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"INTERAÇÕES ENTRE FORMIGAS, FRUTOS E SEMENTES EM SOLO DE CERRADO: O PAPEL DE FORMIGAS NA BIOLOGIA DE SEMENTES E PLÂNTULAS"

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Orientador: Prof. Dr. Paulo Sérgio M. C. de Oliveira

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Dedico este trabalho aos tantos brasileiros anônimos, que mesmo sem serem reconhecidos, sonham e trabalham por um Brasil diferente.

"*Tudo está conectado*"
Alexander von Humboldt

"*La muerte no es verdad cