae sp.3
	8	Galerucinae sp.5
	9	Galerucinae sp.7
	10	Nitidulidae sp.3
	11	<i>Revena rubiginosa</i>
	12	<i>Colopterus</i> sp.1
	13	Scaphidiinae sp.1
	14	Staphilinidae sp.1
	15	<i>Celestes</i> sp.1
	16	<i>Dialomia polyphaga</i>
	17	Galerucinae sp.2
	18	Nitidulidae sp.2
	19	Nitidulidae sp.5
	20	<i>Celestes</i> sp.3
	21	Chrysomelidae sp.6
	22	Nitidulidae sp.4
	23	Galerucinae sp.4
	24	Chrysomelidae sp.1
	25	<i>Cillaeus</i> sp.1
	26	Cucujoidae sp.1
	27	<i>Celetes bipunctatus</i>
	28	<i>Celestes</i> sp.2
	29	<i>Mystrops</i> sp.1
	30	<i>Mystrops</i> sp.3
<b>Cerradão</b>	1	Chrysomelidae sp.8
	2	<i>Cillaeus</i> sp.1
	3	Cucujoidae sp.1
	4	Galerucinae sp.6
	5	Scarabaeidae sp.2
	6	Chrysomelidae sp.4
	7	Chrysomelidae sp.5
	8	<i>Lobiopa</i> sp.1
	9	Nitidulidae sp.5
	10	Galerucinae sp.7
	11	Nitidulidae sp.3

Note: Were considered as generalists those beetle species that use a large proportion of the available plant species, and as specialists the beetle species that visited a relatively small proportion of the available plants.



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## **CAPÍTULO 2**

**A tight plant-animal interaction: Beetle-flower networks**

## **ABSTRACT**

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Plant- animal interactions, such as pollination, are a key ecological processes in many terrestrial communities. The network approach has been useful for investigate the structure and fragility of ecological interactions, and comparative studies of network structure help to uncover community-level patterns of ecological specialization in different types of interspecific interactions. The present study investigate for the first time the community-level patterns of interactions observed in a community composed by beetles associated to flowers in southeastern Brazilian Cerrado. Our goals are to characterize the structure of the beetle-flower community. Based on the ancient evolutionary relationship between flowers and beetles we expected the visitor-flower and pollinator- flower webs present a network characterized by several compartments as a result of high levels of specialization resulting that one or few species of beetles act as an unique floral visitors of a given plant species. We did not find temporal variation in the interaction between beetles and flowers. The connectances to visitor-flower web were 0.063 for the first year and of 0.067 for the second year and to pollinator-plant network were 0.165 and 0.151, respectively. Beetle species visited, most of times, only one and no more than four plant species. Plant species also received few beetle species as floral visitors. These beetle pollinator webs show a compound structure mixing nested and compartmented community network structure, but compartments are much more evident in this system than in any other reported for other systems. The compartments are delimited as subsets of visitor-flower or pollinator species interacting with a core plant species.

**Keywords:** beetle-flower interaction, Cerrado, community ecology, specialization, network interactions

## RESUMO

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### **Uma Rígida Interação Planta-Animal: Redes besouro-planta**

Interações planta-animal, tais como polinização, são a chave de processos ecológicos in muitas comunidades terrestres. O uso de redes tem sido útil para se investigar a estrutura e a fragilidade de interações ecológicas, e estudos comparativos de estrutura de redes ajuda a elucidar padrões ecológicos de especialização em nível de comunidade em diferentes tipos de interações interespecíficas. O presente estudo investiga pela primeira vez padrões de interações em nível de comunidade observados em uma comunidade composta por besouros associados a flores em área de Cerrado no sudeste do Brasil. Nosso objetivo é caracterizar a estrutura da comunidade besouros-flores. Baseado na antiga relação evolucionária entre flores e besouros esperamos que as redes visitantes-flores e polinizadores-flores apresentem uma rede caracterizada por vários compartimentos como consequência dos altos níveis de especialização resultando que uma ou poucas espécies de besouros ajam como únicos visitantes flores de uma dada espécies de planta. Não encontramos variação temporal na interação entre besouros e flores. As conectâncias para rede visitantes-flores foi 0,063 para o primeiro ano e de 0,067 para o segundo ano, e para rede polinizadores-planta a conectância foi de 0,165 e 0,151, respectivamente. Na maioria das vezes, as espécies de besouros visitaram somente uma e não mais que quatro espécies de planta. Espécies de planta também receberam poucas espécies de besouros como visitantes florais. Estas redes de besouros polinizadores mostram uma estrutura de comunidade composta que combina estrutura de rede aninhada e compartimentada, mas compartimentos são muito mais evidentes neste sistema do que o relatado para outros sistemas. Os compartimentos são delimitados como subconjuntos de espécies de visitantes florais ou polinizadores interagindo com espécies de plantas que centralizam as interações.

**Palavras-chave:** interação besouro-flor, Cerrado, ecologia de comunidade, especialização, rede de interações

## INTRODUCTION

Plant-animal interactions, such as pollination, are a key element in many terrestrial communities (Bascompte & Jordano 2007, Memmott *et al.* 2004). The study of who interacts with whom is an important approach for understanding ecological and evolutionary processes (Vazquez *et al.* 2009). Several general topological features of the plant-animal interaction have been described, including the skewed distribution of links per species (Waser *et al.* 1996, Jordano *et al.* 2003, but see Kay & Schemske 2004), the nested organization of the interaction matrix (Bascompte *et al.* 2003), and the frequent occurrence of asymmetric interactions (Vasquez & Aizen, Bascompte *et al.* 2006). Most interactions are likely relatively weak, diffuse (Jordano 1987, Morris 2003) with facultative interactions of high generality being the rule (Jordano 1987).

The network approach has been useful for investigating the structure and fragility of ecological interactions, and comparative studies of network structure help to uncover community-level patterns of ecological specialization in different types of interspecific interactions (Vázquez & Aizen 2004, Bascompte & Jordano 2007, Olesen *et al.* 2007). In natural communities, species and their interactions are often organized as nonrandom networks, showing distinct and repeated complex patterns (Guimarães *et al.* 2007). Recent studies demonstrate that the network structure of plant-animal mutualisms is often nested, whereas antagonistic interactions are usually non-nested (Bascompte *et al.* 2003, Lewinsohn *et al.* 2006).

Together with other properties of mutualistic networks, the extent to which species interactions are organized into modules is termed the modularity of the network. Modularity may reflect habitat heterogeneity, divergent selection regimes, and phylogenetic clustering of closely related species (Lewinsohn *et al.* 2006), leading to nonrandom patterns of interaction and ultimately contributing to the complexity of ecological networks. Modules with their tightly linked species may even be the long-sought key units of coevolution, in which reciprocal selection leads to trait convergence in unrelated species (Thompson 2005). Understanding how species interact and evolve at the modular level may thus be a key to an understanding of trait evolution.

Here, we investigate for the first time the community-level patterns of interactions observed in a community composed by beetles associated to flowers in a Cerrado in southeastern Brazil. Our goals are to characterize the structure of the beetle-flower community. Based on the ancient evolutionary relationship between flowers and beetles as well as high specialization (Bernhardt 2000, Gottsberger & Silberbauer-Gottsberger 2006a, Leavitt & Robertson 2006, Paulino Neto & Teixeira 2006), we expected the flower-visitor web and pollinator-plant web present a net structure

composed by several compartments in which one or few species of beetles act as an unique floral visitors of a given plant species.

## **MATERIAL AND METHODS**

**THE STUDY AREA** – The Cerrado Biome consists of diverse vegetation phytophysiognomies, termed *cerrado sensu lato* (Oliveira-Filho & Ratter 2002). These phytophysiognomies are associated mainly with factors such as soil fertility and drainage, seasonal precipitation and fire regime that create a mosaic represented by open grasslands (*campo limpo*) to dense woodlands (*Cerradão*) classified traditionally by a continuum of recognizable stages areas (Eiten 1972, Gottsberger & Silberbauer Gottsberger 2006b). In this study, we sampled the Itirapina Ecological Station, one of the southern reserves of Brazilian savannas and a Cerrado fragment belongs to the Arruda Botelho Institute (22°00' - 22°15' S and 43°57' - 48°00' W, Durigan *et al.* 2002, Delgado *et al.* 2004). These areas are 730 a.s.l and the climate in this region is mesothermic with a marked dry season from April to September and a wet season between October and March (Delgado *et al.* 2004).

**SAMPLING METHODS** – Data on flower-beetle was collected from April 2006 to February 2008. At intervals of two months during this period, we walked along four trails of 70 m, which were 8 m wide, for each of four Cerrado phytophysiognomies: *campo sujo*, *campo cerrado*, *cerrado sensu stricto*, *cerradão*. In each time, we registered species in flowering, number of flowers recently opened per plant and the occurrence of beetles on flowers in three flowers haphazardly selected from each flowered plant along the trails between 0700am to 0100pm. We registered both all visitor beetles observed in the flowers and we also recorded data just for the pollinators. The effective pollinator identities were assessed observing the behavior, abundance and frequency of each beetle species recorded during these two years of study and also were supported by current literature (see Gottsberger and Silberbauer Gottsberger 2006a). Plants containing just one or two flowers also were considered. During the morning period is possible to census beetle species that arrived during the night before. In the construction of networks, species are nodes and interaction between any species pair is links (Jordano *et al.* 2006). Beetle and flower data were pooled over year during and quantitative interaction webs were built for each year.

DETECTION OF NETWORK STRUCTURE AND ANALYSIS - The network was defined by a matrix describing interactions between species of beetle and species of plants in which flowers were used as resource. It is important to emphasize that beetle-flower interactions do not necessarily imply mutual benefits for both species. In fact, beetle species may act as antagonist in some ecological communities.

A nonrandom interaction matrix can be positioned among three simple configurations: a gradient, a compartmented structure or a nested structure. In a bipartite graph view, the gradient structure appears as a uniformly intermeshed series, without noticeable clusters or discontinuities. An assemblage is considered compartmented when there are recognizable subsets of interacting animals and plants, so that species are more linked within than across subsets. When compartments are of equal dimensions, host ranges and faunas can all be of uniform size, as they are in an ideal gradient, but compartmentalization is readily apparent by their blocked structure and compartments appear in ordered interaction matrices as distinct clusters of cells. Finally, the matrix is perfectly nested if showing a progression of inclusive subsets after ordering rows and columns in decreasing totals. In an ordered bipartite graph, nested structures will appear as link clusters of decreasing density on each side, which need not however be symmetrical in either species number or in link distributions (Lewinsohn *et al.* 2006).

The analyzed matrix consists of quantitative data both from species of beetle visitors and from pollinator beetles recorded to each flowered plant species pooled over all four Cerrado phytophysiognomies for each year. The quantitative data represents the sum of the interaction strength for each beetle species to each visited plant species. We calculated the interaction strength for each beetle species in each observed flower of each plant species using the following formula: beetle abundance/ number of observed flowers x flower abundance in the plant. We multiplied the interaction strength by the flower abundance on the plant to adjust the sampling effort.

We used the Bipartite package (Dormann *et al.* 2009) from R-program version 2.9.1 to analyze our matrices as a bipartite network, generating bipartite graphs of quantitative plant-beetle visitor web, pollinator-plant web and network metrics. We adopted the bipartite web analysis, because according to Lewinsohn *et al.* (2006), it offers several advantages of their own: first, they are often fully resolved, without the problems of uneven resolution which haunt the analysis of complete webs. Second, all links are of a single kind of ecological interaction, which ensures structural integrity as well as similar ecological and evolutionary processes throughout the whole assemblage. We also followed Atmar (1993) and Bascompte *et al.* (2003) just to define the degree of nestedness,  $N$ , as  $N = (100-T)/100$ , with values ranging from 0 to 1 (maximum nestedness). We

adopted the Nestedness Temperature Calculator Program to calculate the matrix temperature ( $T$ ) which values ranging from 0 (perfectly nested) to 100 (perfectly non-nested).

The network approach is just one way of analyze the degree of specialization and generalization in pollinator web systems that also can be evaluated by the direct measures from the frequency distribution of interactions (Kay & Schemske 2004). Thus, we also recorded data from visitor-flower and pollinator-plant interaction to construct the frequency distribution of interactions of the number of visitor taxa per plant taxon and the number of plant taxa per visitor taxon, and also of the number of pollinator taxa per plant taxon and the number of plant taxa per pollinator taxon (Waser *et al.* 1996, Kay & Schemske 2004). For determine the role of beetle on flowers, we recorded beetle visitor behavior during their flower visits checking their possibilities to transfer pollen or to damage floral structure.

## RESULTS

The analysis of the beetle-flower web interaction for both years presented similar species diversity between years: first year 79 species (24 plant species and 55 beetle species; second year 70 species (19 plant species and 51 beetle species). Few interactions were recorded among plant species and beetle in both years. In the first year, from 1320 possible interactions ( $M$ ), only 83 interactions were recorded ( $K$ ) with a mean number of interaction per plant species ( $Kp$ ) of 3.46 (maximum 16) and per beetle species ( $Ka$ ) of 1.51 (maximum 4). In the second, from 969 ( $M$ ), just 65 beetle interaction were observed ( $K$ ) presenting mean number of interaction per plant species ( $Kp$ ) of 3.42 (maximum 14) and per beetle species ( $Ka$ ) of 1.27 (maximum 4) (Table 1). These networks showed a connectance of 0.063 for the first year and of 0.067 for the second year. The networks in both years presented a nested pattern with values of nestedness ( $N$ ) of 0.914 and 0.863 (Table 1). Each visitor species had few interactions with the core of plant species, but these plants interacted with many beetle species resulting a nested pattern (figure 1, 2).

Table 1 – Statistic metrics of visitor-flower and pollinator-plant interaction networks at cerrado of Itirapina from April 2006 to February 2007 (first year) and from April 2007 to February 2008 (second year).

	<i>A</i>	<i>P</i>	<i>S</i>	<i>M</i>	<i>K</i>	<i>K<sub>a</sub></i>	<i>K<sub>p</sub></i>	<i>C</i>	<i>T</i>	<i>N</i>
Visitor-flower web										
1 <sup>st</sup> year	55	24	79	1320	83	1.51	3.46	0.063	10.77°	0.892
2 <sup>nd</sup> year	51	19	70	969	65	1.27	3.42	0.067	15.24°	0.847
Pollinator web										
1 <sup>st</sup> year	32	8	40	256	42	1.31	5.12	0.165	47.20°	0.528
2 <sup>nd</sup> year	38	8	46	304	46	1.21	5.75	0.151	29.83°	0.702

(*A*) number of pollinator species; (*P*) number of plant species; (*S*) species richness; (*M*) matrix size (total number of potential interaction; (*K*) number of interaction recorded; (*K<sub>a</sub>*) mean number of interaction per pollinator species; (*K<sub>p</sub>*) mean number of interaction per plant species; (*C*) connectance or density of the network ; (*T*) matrix temperature; (*N*) nestedness.



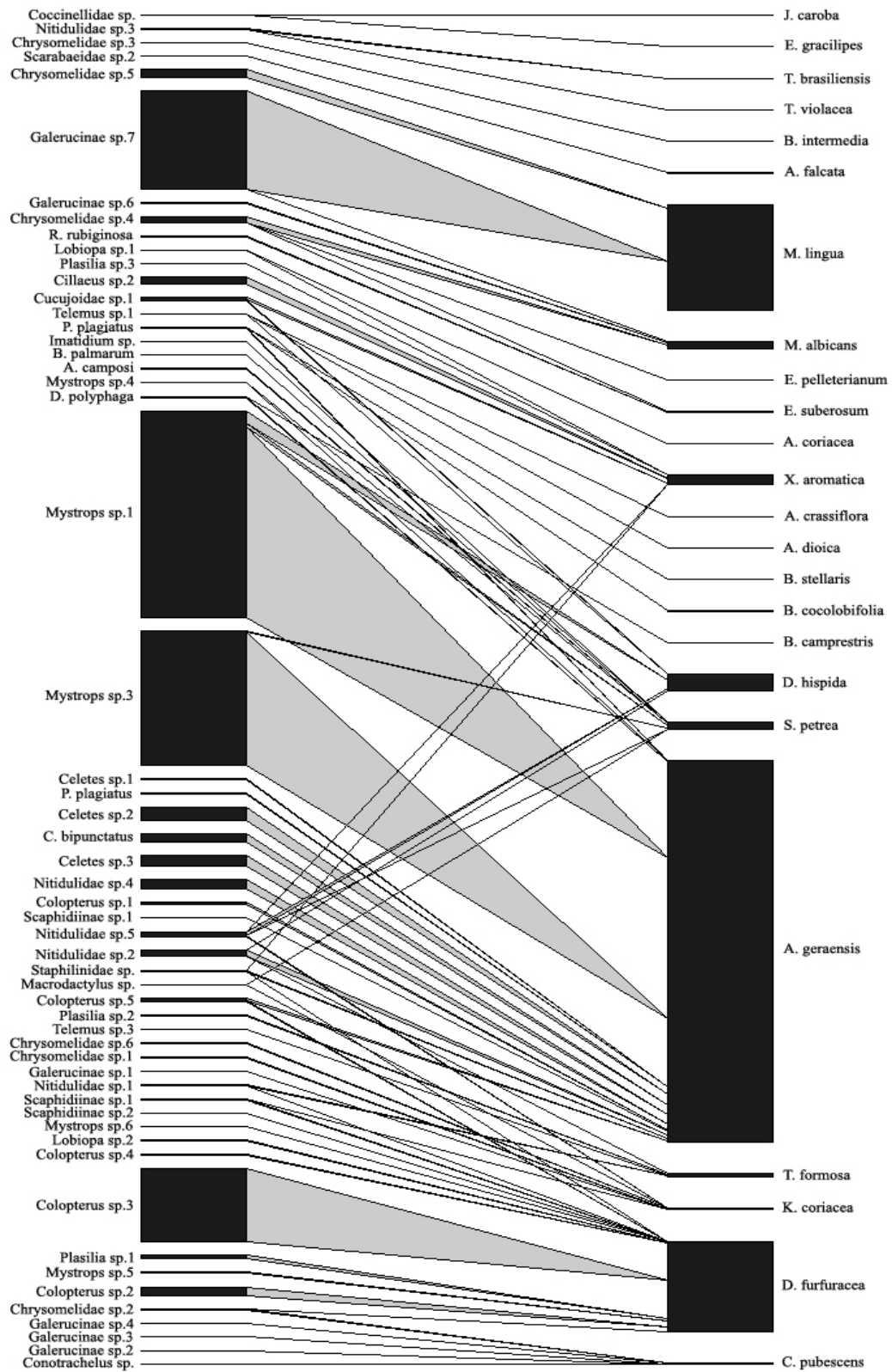


Figure 1 – Quantitative plant-visitor web recorded for cerrado area for the 1st year of study. The species/morphotypes of plant and insect are represented by rectangles. Species identities and respectively family are listed in the appendixes 1 and 2. The width of rectangles represents the whole interaction of each species at the field site. The interactions are shown as connecting triangles which sizes reflect the proportion of all recorded interaction strengths of each partner.

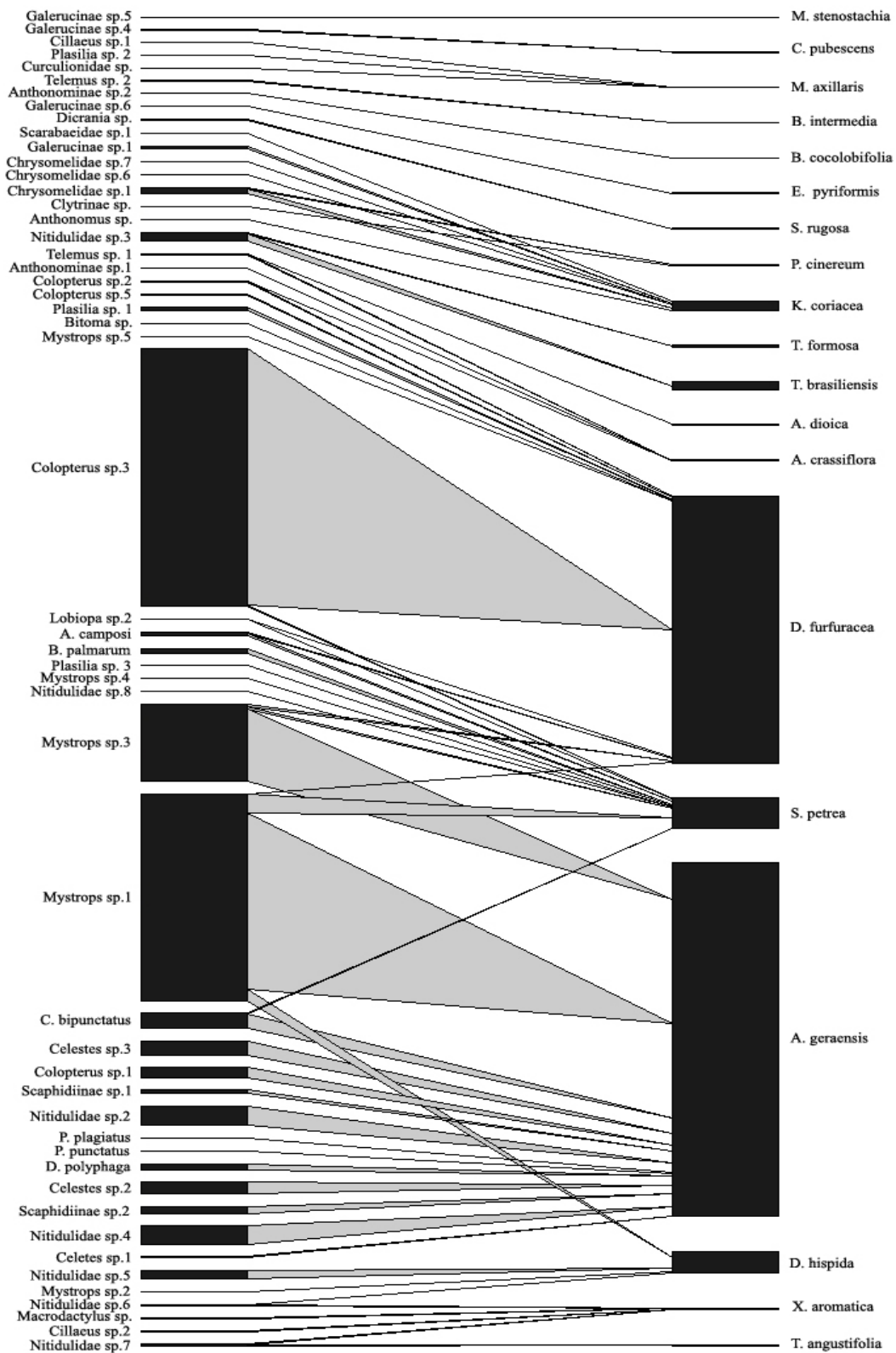


Figure 2 – Quantitative plant-visitor web recorded for cerrado area for the 2nd year of study. The species/morphotypes of plant and insect are represented by rectangles. Species identities and respectively family are listed in the appendixes 1 and 2. The width of rectangles represents the whole interaction of each species at the field site. The interactions are shown as connecting triangles which sizes reflect the proportion of all recorded interaction strengths of each partner.

Additionally, the two palm species together (*Attalea geraensis* and *Syagrus petrea*) comprised 24 from 83 (29.92%) and 23 from 64 (35.94%) of the interactions in the first year and second year, respectively. Moreover, six plants species (*A. geraensis*, *Diospyros hispida*, *Duguetia furfuracea*, *Kielmeyera coriacea*, *Syagrus petrea* and *Xylopia aromatica*) represented 65.06% and 70.31% of the total number of interactions to each year.

The palm species had many visitor interactions and shared some beetle species, but most of their beetle visitors are specialist. *A. geraensis* presented eight and 11 specialist interactions for the first and second year, respectively. *S. petrea* had three and four specialist visitor species for each studied year. These palm species shared four and three beetle species in each year, respectively. Besides, our data to both years showed that this cerrado area had a predominance of mix of nested and compartmented network presenting a weak gradient represented principally by weak strength interactions. In these networks, few plant species centralized most of the beetle interactions and they shared no more than four visitors species with other partners resulting many compartmented groups. The networks in both years were constituted by some compartmented assemblages with species-specific interactions or with a group of visitor species visiting just one plant species (figure 1, 2).

The pollinator-plant networks were smaller than that to visitor-flower with 32 species of beetles and eight species of plants recorded for the first year of study, which comprised 42 interactions among these species ( $K$ ) from 256 that were theoretically possible. The mean number of interactions per plant species ( $Kp$ ) and per pollinator species ( $Ka$ ) were 5.12 (maximum 13) and 1.31 (maximum 4), respectively (Table 1). In the second year, 38 species of beetles and eight species of plants were recorded with only 46 interactions occurring from 304 possible. The mean number of interactions per plant species ( $Kp$ ) and per pollinator species ( $Ka$ ) were 5.75 (maximum 14) and 1.21 (maximum 3), respectively. The pollinator-flower network connectances to both years were 0.165 and 0.151 with values of nestedness ( $N$ ) of 0.528 and 0.702 for the first and second year, respectively (Table 1). The pollinator-plant networks showed a connectance of 0.165 for the first year and of 0.151 for the second year. The networks in both years presented a nested pattern with values of nestedness ( $N$ ) of 0.528 and 0.702 (Table 1). Here the pollinator species also had few interactions with the core of plant species and these plants interacted with many beetle species resulting a nested pattern (figure 3, 4). Moreover, the plant-pollinator web for both years presented many compartments delimited as subsets of beetle visitor-flower species interacting with a core plant species (figure 3, 4).

The degree of specialization and generalization based on the frequency distribution of interactions to the first and second year for the visitor-flower web system showed that 54 and 47%

of plant species were visited just by one visitor species. The pollinator web system presented just 11 and 25% of plant species receiving only one pollinator species. For beetles, we recorded that 62 and 78% of their species visited only one plant species. Moreover, our data just for pollinator web system showed that 81 and 84% of pollinator species interacted with only one plant species (figure 5).

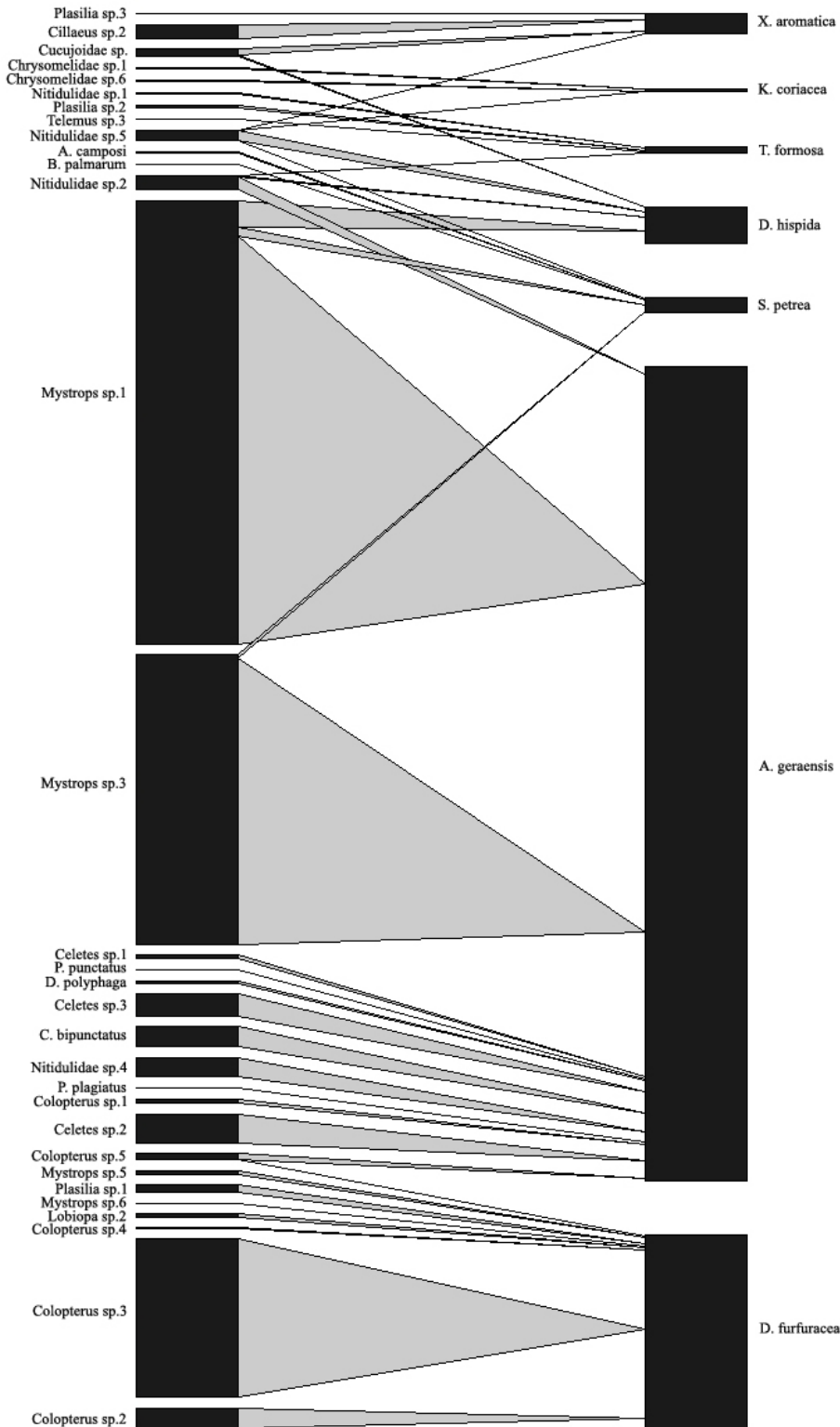


Figure 3 – Quantitative plant-pollinator web recorded for cerrado area for the 1st year of study. The species/morphotypes of plant and insect are represented by rectangles. Species identities and respectively family are listed in the appendixes 1 and 2. The width of rectangles represents the whole interaction of each species at the field site. The interactions are shown as connecting triangles which sizes reflect the proportion of all recorded interaction strengths of each partner.

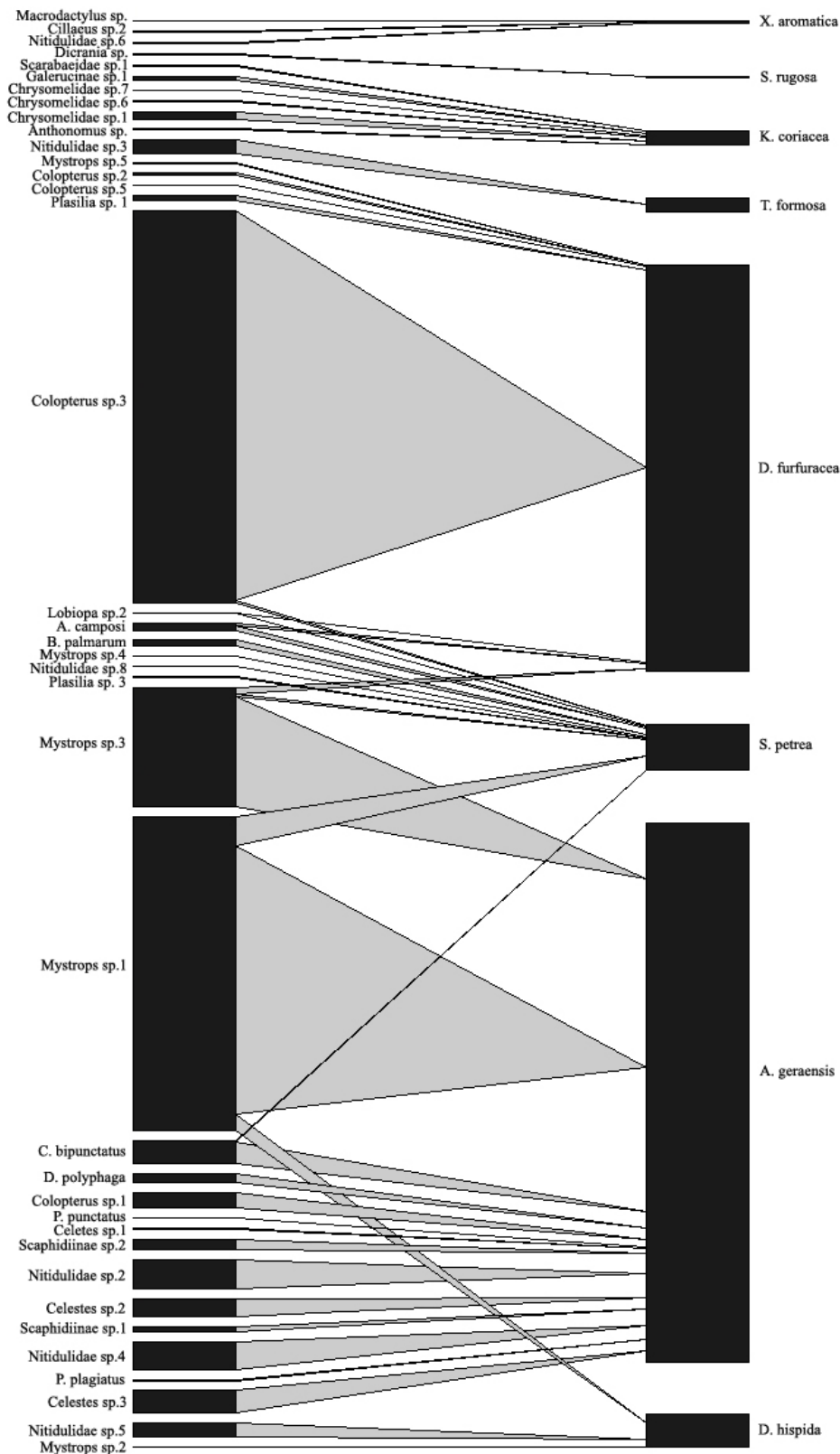


Figure 4 – Quantitative plant-pollinator web recorded for cerrado area for the 2nd year of study. The species/morphotypes of plant and insect are represented by rectangles. Species identities and respectively family are listed in the appendixes 1 and 2. The width of rectangles represents the whole interaction of each species at the field site. The interactions are shown as connecting triangles which sizes reflect the proportion of all recorded interaction strengths of each partner.

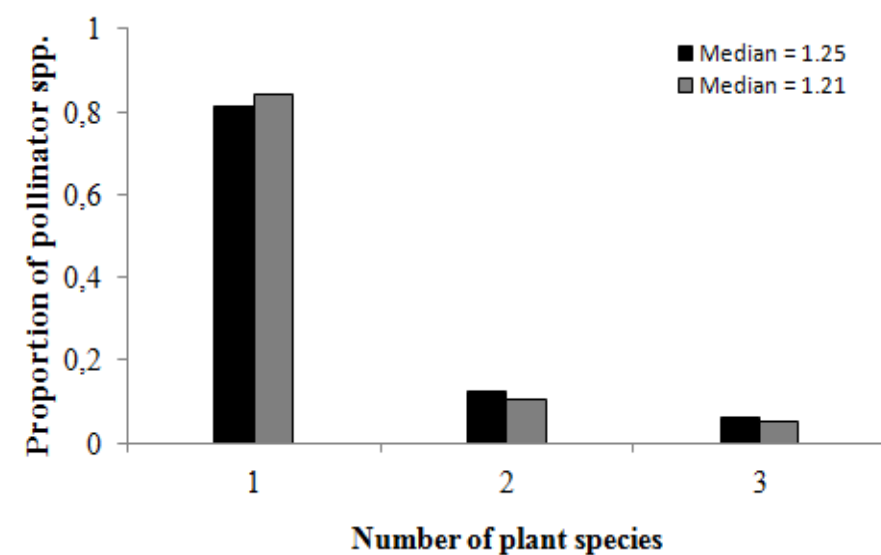
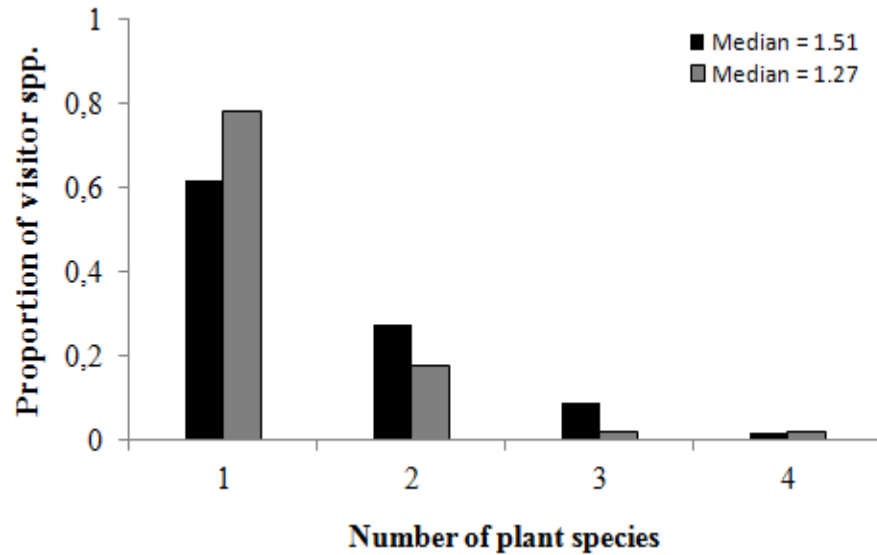
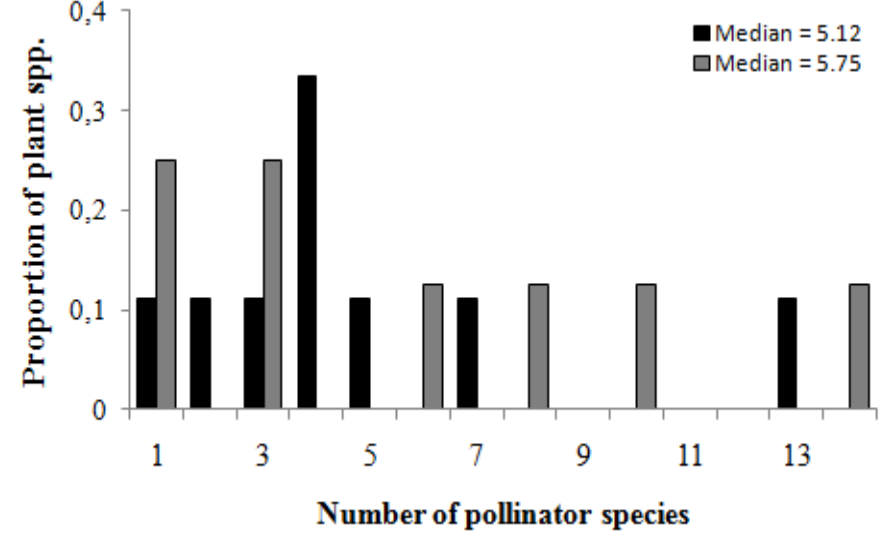
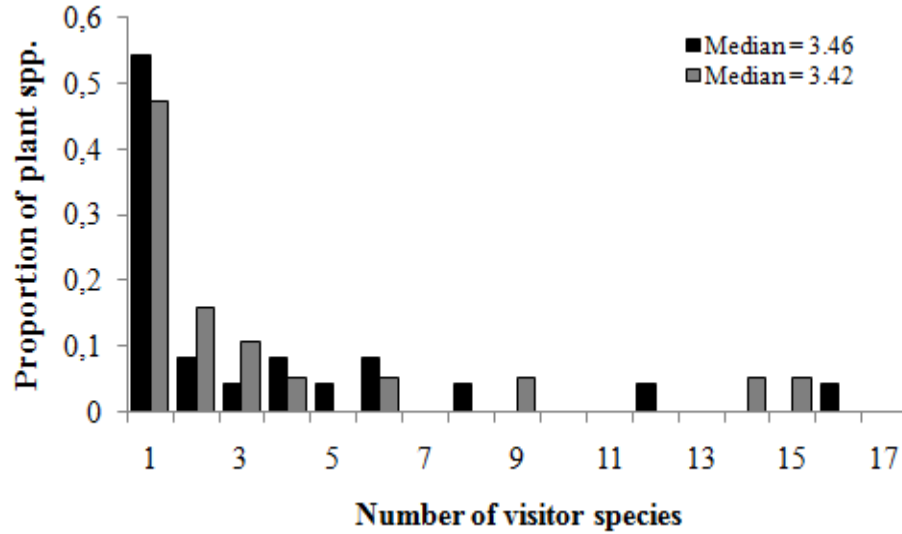


Figure 5 – Frequency distributions of the number of visitor species per plant species and number of plant species per visitor species; and number of pollinator species per plant species and number of plant species per pollinator species for two consecutive years for the cerrado area. First year in black and second year in gray.

## DISCUSSION

In general, both for the visitor-flower and for pollinator-plant web, we found no temporal variation in the web between years. Visitors had more specialized interactions than plant species, independently if beetles act as pollinators or not. However, in spite of pollinator-plant networks show beetle species highly specialist, their plants species had less specialist interactions than in visitor-flower web, presenting higher median number of interactions per plant species and consequently, they presented higher connectance. Data corroborating that plant species are more generalists than pollinator insect species have been recorded in many other pollination systems (Waser *et al.* 1996, Dycks *et al.* 2002, Olesen & Jordano 2002). According to Waser *et al.* (1996), there are invariably much more insect species than plant species, and most of these insects present a degree of generality in their foraging choice.

The visitor-flower networks are higher diversity and number of interactions, present more interactions and less number of interaction per plant species resulting in lower connectance. On the other hand, our study reveals a bigger proportion of beetle species per plant taxon in the pollinator networks resulting in higher connectance. These data corroborates Jordano (1987) discussing that the level of connectedness in community of pollinator-plant mutualists decreases with increasing species richness. The lower median number of interaction per plant species in visitor-flower web is consequence of record of sporadic and occasional floral beetle visits that most of times no favor the pollen transference. As these beetle species were recorded just once in the flowers, they represent untruthfully a false specialized interaction resulting in lower connectance. However, for the plant the most important are the specialized interaction with abundant and assiduous pollinators. On the other side, we also considered all connectances recorded here can be considered extremely low in comparison with that found or discussed in many others studies (Olesen & Jordano 2002, Kay & Schemske 2004), indicating that this beetle-flower web interaction presents very close interaction with plants. Beetle species were considered extremely specialized visitors, most of times, only one and no more than four plant species and most of plant species presenting few visitor species, many of them also are specialists.

The higher connectance in pollinator-flower web probably is due to some generalist plants species that are visited by sets of specialist pollinators as discussed by Bascompte and Jordano (2007), which use their flowers for feeding, shelter, copulation and oviposition (Gottsberger and



Silberbauer Gottsberger 2006a, Paulino Neto *et al.* chapter 3). Thus, in these systems, some plant species present several pollinator species but most of these pollinators are specialists, abundant and reliable.

A nestedness network structure occurs when specialists interact with species that form a well-defined subsets of species with which generalists interact (Bascompte and Jordano (2007). Moreover, a nested matrix in the plant-animal mutualistic networks presents two main proprieties. First, there is a core of generalist plants and animals that interact among themselves and consequentially, a few species are involved in a large number of interactions, which introduces a functional redundancy and the possibility for alternative routes for system persistence if some of these interactions disappear. Second, there were asymmetries in the level of specialization and specialists tend to interact with most of most of generalist species (Vazquez & Aizen 2004). Generalists tend to be more abundant, less-fluctuating species in comparison with specialists because generalists rely on so many other species (Bascompte and Jordano 2007). Then, in this present study, some few plant species, as *A. geraensis*, *C. pubescens*, *D. furfuracea*, *D. hispida*, *K. coriacea*, *S. petrea*, *T. formosa* and *X. aromatica*, involved in many interactions were considered a core and are the main responsible by the nested pattern found to all interaction networks studied here. According to Lewinsohn *et al.* (2006), an assemblage is compartmented if there are recognizable subsets of interacting animals and plants, so that species are more linked within than across subsets. Hence, these visitor-flower and pollinator-plant webs show a compound structure combining a nested and a compartmented network structure, but with predominance of the compartmented pattern. The compartmented patterns recorded here are resultant from both of the many species-specific interactions between beetle and plant species and of the those plant species above cited arising from several specialized beetle species that interact more with the core plant species than with other plant species. Therefore, compartments here are delimited as subsets of flower visitors or pollinator species interacting with a core plant species, presenting network compartmentalization similar to that discussed by Lewinsohn *et al.* (2006) and Bascompte and Jordano (2007).

In the visitor's perspective, the evaluation of the degree of specialization utilizing the frequency distribution of interactions in visitor-flower webs indicates a specialized interaction system with 62-84% of the beetle species visiting only one plant species and median number lower than 1.5 visitor taxa per plant taxon. In comparison with other similar studies focusing in

different insect orders, we considered this interaction web between beetles and flowers as the most specialist flower-visitor and pollinator web already studied (see Bronstein 1995, Waser *et al.* 1996, Jordano 1987, Kay & Schemske 2004 for other systems). Additionally, from the point of view of plants, the visitor-flower webs for both years also were considered specialist and in general, they present half of the plants species interacting with one beetle species. It is important reinforce that even considering the temporal and spatial heterogeneity, once we sampled several cerrado phytophysiognomies and two whole years covering twice every season, a generalized web visitor-flower interactions were not recorded (Herrera 1988, Waser *et al.* 1996, Price *et al.* 2005, Petanidou *et al.* 2008). Thus, both visitor-flower and pollinator-plant webs may be denominated as highly specialist from the beetle perspective whereas that these beetle fauna visited flowers of very few plant taxa over time and space. Nevertheless, the pollinator web system presented a pattern of pervasive generalization with just 11-25% of plant species receiving only one pollinator species and medians between 5-6 pollinator species per plant taxon for both years.

Besides specialization is considered rare in tropical forests and more common in temperate regions and predominant in deserts (Waser *et al.* 1996, & Jordano 2002), our study showed that the Cerrado, a typical tropical habitat, presents an interaction system between beetles species and flowers of plant species with highly specificity. Further studies are necessary to verify if this pollinator specialization degree found in this present work is related with the geographic location of the studied area (Olesen & Jordano 2002), if other biotic and abiotic aspects drive its evolution of high specialist pollinator-plant interactions (Thompson 1994), or if there is phyllogenetic background promoting specializations and generalizations in plant-pollinator systems (Waser *et al.* 1996).

## GENERAL CONCLUSIONS

Our results indicated that in the visitor-flower webs to both years most of the beetle species, both in richness as in abundance, were effective pollinator species. Pollinators were more specialists and had higher interaction strengths than those visitor beetles that visited flowers not exhibiting behavior that favor the pollen transference or sporadically observed in flowers. On the other hand, both in visitor and pollinator web most of the beetle species were specialists, but with higher specificity in the pollinator web.

This is the first study describing the pattern of plant-pollinator mutualistic network for cantharophylous systems. In these beetle interaction systems (visitor and pollination net work) most of the involved beetle species are highly specialist, presenting one-to-one interaction with plant species or visiting few plant species that most of the time are phylogenetically related. Most of this high specificity level of beetle-pollinator species can be explain by the close interactions of many beetle species with plants that they pollinate. Usually, these beetle species use the flowers not only to feed pollen and petals but also to copulate and to lay eggs inside the flowers whose larvae may feed of petals, anthers, ovules, fruit pulp or seeds (Gottsberger and Silberbauer Gottsberger 2006, Paulino Neto & Teixeira 2006, Paulino Neto *et al.* chapter 3).

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Appendix 1 – Plant species

<b>ANNONACEAE</b>	<b>MIMOSACEAE</b>
<i>Annona coriacea</i>	<i>Anadenanthera falcata</i>
<i>Annona crassiflora</i>	<i>Senna rugosa</i>
<i>Annona dioica</i>	<b>MYRTACEAE</b>
<i>Duguetia furfuracea</i>	<i>Campomanesia pubescens</i>
<i>Xylopia aromatica</i>	<i>Eugenia pyriformis</i>
<b>APOCYNACEAE</b>	<i>Myrcia lingua</i>
<i>Temnadenia violacea</i>	<i>Psidium cinereum</i>
<b>ARECACEAE</b>	<b>RUBIACEAE</b>
<i>Attalea geraensis</i>	<i>Tocoyena brasiliensis</i>
<i>Syagrus petrea</i>	<i>Tocoyena formosa</i>
<b>BIGNONIACEAE</b>	<b>SAPINDACEAE</b>
<i>Jacaranda caroba</i>	<i>Talisia angustifolia</i>
<i>Memora axillaris</i>	
<b>CLUSIACEAE</b>	
<i>Kielmeyera coriacea</i>	
<b>EBENACEAE</b>	
<i>Diospyros hispida</i>	
<b>ERYTHROXYLACEAE</b>	
<i>Erythroxylum pelleterianum</i>	
<i>Erythroxylum suberosum</i>	
<b>MALPIGHIACEAE</b>	
<i>Banisteriopsis campestris</i>	
<i>Banisteriopsis stellaris</i>	
<i>Byrsonima coccolobifolia</i>	
<i>Byrsonima intermedia</i>	
<b>MALVACEAE</b>	
<i>Eriotheca gracilipes</i>	
<b>MELASTOMATACEAE</b>	
<i>Miconia albicans</i>	
<i>Miconia stenostachia</i>	

Appendix 2 – Beetle species

<b>CHRYSOMELIDAE</b>	<i>Celetes</i> sp.3
<i>Clytrinae</i> sp.	<i>Conotrachelus</i> sp.
Chrysomelidae sp.1	Curculionidae sp.
Chrysomelidae sp.2	<i>Dialomia polyphaga</i>
Chrysomelidae sp.3	<i>Palmocentrinus cf. punctatus</i>
Chrysomelidae sp.4	<i>Parisoschoenus plagiatus</i>
Chrysomelidae sp.5	<i>Plasilia</i> sp.1
Chrysomelidae sp.6	<i>Plasilia</i> sp.2
Chrysomelidae sp.7	<i>Plasilia</i> sp.3
Galerucinae sp.1	<i>Revena rubiginosa</i>
Galerucinae sp.2	<i>Telemus</i> sp.1
Galerucinae sp.3	<i>Telemus</i> sp. 2
Galerucinae sp.4	<i>Telemus</i> sp.3
Galerucinae sp.5	<b>NITIDULIDAE</b>
Galerucinae sp.6	<i>Cillaeus</i> sp.1
Galerucinae sp.7	<i>Cillaeus</i> sp.2
<i>Imatidium</i> sp.	<i>Colopterus</i> sp.1
<b>COCCINELLIDAE</b>	<i>Colopterus</i> sp.2
Coccinellidae sp.	<i>Colopterus</i> sp.3
<b>COLLYDIIDAE</b>	<i>Colopterus</i> sp.4
<i>Bitoma palmarum</i>	<i>Colopterus</i> sp.5
<i>Bitoma</i> sp.	<i>Lobiopa</i> sp.1
<b>CUCUJOIDAE</b>	<i>Lobiopa</i> sp.2
Cucujoidae sp.	<i>Mystrops</i> sp.1
<b>CURCULIONIDAE</b>	<i>Mystrops</i> sp.2
<i>Anchylorhynchus cf. camposi</i>	<i>Mystrops</i> sp.3
Anthonominae sp.1	<i>Mystrops</i> sp.4
Anthonominae sp.2	<i>Mystrops</i> sp.5
<i>Anthonomus</i> sp.	<i>Mystrops</i> sp.6
<i>Celetes bipunctatus</i>	Nitidulidae sp.1
<i>Celetes</i> sp.1	Nitidulidae sp.2
<i>Celetes</i> sp.2	Nitidulidae sp.3
Nitidulidae sp.4	
Nitidulidae sp.5	
Nitidulidae sp.6	
Nitidulidae sp.7	
Nitidulidae sp.8	
<b>SCARABAEIDAE</b>	
<i>Dicrania</i> sp.	
Scarabaeidae sp.1	
Scarabaeidae sp.2	
<b>STAPHILINIDAE</b>	
Scaphidiinae sp.1	
Scaphidiinae sp.2	
Staphilinidae sp.	



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## **CAPÍTULO 3**

**Floral Traits, Beetle pollination and Reproductive**

**Output of *Duguetia furfuracea* (Annonaceae):**

**A Tropical Nursery Pollination System**

## ABSTRACT

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Reciprocal exploitations between insects and plants are among the most studied interactions to discuss the evolution of mutualisms. The result of the interaction and of the evolution of phenotypic traits of plants and insects is affected by the conflicts between partners, in which each partner is selected to increase its own benefits and reduce the costs of the interaction. Considering that pollinator beetles species of Annonaceae exhibit mating behavior and oviposition inside of floral chamber, we may expect that the interaction between beetles and flowers share the interface of mutualism and parasitism. The objective of this study is to describe the reproductive and natural history traits of the partners involved in the *D. furfuraceae*- beetle interaction to verify the role of beetles in this system and to reveal the occurrence of evolutionary conflicts on this interaction. *D. furfuracea* had an extended flowering season blossoming from September to May, but at least there were some few plants with flowers every time along the year. Fruits were year round with a peak in fruit production from March to November. *D. furfuracea* was considered self-incompatible species that presented both pollen limitation and resource limitation in this population. There were two specialized floral visitant guilds, one composed by the curculionid *Plasilia* sp. and another by nitidulid beetles. *Plasilia* sp. larvae developed into of *D. furfuracea* fruits and they were seed consumers. In this pollination system, the floral visitors presented high specificity, which all phases of their life cycle are associated with the plant. *D. furfuracea* pollination system constitutes the first obligate pollination mutualism case involving different guilds of floral visitors and also the first pollination system combining predictable and reliable pollinators, seed consumption, pollen and resource limitation resulting in a complex and efficient mechanism to regulate the seed consuming by floral visitor's population and to optimize the plant fitness.

**Keywords:** Annonaceae, Brazilian Savanna, cantharophily, Cerrado, Curculionidae, mutualism, Nitidulidae, parasitism, seed consumption.

## RESUMO

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### **Atributos florais, polinização por besouros e sucesso reprodutivo de *D. furfuracea*: um sistema tropical cujos besouros utilizam estruturas florais para sua reprodução**

Explorações recíprocas entre insetos e plantas são as interações mais estudadas para discutir a evolução de mutualismos. O resultado da interação e da evolução de atributos fenotípicos de plantas e insetos é afetado pelos conflitos entre participantes, nos quais cada participante é selecionado para aumentar seus próprios benefícios e reduzir os custos da interação. Considerando que espécies de besouros polinizadores de Annonaceae copulam e ovipõem no interior da câmara floral, nós podemos esperar que a interação entre besouros e flores compartilhe a interface entre mutualismo e parasitismo. O objetivo deste estudo é descrever o sistema reprodutivo e fatores da história natural dos participantes envolvidos na interação *D. furfuracea*-besouros para verificar a função dos besouros neste sistema e para demonstrar a ocorrência de conflitos evolucionários nesta interação. *D. furfuracea* apresentou um extenso período de floração com pico de floração entre setembro e maio, mas ao longo do ano foram encontradas pelo menos algumas poucas plantas florindo. Frutos ocorrem o ano todo com pico de frutificação entre março e novembro. *D. furfuracea* foi considerada como espécie auto-incompatível que apresentou tanto limitação polínica quanto limitação de recursos nesta população. Há duas guildas especializadas de visitantes florais, uma composta pelo curculionídeo *Plasilia* sp. e outra composta por besouros nitidulídeos. Larvas de *Plasilia* sp. são consumidores de sementes. Neste sistema de polinização os visitantes florais apresentaram alta especificidade, no qual todas as fases do ciclo de vida estão associadas com a planta. O sistema de polinização de *D. furfuracea* consiste no primeiro caso de polinização mutualística obrigatória envolvendo diferentes guildas de visitantes florais e também no primeiro sistema de polinização combinando previsíveis e confiáveis polinizadores, consumo de sementes, limitação polínica e de recursos resultando em um complexo e eficiente mecanismo para regular a população dos visitantes florais consumidores de sementes e para otimizar o sucesso reprodutivo da planta.

**Palavras-chave:** Annonaceae, savana brasileira, Cantarofilia, Cerrado, Curculionidae, mutualism, Nitidulidae, parasitismo, consumo de sementes.

## INTRODUCTION

Reciprocal exploitations between insects and plants are among the most studied interactions to discuss the evolution of mutualisms (Bronstein 1994, Bronstein et al. 2006). The result of the interaction and the evolution of phenotypic traits of plants and insects are affected by the existence of conflicts between partners, in which each partner is selected to increase its own benefits and reduce the costs of the interaction (Bronstein 1994, Strauss and Irwin 2004). Nursery pollination system, in which pollinating species reproduce in reproductive structures of their host plant, has been considered model system to study the evolution of mutualism because it offers the possibility to evaluate the net result of the conflicts of interests between partners by measurement the partners' fitness (Thompson & Pellmyr 1992, Holland & DeAngelis 2001).

Among pollination systems, beetles have an old evolutionary history with angiosperms, acting as pollinators in some early as well as highly evolved angiosperms (Endress 1994). However, beetle activity on flowers can also destroys floral parts and it is surprising that only four cases of nursery pollination had been described between flowering plants and beetles up to now (Dufay & Anstett 2003). Most species in the family Annonaceae, with approximately 2500 species distributed mainly in tropical regions, has flowers pollinated by beetles. Based on the size of floral chamber, time of anthesis and beetle size and behavior, small and large specialized beetle pollination system can be distinguished (Gottsberger 1999). There are suggestions that beetle activity might not result in negative effects on pollination, contrary to the evidence that flowers could be heavily gnawed or even totally consumed by beetles (Gottsberger 1999).

Considering that mating and oviposition of beetles could occur during the flowering period of Annonaceae species with records of mating behavior and oviposition of beetles inside floral chamber (Gottsberger & Silberbauer Gottsberger 2006a), one might expect that the interaction between beetles and flowers shares the interface of mutualism and parasitism. The net result of it depends on the level of pollination, oviposition and development of flowers into a fruit. In order to contribute to the understanding of the interaction between flowers and beetles and the possibility to add a tropical system with measureable traits to study the evolution of mutualism, the objective of this study is to describe the reproductive and natural history traits of the partners involved in the *Duguetia furfuraceae* (A.St.-Hil.) Benth. & Hook.f. – beetle

interaction in order to verify the role of beetles in this system and to reveal the occurrence of evolutionary conflicts on this interaction.

## MATERIAL AND METHODS

STUDY SITE AND SYSTEM – Cerrado, the Brazilian savanna, comprises a mosaic of vegetational physiognomies determined mainly by fertility and drainage of soils and fire regime. A gradient of habitats between two extremes can be found in Cerrado - open grasslands or “campo limpo” and dense woodlands or “Cerradão” (Oliveira-Filho & Ratter 2002, Gottsberger & Silberbauer Gottsberger 2006a). Among the few areas of Cerrado in São Paulo, Brazil, the neighboring areas the “Estação Ecológica de Itirapina” (22°12’ S, 47°55’ W) and the “Instituto Arruda Botelho” (22°10’S , 47°57’W) contain in assemblage all possible vegetational Cerrado physiognomies in an area of 2,566 ha - “campo limpo”, “campo sujo”, “campo cerrado”, “cerrado *sensu stricto*” and “Cerradão” (for additional information on composition and structure of vegetational physiognomies, see Oliveira-Filho & Ratter 2002, Gottsberger & Silberbauer Gottsberger 2006a, Figure 1). Both sites are under a dry and wet season from April to September, and from October to March, respectively. Data on meteorology from Instituto Florestal of Itirapina for the period comprehended between January 2005 and December 2007 showed mean monthly temperature, pluviosity and humidity in the dry season ranging from 16.2 to 23.3°C, 0 to 142 mm and 36.4 to 62.6% while in the wet season varying from 16.7 to 25.4°C, 81 to 492 mm and 45.4 to 80.4%, respectively.

The family Annonaceae contains several genera and species distributed in Cerrado, and several aspects of the floral biology and pollination of these species were studied (Gottsberger & Silberbauer Gottsberger 2006a, b). Among the species, *D. furfuracea* can be easily found, occurring in high abundance and distributed in all Cerrado physiognomies (Durigan *et al.* 2002, 2004, Gottsberger & Silberbauer Gottsberger 2006b). Additionally, its small size (up to 2m), growth form and flower structure that permits several species of beetles to enter the floral chamber (Gottsberger 1999) makes us select it for the purpose of this study.



Figure 1 - A-D. Studied cerrado phytophysiognomies: (A) Campo sujo; (B) Campo cerrado; (C) Cerrado *sensu stricto*; (D) Cerradão.

FLORAL AND FRUIT PHENOLOGY – To access the flower and fruit phenology of the cerrado of Itirapina we recorded of the numbers of flowers and fruits of *D. furfuracea* individuals along four trails of 70 m, which were 8 m wide in the phytophysiognomies of campo sujo (Figure 1A), campo cerrado (Figure 1B), cerrado *s.s.* (Figure 1C) and cerradão (Figure 1D) every two months from April 2006 to March 2008 and we pooled these data to represent the area in a better way. Additionally, the beetle occurrence was registered verifying the number and identity of beetles inside of floral chamber in at least three haphazardly selected flowers on each plant.

## ANTHESIS AND FLORAL TRAITS

The observation of floral biology and floral traits were made on 188 flowers from 32 individual plants to verify details of the events of anthesis. We recorded the number of stigmas and carpels in 16 flowers, and made floral measurements on 23 flowers and on 9 buds in the pre-anthesis stage from different plants. We also carried observations in 28 flowers to verify the relation between floral rhythm and beetle visitation. The stigmas of Annonaceae are receptive when they produce exudate (Gottsberger 1989a, 1994). We therefore recorded stigmatic receptivity visually using a magnifying glass. We also determined stigmatic receptivity by performing hand cross-pollinations in 108 flowers during each of two stages: early anthesis (when flowers had green-reddish or orange petals, and stigmas had low exudate production and no scent), and later anthesis or “pistillate stage” (orange or dark red petals, stigmas with higher exudate production, and intense odor emission) totalizing 206 pollinations. Both treatments were equally distributed among 36 plants.

## BREEDING SYSTEM

To evaluate the effective role of the beetles that visit the *D. furfuracea* flowers, we protected buds with bridal-veil netting prior to anthesis to avoid any floral visitation of 36 plants in 2006, and 44 plants 2008. In these plants we performed five treatments: 1) bagging and emasculation (to test the apomixis); 2) bagging only (to test for spontaneous self-pollination); 3) hand self-pollination; 4) cross-pollination; and 5) open control (to assess natural pollination, and to compare with self- and cross- treatments to detect pollen limitation). Pollinations were performed during the pistillate period using fresh pollen. The self-pollinations were done with recently fallen pollen collected in the floral chamber, simulating the natural conditions under which self-pollinations likely occur. Emasculations were performed on pre-anthesis floral buds. Fruit set among these treatments were compared using a Chi-square test ( $\alpha = 5\%$ , Sokal & Rohlf 1981). We calculated the index of self-incompatibility - ISI (sensu Bullock 1985, i.e. percentage of selfed fruits/crossed fruits;  $ISI \leq 0.25$  is considered self-incompatible) to determine the predominant breeding system. We also calculated the reproductive efficacy (sensu Ruiz & Arroyo 1978, i.e. percentage of natural fruit set/hand crossed fruits). According to Ruiz and Arroyo (1978), the reproductive efficacy estimates the fruit set under maximal conditions of pollination and also provides a direct indication of the pollinator efficiency by comparison of

fruit set under open pollination fruit set with controlled cross-pollination of previously bagged flowers.

Additionally, we done an experiment combining pollen supplementation and pollination under natural conditions with open pollination and exclusion of *Plasilia* sp. (Curculionidae) to evaluate the pollen and resource limitation, and the role of this curculionid beetle visitor on the fruit and seed set of *D. furfuracea* through fruit and seed set and fruit abortion. Each one of these four treatments constituted of 40 flowers that, subsequently, were bagged to avoid another possible interactions. Also was calculated the ratio of benefit to cost for the *D. furfuracea*/beetle interaction (seed set percentage from pollination/ seed consumption percentage from pollinators larvae).

#### FLORAL VISITORS AND THEIR ROLE

During the flowering peak of *D. furfuracea* in December 2007, census of floral visitors were made in three flowers per plants (n = 38 plants). The observations occurred during 7 consecutive days between 0700am to 0100pm, time interval in which beetle species that arrived in previously night are still into the floral chamber.

We carried a census on floral visitors during two entire years at intervals of two months from March 2006 to February 2008 in the all cerrado areas above cited. In this census, the occurrence of beetles on flowers was assessed in three flowers haphazardly selected from each of the 38 plants along the trails totaling 148 flowers in which the number of beetles present was sampled also to determine their identification. The observations occurred between 0700am to 0100pm, time interval in which beetle species that arrived in previously night are still into the floral chamber. Additionally, to more accurately determine the beetles' visitation period, we carried out nocturnal and morning census every 2 h in 329 flowers spread over 19 plants distributed campo sujo and campo cerrado vegetation during six consecutive days during the flowering peak of *D. furfuracea* (December 2007). This second census was restricted just to campo sujo and campo cerrado because were the areas where *D. furfuracea* occurred with biggest abundance. The results of both floral visitor's census were pooled.

Since there were clearly two distinct groups of beetle visitors that showed different sizes and behaviors, we did selective exclusion experiment to these beetle groups to verify its role for the reproductive output of *D. furfuracea*. To register differences in the behavior of these groups



we carefully followed 30 marked flowers in eight plants every day from the beginning of anthesis to three days after senescence. We observed these same flowers 3 months later to measure fruit and seed set as well to measure the amount of seeds consumed by beetles. The exclusion experiment consisted of 80 flowers (naturally pollinated) in two groups. For the exclusion treatment, 40 flowers were bagged with bridal-veil netting (1-mm mesh) to prevent visitation by the Curculionidae species, but allowing all nitidulid species. The open control was composed of another 40 flowers open to natural visitation by all beetles species. We recorded fruit set of these two groups of flowers at the beginning of development (one month after flowering) and in advanced development (3 months later) to determine the reproductive consequences of curculionid exclusion. Additionally, we collected 34 developed fruits on natural conditions to verify the seed consumption and another 31 no damaged fruits developed under natural conditions to check the seed production without seed consumption by beetles. Moreover, we collected others 21 fruits that were kept in plastic containers until the beetle emergence to verify the seed-consumer species. We also calculated the ratio of benefit to cost for the *D. furfuracea*/beetle interaction as percent seed set contributed by adult beetle pollinators divided by percent fruit destroyed by beetle larvae.

Beetles specimens were collected, determined, and deposited as vouchers at the Museum de Zoologia of the Universidade de São Paulo.

## RESULTS

### FLOWER AND FRUIT PHENOLOGY

*Duguetia furfuracea* has an extended flowering season blossoming from September to May with highest abundance of flowers between December and February. However, it is possible find at least some few plants with flowers every time along the year. Fruits were year round with a peak in fruit production from March to November. In the studied population, individuals presented distinct flowering rhythm, with some plants start flowering, other presenting just fruits, or other both (Figure 2).

Successfully pollinated pistils started to develop fruit, but their growth was irregular, with fruits initiated at the same time displaying different sizes during development. Fruits are mature

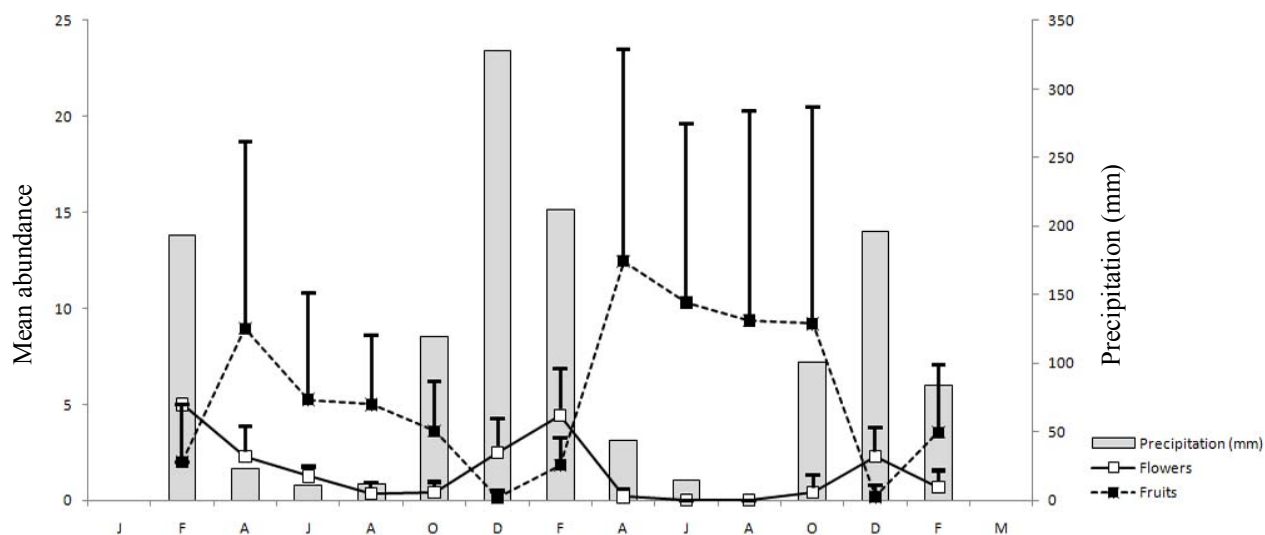


Figure 2 - Flower and fruit phenology of *D. furfuracea* (Annonaceae) and precipitation in four Cerrado phytophysiognomies (Campo sujo, Campo cerrado, Cerrado *sensu stricto*, Cerradão) at Itirapina from March 2006 to March 2008.

six-eight months later, between the end of the dry season and beginning of the wet season, when they become soft and dark yellowish and emit a strong scent. It is possible find mature fruits, flowers and pollinators during all months of the year because plants may bloom all year, in spite of producing fewer flowers during the dry season.

#### ANTHESIS AND FLORAL TRAITS

Buds achieved their greatest diameters ( $1.31 \pm 0.17$  cm (mean  $\pm$  SD;  $n = 9$ )) before anthesis. The length and width of petals, when they were totally expanded and the flowers were attracting beetles, were  $2.75 \pm 0.44$  cm and  $1.29 \pm 0.24$  cm (mean  $\pm$  SD;  $n = 23$ ), respectively. The base of three inner petals is fleshy, with many papillae, corrugated and purple, but the petals are more delicate and reddish-orange or purple at their extremity. The three external petals are very delicate (with no corrugations), and range from red-orange to purple. The inner petals form a floral chamber presenting a small orifice used by the beetles to access its interior (Figure 3A). This chamber has a centrally located androecium composed of many stamens ( $137.68 \pm 15.67$ ; mean  $\pm$  SD;  $n = 16$ ) that are strongly adherent to each other, and arranged helicoidally

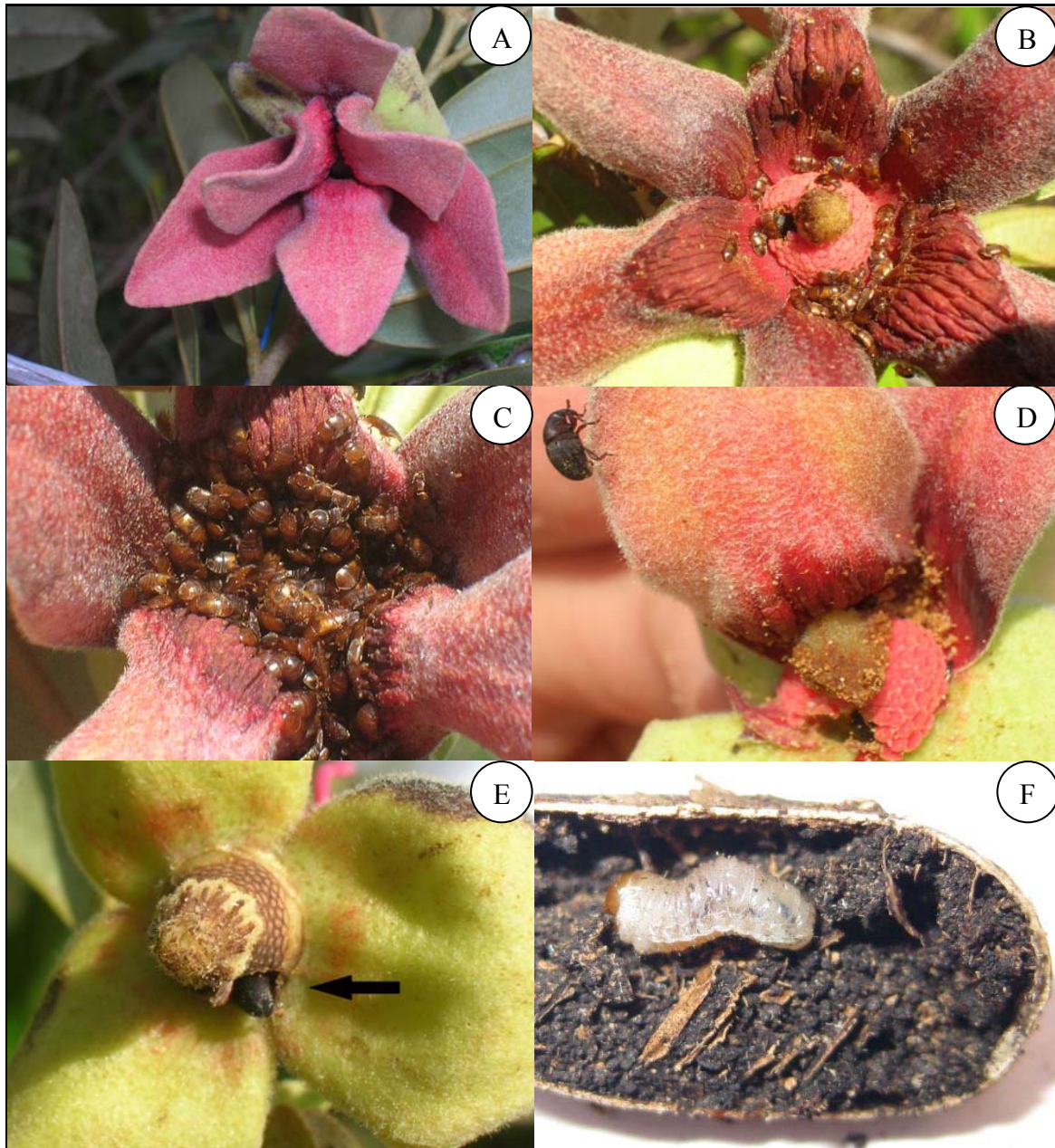


Figure 3 - A-H. *Duguetia furfuracea* (Annonaceae) floral structures and interactions: (A) Access to floral chamber in frontal view; (B) Inner perspective of floral chamber, flower structures and visitors; (C) overlap between female and male phases; (D) A *Plasilia* sp. (Curculionidae) beetle on petal and another making a hole in ovary; (E) Floral chamber full of beetle visitors, mainly *Colopterus* sp.3 (Nitidulidae); (F) *Lobiopa* sp. (Nitidulidae) on a inner petal of *D. furfuracea*; (G) Flower after senescence and *Plasilia* sp. still boring a hole in ovary; (H) One *Plasilia* sp. larva inside of *D. furfuracea* ovary. Figures B and D had petals manually opened to make pictures.

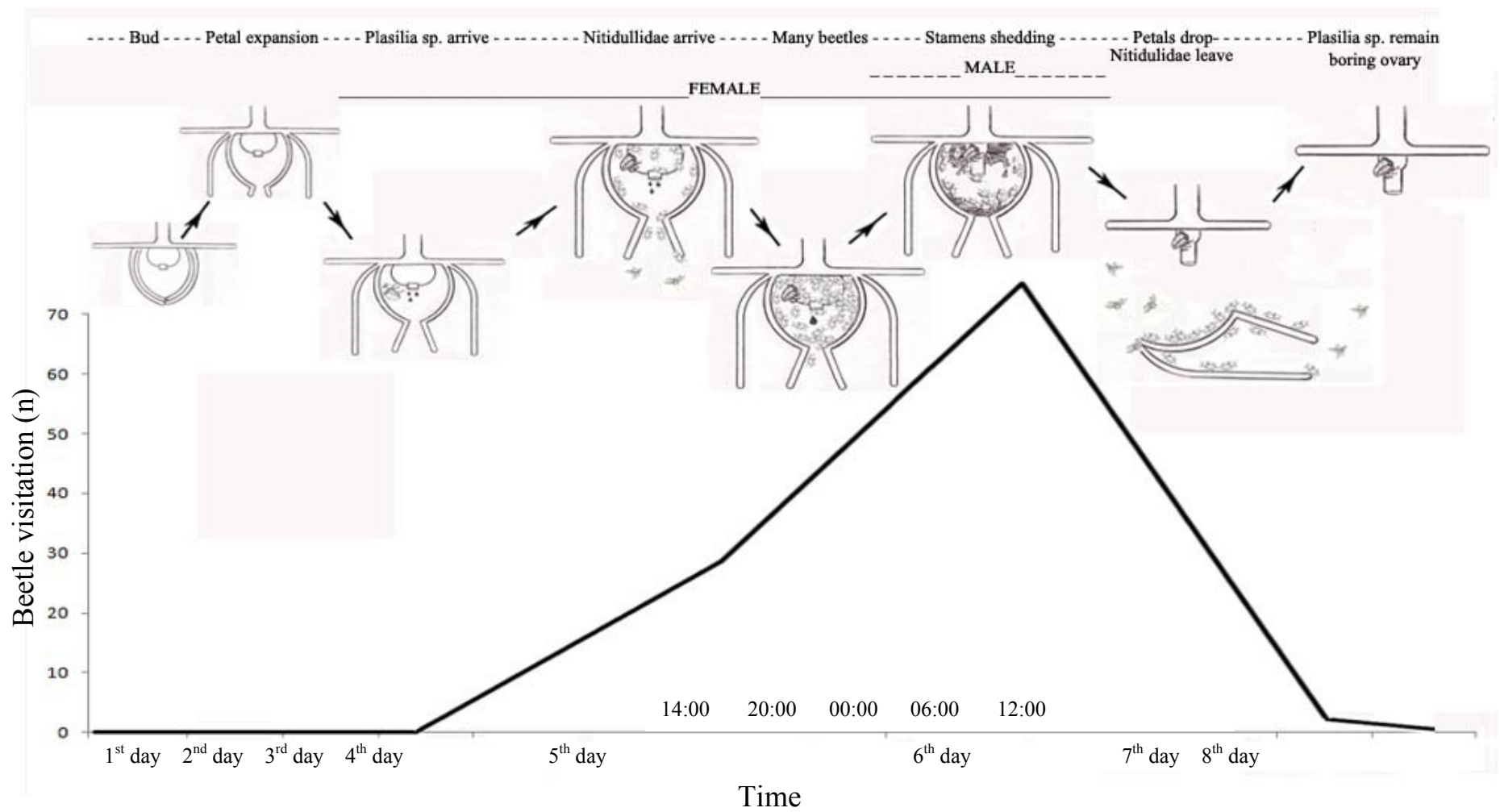


Figure 4 - Floral and beetle visitation chronology in *D. furfuracea* flowers in cerrado of Ecological Station at Itirapina-SP.

around the carpels ( $66 \pm 12.21$ ;  $n = 16$ ). This arrangement favors the contact of pollinators with both male and female structures during the period they stay inside the floral chamber gnawing petals, eating pollen and/or copulating (Figure 3B).

Flowers are perfect and distinctly protogynous, with the total period from bud opening to floral senescence of seven to eight days. Buds in pre-anthesis open with a split of the three green sepals, then the six petals start their expansion. In the beginning of the anthesis process petals vary between green-reddish and orange in color, and their stigmas have low exudate production. Flowers spent about five to six and six to seven days after the split of the sepals to reach pistillate (Figure 3B; 4) and staminate stages (Figure 4), respectively. During these stages, there was greater production of bright viscous exudate, intense odor emission, and more intense petal displayed color, ranging between dark red and purple. Pollinators were observed only in the pistillate and staminate stages.

The comparison of fruit set between flowers hand cross-pollinated under their early anthesis (fourth day) and late anthesis (fifth day) showed that only late flowers were receptive. Pistillate phase pollinations of 108 flowers yielded eleven fruit (10.19%) whereas early anthesis pollinations yielded only one fruit from 108 pollinated (0.92%) ( $t = 3.02$ ;  $df = 214$ ;  $p = 0.002$ ). These results indicate that flowers were not receptive in the early anthesis stage, but were receptive in the pistillate stage.

*D. furfuracea* has one-night flowering rhythm. In the fourth day starts the exudate production, but the pistillate stage begins after 5:00 pm of the fifth day when exhibited greater exudate production, stronger odor, and more intense flower color with petals little curled back (Figure 4). At this time, Nitidulidae beetles were observed arriving in the flowers (Figure 3B). The anthesis processes were predominantly nocturnal, but we verified anthesis during the day, principally during cloudy, rainy or cold days; anthesis is apparently dependent upon temperature. In general, a very pleasant, sweet odor reminiscent of its mature fruit was produced from the beginning of the afternoon to noon of the fourth day, but the scent was more intense during the night (8:00 pm first day- 6:00 am) of the fifth day (Figure 4), when there was higher beetle visitation (Figure 3C). There was exudate production from morning of fourth day to the end of the afternoon of the sixth day. Pollen release occurred mainly from 9:00 am to 6:00 pm of the sixth day. Thus, there was overlap of the pistillate and staminate stages in individual flowers, and natural self-pollination was frequent in this species as the anthers fell and they were kept inside

the floral chamber where self-pollen contacts receptive stigmas (Figure 4). Apparently, under natural conditions, even when beetles pollinated flowers there was some self-pollen deposition. At the end of the staminate phase, flowers withered slowly and scent production ceased, and the petals dropped. Sepals remained on the flowers for several days more (Figure 4).

## BREEDING SYSTEM

This species presented a reproductive efficacy of 0.70, with very low fruit set under natural conditions as in hand cross-pollinations. The emasculation treatment no produced fruits and the fruit set of spontaneous (bagged) and hand self-pollinated was very low and they were clearly the same. *D. furfuracea* presented an ISI (*sensu* Bullock 1985) of 0.13, although some fruit were formed from spontaneous and hand self-pollination, emasculated flowers produced no fruits, and we consider this a non-apomictic and preferentially self-incompatible species. Flowers from outcross pollen supplementation and that under natural pollination exhibited similar fruit set (7.17% and 5.02%, respectively) presenting no difference ( $\chi^2 = 0.9624$ ,  $df = 1$ ,  $p > 0.1$ ) suggesting that no occur pollen limitation in this population (Table 1). However, in the experiment with exclusion and no exclusion of *Plasilia* sp. both treatments with supplementary pollen addition produced higher fruit set and higher seed set (Table 2), indicating strongly that this population is really pollen limited.

Table 1 - Results of the hand pollination treatments in *D. furfuracea* from a Cerrado in southeastern Brazil, after emasculation. self-, cross- and open pollinations (fruit set (%) and fruits formed/treated flowers). Index of self-incompatibility (ISI *sensu* Bullock 1985). Reproductive efficacy *sensu* Ruiz and Arroyo (1978).  $ISI \leq 0.25$  is considered self-incompatible.

Emasculation	Self-pollination		Cross-pollination	Open pollination (Control)	Reproductive efficacy	ISI
	Spontaneous	Hand				
0 (0/45)	0.97 (3/310)	0.95 (2/210)	7.17 (17/237)	5.02 (12/239)	0.70	0.13
* n = 13	n = 37	n = 23	n = 42	n = 35	-----	-----

\* n – Number of individuals

Fruits start to grow within three to six days after petals fall, and may show fast initial development during the first month, followed by slow development to maturation over nearly an entire year. Thus, for most *D. furfuracea* reproductive individuals, fruit maturation occurs towards the beginning of the following wet season. Just as some flowering individuals were recorded throughout the entire year, some mature fruits could also be found the entire year. *D. furfuracea* presented a high fruit abortion rate no inferior to 54.54%, except for one treatment. Additionally, the similar fruit sets and abortion rate under pollen supplementation with *Plasilia* sp. and pollen supplementation with *Plasilia* sp. exclusion (Table 2).

#### FLORAL VISITORS AND THEIR ROLE

Just beetles were observed visiting *D. furfuracea* flowers and among them, two groups are ecologically very important for *D. furfuracea* reproduction: Curculionidae and Nitidulidae. During the flowering peak, we recorded 48,217 beetles inside floral chambers of 478 flowers of *D. furfuracea*. These visitors were very common and occurred in the most of the 478 observed flowers. The floral visitor fauna was composed exclusively of beetle species of two main families: Nitidulidae (99.3%), Curculionidae (0.54%), Scarabaeidae (0.004%), Staphylinidae (0.01%), Tenebrionidae (0.002%) and few unidentified beetles (0.13%). Nitidulidae was represented mostly by *Colopterus* sp.3 (98.2%; Figure 3C), followed by other *Colopterus* species and *Lobiopa* sp. and Curculionidae by a single species, *Plasilia* sp. (Figure 3D). On the other hand, the most frequent visitor was the nitidulid *Colopterus* sp. 3 (92.78%). Although the curculionid *Plasilia* sp. was much less abundant than nitidulid species, *Plasilia* sp. was very frequently observed in the flowers (44.92%). The other nitidulid species, Scarabaeidae, Staphylinidae, Tenebrionidae and indeterminate species were much less abundant and were less frequently encountered in *D. furfuracea* flowers (Table 3).

Beetles enter the flower in the middle of the afternoon of the fifth day when stigmas become receptive and emit odor (Figure 3B; 4). The period with highest visitation was from 8:00 pm to 10:00 am ( $100.99 \pm 109.57$ ; mean  $\pm$  SD; n = 478), going from 0 to 521 visitors in a single flower (Figure 3C; 4).

The curculionid beetles, *Plasilia* sp., showed dramatically different behavior compared with the other beetle species. From four observations on 30 flowers, this species arrived in the

Table 2 - Results of the exclusion and no exclusion of *Plasilia* sp. under natural pollination and hand pollen supplementation treatments for fruit set (fruit set (%) and (fruits formed/treated flowers), abortion (fruit abortion (%) and (fruit aborted/initial fruit set), seed consumption (seed consumption (%) (consumed seeds/seed set) in *D. Furfuracea* from a Cerrado in south-eastern Brazil. Ratio of benefit to cost for the *D. furfuracea*/beetle interaction (seed set percentage from pollination/seed consumption percentage from pollinators larvae).

Treatments	Fruit set			Seed set			Benefit: cost ratio
	Initial fruit set	Fruit abortion	Final fruit set	Total Seed set	Intact Seeds	Seed consumption	
<i>Open pollination</i>							
Natural pollination	12.5 (5/40)	80.0 (4/5)	2.5 (1/40)	19	78.94 (15/19)	21.06 (4/19)	3.75
Pollen supplementation	32.5 (13/40)	61.54 (8/13)	12.5 (5/40)	30	20.0 (6/30)	80.0 (24/30)	0.25
<i>Plasilia</i> sp. exclusion							
Natural pollination	5.0 (2/40)	0 (0/2)	5.0 (2/40)	19	100.0 (19/19)	0 (0/19)	
Pollen supplementation	27.5 (11/40)	54.54 (6/11)	12.5 (5/40)	56	100.0 (56/56)	0 (0/56)	



Table 3 – Flower visitors species and respective family with relative (%) and absolute abundance and occurrence in *D. Furfuracea* from a Cerrado in south-eastern Brazil from bimensal census along two consecutive years (March 2006 and February 2008).

Family	Abundance % (n)	Occurrence in Flowers % (n)	Species	Mean ± SD	Abundance % (n)	Occurrence in Flowers % (n)
CURCULIONIDAE	0.54 (263)	45.19 (169)	<i>Plasilia</i> sp.	0.55 ± 0.97	0.54 (263)	44.92 (168)
NITIDULIDAE	99.3 (47935)	94.12 (352)	Nitidulidae sp.1	0.004 ± 0.09	0.004 (2)	0.27 (1)
			Nitidulidae sp.2	0.04 ± 0.37	0.037 (18)	1.87 (7)
			<i>Colopterus</i> sp.1	0.25 ± 0.79	0.25 (121)	17.65 (66)
			<i>Colopterus</i> sp.2	0.46 ± 3.83	0.46 (222)	14.44 (54)
			<i>Colopterus</i> sp.3	99.17 ± 108.97	98.2 (47404)	92.78 (347)
			<i>Colopterus</i> sp.4	0.02 ± 0.32	0.016 (8)	0.54 (2)
			<i>Lobiopa</i> sp.	0.19 ± 0.72	0.19 (90)	15.77 (59)
			<i>Mystrops</i> sp.1	0.09 ± 0.85	0.087 (42)	1.87 (7)
			<i>Mystrops</i> sp.2	0.004 ± 0.09	0.005 (2)	0.27 (1)
			<i>Mystrops</i> sp.3	0.05 ± 1.19	0.06 (26)	0.27 (1)
			SCARABAEIDAE	0.004 (2)	0.53 (2)	Scarabaeidae sp.
STAPHYLINIDAE	0.01 (7)	1.07 (4)	<i>Scaphidiinae</i> sp.	0.002 ± 0.05	0.012 (6)	0.27 (1)
			Staphilynidae sp.	0.004 ± 0.06	0.002 (1)	0.54 (2)
TENEBRIONIDAE	0.002 (1)	0.27 (1)	Tenebrionidae sp.	0.002 ± 0.05	0.002 (1)	0.27 (1)
OTHERS	0.13 (65)	2.41 (9)	Others	0.14 ± 0.40	0.13 (65)	15.24 (57)
Total	100% (48273)				100% (48273)	----- (374)

flower before the pistillate stage, when the petals were not yet completely expanded and there was neither odor emitted nor exudate produced. Additionally, only this curculionid species remained in the flowers after their senescence (21; n = 30; Figure 3E; 4) and just this beetle species visited flowers after the petals and stamens had fallen (4; n = 30). The typical behavior of *Plasilia* sp. was to bore a hole in the ovary to eat ovules and to lay its eggs inside immediately after they penetrate in the floral chamber (Figure 3B; 3E; 4). *Plasilia* sp. always exhibited this behavior, even after floral senescence. *Plasilia* sp. not exhibited behavior typical of effective pollinator since they just walked over reproductive organs when arrived in the flowers when they still were beginning the anthesis process, exhibiting only their singular behavior of boring into the ovary to eat and oviposit. In addition, *Plasilia* sp. larvae were found consuming *D. furfuracea* seeds (Figure 3F) and two *Plasilia* adults emerged from 21 ripe fruits indicating that all phases of the life history of this curculionid beetle species are associated with *D. furfuracea*.

Considering the relative abundance of each species in relation to the total number of visitors recorded in *D. furfuracea* flowers, the main beetle species were *Colopterus* sp.3 (98.9%,  $99.17 \pm 108.97$ ; visitation %, mean  $\pm$  SD; n = 478), followed by *Plasilia* sp. (0.54%,  $0.55 \pm 0.97$ ; visitation %, mean  $\pm$  SD; n = 478), and *Colopterus* sp.2 (0.46%,  $0.46 \pm 3.83$ ; visitation %, mean  $\pm$  SD; n = 329). Thus, *Colopterus* sp.3 was the species that visited flower in greatest abundance (Figure 3C), whereas all others species presented low relative frequency of visitation. On the other hand, when we considered just their presence in the flowers (occurrence), *Colopterus* sp.3 and *Plasilia* sp. were species with higher percentages of occurrence (92.78 and 44.92%, respectively). Thus, in spite of their lower abundance, *Plasilia* sp. is very frequent visitor in *D. furfuracea* flowers. Moreover, nitidulid species in general and more specifically *Colopterus* sp.3 arrived in the floral chamber when the flowers were receptive and they exhibited a behavior of walk over the reproductive organs depositing pollen on the stigmas and they always left the flower after the staminate phase with a lot of pollen adhered in their bodies to visit new open flowers begging the pistillate phase favoring the pollination.

Co-pollinators were absent in this pollination system, as only beetles were observed pollinating flowers or collecting pollen. Though there were many beetle species involved in this pollination system, all species exhibited the same behavior, working as a unique group of pollinators. Only one beetle, the curculionid *Plasilia* sp., did not act just as pollinator, demonstrating also a seed-consuming and egg-laying behavior (Figure 3B; 3E; 3F; 4).

Moreover, open pollination treatments had same seed set under *Plasilia* exclusion and 21.06% of seed consumption with *Plasilia* visitation. Both pollen supplementation treatments had bigger fruit set than open pollination. An interesting result is that, in spite of the bigger seed set, the pollen supplementation treatment with *Plasilia* showed a higher seed consumption than the treatment with *Plasilia* in natural conditions (Table 2). Additionally, the seed set of fruits not visited by *Plasilia* sp. under natural conditions was  $16.77 \pm 5.63$  (mean  $\pm$  SD; n = 31). Thus, considering that *Plasilia* sp. larvae is responsible for 21.06% of the seed consumption, this beetle species interaction results a cost of 3.53 seeds from among 16.77 seeds of each fruit. The ratio of benefit to cost to the beetle pollinators from its interaction with *D. furfuracea* is about 3.75 (i.e., 78.94% fruit set from beetle pollination/ 21.06% fruit destruction by beetle larvae) and the benefit: cost ratio to pollen supplementation under natural presence of *Plasilia* sp decreased dramatically to 0.25 (Table 2).

## DISCUSSION

*Duguetia furfuracea* flowering is concentrate in the wet season, as in others Annonaceae species from cerrado area (Paulino Neto 1999, Gottsberger & Siberbauer-Gottsberger 2006a), what is predictable in most of pollination systems occurring in open cerrado areas (Gottsberger & Siberbauer-Gottsberger 2006a, b) which plants characteristic of these areas as *D. furfuracea* usually growth under limited nutritional levels and presenting alternation of periods of water excess and deficit water stress (Furley & Ratter 1988, Oliveira-Filho & Ratter 2002). In spite of the flowering peak in wet season, along of dry season, there are some *D. furfuracea* plants producing some flowers and this may be an evolutionary strategy to keep this high specialist pollinator fauna. Additionally, our data indicates that the flowering is concentrate in wet season and subsequent fruit development occurs in dry season, under water stressed conditions (Oliveira-Filho & Ratter 2002 ) as also registered to another annonaceous species of cerrado areas (Paulino Neto 1999).

The flower morphology of the *D. furfuracea*, including color, shape, and the fleshy and corrugate inner petals presenting many papillae, is very similar to other neotropical *Duguetia* species (Webber 1996, Paulino Neto 1999, Paulino Neto & Oliveira 2006). It also exhibits high beetle specialization found in several annonaceous species like not producing nectar, offering

only pollen and edible petals as food for its floral visitors and a floral chamber to shelter them from natural enemies and bad weather (Gottsberger 1988, 1989a, Gottsberger & Siberbauer-Gottsberger 2006a). The arrangement of stamens and pistils in the center of the floral chamber favors the contact of pollinators with both structures during each functional stage as they gnaw petals and walk over the reproductive structures. Additionally, protogyny also favors natural cross pollination since pollinators covered with pollen from the flowers visited the previous night arrive in the recently opened flowers during the pistillate stage promoting active pollen transfer when they walk over stigmas producing a sticky and viscous exudate. In the staminate stage, the pollinators already have sticky exudate on their bodies, and the release of pollen and the retention of this pollen inside the floral chamber helps the beetles leave the senescent flower covered with pollen. There is synchronized senescence and anthesis in the population, with the old flowers falling at the same time that recently opened flowers are receptive, emitting strong odor to attract the pollinators.

On the other hand, the overlap of pistillate and staminate stages allows a spontaneous self-pollination as registered in our pollination experiments and in others Annonaceae species, though this species displays lower fruit set (Paulino Neto 1999, Tomé 1999). However, the low fruit set from spontaneous self-pollination found here also may be consequence of resource limitation, probably it can be higher in fertile areas. The absence of fruit set from spontaneous self-pollination recorded for some Annonaceae species (Paulino Neto 1999, Kill & Costa 2003) can be resultant of the resource limitation and further studies are necessary to verify the consequences of resource limitation on the breeding system.

*Duguetia furfuracea* constitutes xenogamous species producing fruits mainly through cross-pollination and it is highly dependent of its pollinator species to reproduction. The greater cross-pollination fruit set, highly specialized floral morphology (floral chamber, higher odor emission during in the pistillate stage, thermogenesis), marked protogyny, the synchrony between the pistillate stage and pollinator visitation indicated a priori an out crossing breeding system.

If the fraction of flowers pollinated is less than maximum fruit set possible in relation to resource availability, then fruit set is pollen limited (Holland & DeAngelis 2006). Thus, when fruit production under natural conditions is lower than that resultant from hand cross-pollination, the former can be interpreted as being pollen limited (Duncan *et al.* 2004). Pollen limitation results in a low fruit production due to insufficient pollination service resultant from the

reduction of diversity and abundance of floral visitors (Steffan-Dewenter *et al.* 2002, Lopes & Buzato 2007) or reduction in pollen supply and/or mate density when plant or flower occur in lower densities (Duncan *et al.* 2004, Lopes & Buzato 2007). Nevertheless, the *D. furfuracea* reproductive efficacy can be considered high in comparison with other Cerrado plant species (see. Oliveira & Sazima 1990, Oliveira *et al.* 1992, 2004) and there was no difference between the fruit set from natural conditions (open pollination) and from hand cross-pollination suggesting that the pollination did not limit the reproductive output of the studied population. However, the lower seed set under natural conditions in comparison with pollen supplementation seed set suggests that this *D. furfuracea* population is pollen limited. As seed set constitutes a better evaluation unit of the plant fitness, we considered this *D. furfuracea* population as pollen limited.

The resource limitation occurs when the fraction of flowers pollinated is higher than maximum fruit set possible given resource availability. In this manner, fruit abortion is the fraction of pollinated flowers that do not set fruit due to limited resources for fruit development and maturation (Holland & DeAngelis 2006). How there was a similar abortion rate (higher than 54% ) both fruits under natural conditions and those pollen supplemented where there was *Plasilia* exclusion resulting in similar fruit amount indicated that the main abortion reason is resource limitation and not the seed consumption by *Plasilia* larvae. Selective abortion commonly occurs in another obligate pollination mutualisms when resources limit the fruit set that may simultaneously reduce the costs to the plant and maintain the seed consuming pollinator population when fruits with low egg/larva numbers are retained (Holland & Fleming 1999). In spite of *D. furfuracea* constitutes a resource limited obligate seed-consuming pollination system, the similar fruit sets and abortion rate with or not *Plasilia* sp. exclusion both under pollen supplementation or open pollination indicated that there was no selective abortion of fruit containing their larvae or with ovary bored and with eggs laid by this beetle species. According to Pellmyr and Huth (1994), the selective abortion could be a mechanism to avoid the overexploitation by seed consuming pollinators, but this mechanism apparently no occurs in *D. furfuracea*/beetle system.

However, how active pollination and flower oviposition are tightly coupled, evolved behaviors of mutualistic beetles in this pollinating seed-eating mutualism, the resource limitation and fruit abortion can be regulating the seed consuming pollinators beetle population, as also

discussed by Holland and DeAngelis (2006). Selective abortion and random abortion may result in similar seed-eating pollinator mortality. Then, there may be an evolutionary stable strategy of excess flower production with subsequent resource limited fruit set and fruit abortion, to maximize plant fitness through the regulation of the pollinator density. According to Shapiro and Addicott (2004), if hermaphroditic plants produce excess flowers and have limited resources available for fruit maturation, then the fruit set should be theoretically predicted to remain relatively invariable. In *D. furfuracea* there is an extensive flowering season with excess flower production, resource limitation, also, it consists in a pollinating seed-eating mutualism and *Plasilia* sp. visits flowers exhibiting high occurrence but low density. Thus, as in Yucca/Yucca moth and Senita/Senita moth, the random abortion resulting from excess flower production and resource-limited fruit set can maintain the seed-consuming pollinator population at low enough numbers that the pollinator population will not saturate the flowers with their eggs and larvae.

In addition, resource limitation, high pollinator-plant specialization, mutualistic interactions apparently coevolved, self-incompatible and oviposition into flowers by effective pollinators also was verified to others pollinating seed-consuming systems that in general also occur in arid regions like figs and fig wasps (Janzen 1979, Bronstein 1987, 1988, Addicott *et al.* 1990), yucca and yucca moths (Addicott 1986, Pellmyr *et al.* 1996, Pellmyr 2003), Senita cacti and Senita moths (Fleming & Holland 1998, Holland & Fleming 1999, 2002), *Trollius europaeus* and *Chiastocheta* flies (Pellmyr *et al.* 1996), *Lithophragma* and *Greya* moths (Pellmyr *et al.* 1996). As the fruit set of this population as well as many others cerrado species (Oliveira & Sazima 1990, Oliveira & Gibbs 2002, Oliveira *et al.* 1992, 2004) is very low, indicating that abiotic factors (e.g. water and soil fertility) can be limiting the development of a higher quantity of fruits since the Cerrado presents soil typically dry, poor in nutrient and very acid because the high aluminium presence (Gottsberger & Siberbauer-Gottsberger 2006a). Then, is very probable that *D. furfuracea* and the others pollinating seed-consuming systems discussed above evolved under rare and very similar ecological conditions that molded very specialized and complex plant pollinator interaction.

Nevertheless, there is one essential difference between *D. furfuracea* and all others pollinating seed-consuming interactions here discussed: there are two specialized floral visitant guilds. The first guild is composed just by the curculionid *Plasilia* sp. that apparently presents no relevance as pollinator since that arrives in the flowers some days before the pistillate stage.

These curculionid species no exhibits any pollination behavior, spends all time boring a hole in the ovary to eat ovules, lays its eggs, and leaves the floral receptacle few days after the floral senescence. The *Plasilia* sp. behavior indicates that this beetle species visits *D. furfuracea* flowers only interested in eat the ovules and oviposit and pollen or eatable petals appears no constitute a attractive. The second guild is composed by beetle pollinators that consists of Nitidulidae beetles species and very sporadic staphylinids. The pollination guild show high specificity (Paulino Neto & Buzato, unpublished data) and is very assiduous and abundant visitors, being observed in almost all flowers in pistillate stage in the study area (Hipólito F. P. N. pers. observ.) like observed in the pollinating seed-consuming systems before discussed.

The floral visitation in *D. furfuracea* was lower in the dry season and very intense during the wet season (flowering period) when frequently were registered hundred beetles visiting a single flower. Despite the variation in the absolute abundance, this visitant fauna presented practically of the same species composition and relative abundance as much in the census along two consecutive years as in the census conducted during the flowering peak. In addition, in an obligate pollination mutualism should present a specialization of the plant on a pollinator or vice-versa (Waser *et al.* 1996). In order to favor the evolution from a pollination system to obligate pollination mutualism, the pollinator's generations per flowering season should reflect duration of flowering season (Addicott *et al.* 1990, Waser *et al.* 1996). Thus *D. furfuracea* pollination system satisfy these premises since its floral visitors present high specificity with all phases of their life history being associated with the plant. In addition, they are present along the whole year with each species occurring with the same relative proportion along of the year evidencing a strong association between all life stages of its floral visitors and a synchronism between these partners.

Among the floral visitors, *Colopterus* sp.3 was considered the main visitor because it presented a relative abundance bigger than 98% and it occurred in about 92% of the visited flowers consisting in the visitant species more abundant and frequent in *D. furfuracea* in whole year. Additionally, whereas *Colopterus* sp.3 exhibits behavior that favors the pollination resulting in an active pollination and it is the more abundant and frequent visitant we considered it the main pollinator. Thus, as discussed by Addicott *et al.* (1990) and Holland & Fleming (1999), obligate pollination mutualism request a effective pollinator whose population dynamics are predictable and all pollinator species of *D. furfuracea* are presents along the whole flowering,

specially *Colopterus* sp.3. However, excepting *Plasilia* sp., the others species also were considered pollinators and they also constitute in an assiduous flower visitors along the year.

Our extensive field observations and experiments allow us classify the main visitors species in two guilds since each group exhibit a very distinct behavior when they visit the flowers and their visits affect the *D. furfuracea* fitness of opposite way. The first guild is composed just by *Plasilia* sp. that constitutes the unique curculionid species observed in *D. furfuracea*. This species stayed whole time boring a hole in the ovary to eat ovules and lay eggs inside. It was the unique floral visitant of *D. furfuracea* observed when flowers no offered the floral resource looked for its pollinators as petals, heat and pollen. In addition, the fact of beetles stayed boring a hole in the floral receptacle even after the senescence indicates that resources used by *Plasilia* sp. are more related with their reproduction than their feeding. Thus, *Plasilia* sp. adult no showed behavior that favors the pollination and it was considered florivorous whose progeny consumes seed of pollinated flowers resulting in higher fruit abortion that just very sporadically may promote some pollination.

The second visitor's guild is composed by pollinator species and it comprehend mainly by Nitidulidae species, but with Scarabaeidae, Staphylinidae and Tenebrionidae sporadically occurring. All these others beetles species visit *D. furfuracea* flowers mainly to feed petals and anthers as observed to others annonaceous (Gottsberger 1989a, 1994, Paulino Neto 1999, Gottsberger & Silberbauer-Gottsberger 2006a). *Colopterus* spp. was the more abundante visitors of *D. furfuracea* flowers and they visited the flowers only during the pistillate and staminate stages as also registered in *Duguetia lanceolata* St.Hil. (Paulino Neto 1999) and others annonaceous species from different gender (Gottsberger 1989a, 1994, Webber 1996, Paulino Neto 1999, Gottsberger & Silberbauer-Gottsberger 2006a). The beetles from this second guild arrive in the floral chamber in the begging of pistillate stage and actively pollinate flowers walking or staying over the stigmas as described for visitors of the most Annonaceae (Gottsberger 1989a, 1989b, 1994, Webber 1996, Paulino Neto 1999, Gottsberger & Silberbauer-Gottsberger 2006a). After staminate stage, these beetles usually dirty with pollen leave the senescent flower with pollen adhered in their bodies to visit others flowers initiating the pistillate stage and observed to almost all annonaceous species (Gottsberger 1989a, 1994, Paulino Neto 1999, Gottsberger & Silberbauer-Gottsberger 2006a). Thus, our data show that all these species may be considered pollinators since they present behavior favoring the pollination and their



flower visitation increased the fruit set. However, *Colopterus* species and among them, *Colopterus* sp.3 undoubtedly consists in the main pollinators and they are fundamental to fitness of *D. furfuracea*. However, undoubtedly, *Colopterus* sp.3 is very predictable visitors, which were present in almost all visited flowers, constitutes the principal flower visitor, representing 88.97% of visits. Co-pollinator are absent in this beetle-Duguetia pollination that have only beetles species involved. Although, there are several species visiting *D. furfuracea* flowers, all species are beetles that present a convergent behavior, act as one group of pollinators, being considered as pollinator guild.

Thus, as discussed by Addicott *et al.* (1990) to yucca/ yucca moth and by Holland and Fleming (1999) to senita/ senita moth systems, appropriate life history traits must be present for the development of obligate mutualism in an ecological interaction systems. *D. furfuracea*/ beetle presents convergent life history traits with both systems, such as: 1) long lived plant populations presenting many reproductive episodes; 2) pollinator's generation time cannot be longer than the duration a flowering season; 3) at least one effective pollinator whose population dynamics are predictable; 4) synchrony between flowering phenology and a pollinators life cycle; 5) resource limited fruit set with water likely being the limiting resource; flowers are self-incompatible and produce little or no nectar; 6) co-pollinators are temporally unreliable (yucca) or absent (senita and *D. furfuracea*); 7) larvae destroy 20-30% of the seed crop resulting benefit: cost ratios of 2-5. However, *D. furfuracea*/ beetle system is closer to the senita/ senita moth system since also presents 1) an extended flowering season; 2) pollinators have several generations per year. So many shared characteristics in these independently derived mutualisms indicates that they evolved under similar selection pressures and according to Fleming and Holland (1998), nocturnal anthesis, self-incompatibility and resource limited fruit set are very important in the evolution of these obligate mutualisms. Thus, we considered the *D. furfuracea* pollination system a new case of obligate mutualism since that it presents all life history traits necessary to the development of an obligate mutualism and still constitutes an ancient relationship between beetles and most annonaceous species evolved many floral adaptations (Gottsberger 1988, 1989a, Webber 1996, Bernhardt 2000, Siberbauer-Gottsberger 2006a).

Second Holland and Fleming (1999), the oviposition in flowers and subsequent predispersal seed consumption by larvae of adults that pollinated flowers is extremely rare. Known cases of both pollinating and seed-consuming interactions between plants and their

pollinators include *Silene vulgaris* and *Hadena* moths (Pettersson 1992a), *Lithophragma* and *Greya* moths (Thompson & Pellmyr 1992), *Trollius* spp. and globeflower flies (Pellmyr 1989), *Ficus* and fig wasps (Janzen 1979, Bronstein 1987, 1988), *Yucca* and yucca moths (Addicott 1986, Pellmyr *et al.* 1996, Pellmyr 2003) and Senita cactus and Senita moths (Fleming & Holland 1998, Holland & Fleming 1999, 2002). We also considered this very coevolved pollination system as new pollinating seed-consuming mutualism because in spite of oviposition have been recorded just to *Plasilia* sp., it is very probably that oviposition into the floral chamber also occur to *Colopterus* species and the others pollinators species since that this constitutes a very spread behavior in annonaceous pollination systems (Siberbauer-Gottsberger 2006a, pers. obs.), but this aspect requires more investigation in the specific *D. furfuracea* case.

In some obligate pollination mutualisms, selective abortion of fruits of fruits containing many eggs or larvae should occur when the fruit set is resource limited. Selective abortion simultaneously may reduce the costs to the host plant and maintain the pollinator population when fruits with low egg/larva numbers are retained. In this situation, selective abortion constitutes an ecological mechanism to inhibit over exploitation in seed-consuming pollination systems (Holland & Fleming 1999). On the other hand, in some situations, the selective abortion of fruit based on pollination quality and quantity is plausible since this provides a strong selective pressure for the evolution of active pollination. In this case, flowers with greater pollination quality and quantity should be, preferentially retained by plants where resources limit fruit production with subsequent increase of the progeny survival of active pollinators (Pellmyr *et al.* 1996). In *D. furfuracea*, pollen supplementation results higher seed set/fruit becomes evident the pollen limitation, but this increase of seed set also results in a seed consuming amplification resulting in a strong decrease of the intact and healthy seed. Then, a higher pollinator efficiency would reduce the benefit to cost ratio from 3.75 to 0.25 because the *Plasilia* sp. interaction under natural conditions. However, it worth stick out that in *D. furfuracea* populations where there was no *Plasilia* sp. or another seed consumers, the higher pollinator efficiency could significantly increase the plant fitness since the seed set would increase almost four times and wouldn't there be seed consumption.

Thus, the *D. furfuracea* pollination system satisfy all evolutive traits request in an obligate mutualism and both beetle visitors guilds oviposit into *D. furfuracea* flowers where adults confer a positive effect during the pollination and their progeny produce a cost to the plant by partial

seed consumption or inducing fruit abortion. However, this system presents a special particularity in comparison with others pollinating seed-consuming mutualism because have a florivory guild composed by the curculionid *Plasilia* sp. that is so specialized and predictable like the main pollinators and that impose the higher cost to the plant fitness. On the other hand, this pollination system appear be in a ecological equilibrium since *Plasilia* sp. always occur in low abundance in each visited flower and the main pollinators are much higher abundante along whole flowering season. This *D. furfuracea* population presents an extensive flowering season with production of a huge quantity of flowers along the year among theirs countless individuals indicating that this probable constitutes an evolutive strategy to support as the pollinators as the florivorous beetles. In addition, how *D. furfuracea* is a species abundante in the studied area and its fruits were observed along whole period of study, its population as a whole appears presents a satisfactory reproduction rate and it appears be adapted simultaneously to a high cost imposed by resource limitation characteristic of most cerrado areas and to their seed consumers pollinators.

In conclusion, *D. furfuracea* pollination system constitutes the first obligate pollination mutualism case involving different guilds of visitors and also the first pollination system combining a predictable and reliable pollinators , seed consumption, resource and pollen limitation resulting in a complex and efficient mechanism to regulate the seed consuming visitor`s population and optimize the plant fitness.

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## **CAPÍTULO 4**

### **Considerações finais**



Uma alta proporção de espécies de plantas tem suas flores visitadas por besouros em todas quatro fitofisionomias de Cerrado estudadas (12-40%) indicando que os dados disponíveis até o momento subestimam a ocorrência de cantarofilia para áreas de Cerrado.

O Cerrado *s. s.* e Cerradão, fitofisionomias de cerrado com maior abundância de plantas arbóreas, foram as áreas que apresentaram maior proporção de espécies de plantas visitadas por besouros.

O número de espécies de besouros varia entre fitofisionomias e alto número de espécies ocorreu em áreas de Campo cerrado e Cerrado. Adicionalmente, não encontramos relação direta entre abundância de flores e besouros para a escala estudada dentro de cada fisionomia.

Este consiste no primeiro estudo focando toda uma comunidade de besouros associados a flores.

Não houve variação temporal entre anos tanto para as redes de visitantes-flores ou para redes de polinizadores-plantas.

Redes de polinizadores-plantas tiveram espécies de besouros altamente especialistas, mas as espécies de plantas foram menos especialistas que plantas da rede visitantes-flores, apresentando maior média de interações por espécies de planta. Portanto, as redes de polinizadores-plantas apresentaram maior conectância que as redes de visitantes-flores. A menor conectância obtida para as redes visitantes-flores é resultante do registro de visitantes florais ocasionais que muito esporadicamente visitaram flores, na maioria das vezes, ao visitarem flores, não exibiam comportamento que favorecesse a transferência de pólen.

*Attalea geraensis*, *C. pubescens*, *D. furfuracea*, *D. hispida*, *K. coriacea*, *S. petrea*, *T. formosa* e *X. aromatica* consistem em espécies de planta envolvidas em muitas interações e foram consideradas espécies centrais sendo as principais responsáveis pelo padrão aninhado registrado para as redes de interações estudadas no presente trabalho.

Tanto as redes de visitantes-flores, como a rede de polinizadores-plantas evidenciaram uma estrutura composta combinando estrutura de rede aninhada com compartimentada, mas com predomínio do padrão compartimentado. Estes compartimentos são resultantes tanto das muitas interações espécie-específica entre espécies de besouros e plantas, como daquelas espécies de planta que interagem com várias espécies especialistas de besouros. Portanto, os compartimentos são definidos como subconjuntos de espécies de visitantes florais ou de polinizadores interagindo com espécies centrais de plantas.

A frequência de distribuição de interações indica que as redes visitantes-flores constituem um sistema de interação muito especializado com 62-84% das espécies de besouros visitando apenas uma espécie de planta e número médio de espécies de visitantes florais por espécies de planta menos que 1,5. Em comparação com estudos semelhantes para diferentes ordens de inseto, nós consideramos esta rede de interações entre besouros e flores como a rede flores-besouros e polinizadores-plantas como a mais especializada já estudada.

De modo geral, focando atenção nos besouros, ambas as redes, visitantes-flores e polinizadores-plantas foram definidas como altamente especializadas já que visitaram flores poucas espécies de planta tanto para comparações entre anos, como entre fitofisionomias.

Embora especialização seja considerada rara em florestas tropicais e mais comuns em regiões temperadas e predomina em áreas de deserto, o presente estudo mostrou que o Cerrado, habitat tipicamente tropical, apresentou sistemas de interação entre besouros e flores de espécies de planta com alta especificidade.

Dentre as várias espécies compreendidas nestas redes de interação de alta especificidade, merece desta *D. furfuracea* que apresenta uma fauna composta por várias espécies de besouros polinizadores altamente especialistas e apesar de apresentar pico de floração na estação chuvosa, floresce o ano todo como estratégia para manter seus polinizadores.

*Duguetia furfuracea* é uma espécie auto-incompatível, portanto altamente dependente de seus polinizadores para reprodução e cuja população estudada apresentou tanto limitação polínica quanto limitação de recursos no solo.

Há duas guildas especializadas de besouros interagindo com *D. furfuracea*. A primeira é composta por uma única espécie de curculionídeo (*Plasilia* sp.) que visitam suas flores em baixa abundância (média de 0,55 besouros por flor) mas com alta frequência de ocorrência (44,9% das flores). Os adultos ovipõem no interior do ovário e suas larvas consomem as sementes dos frutos que completam desenvolvimento. A segunda guilda é composta por várias espécies de besouros nitidulídeos, principalmente *Colopterus* sp.3, que visitam as flores em grande abundância (média de 99 besouros por flor) e também com alta ocorrência de visitação (92% das flores) que efetivamente promovem a polinização.

O sistema de polinização de *D. furfuracea* consiste no primeiro caso de polinização mutualística obrigatória envolvendo diferentes guildas de visitantes florais. Este também é o primeiro sistema de polinização combinando polinizadores previsíveis e confiáveis, consumo de sementes, limitação polínica e limitação por recursos, o que resulta em um complexo e eficiente mecanismo para regulação da população do visitante floral consumidor de sementes e para otimizar o sucesso reprodutivo da planta.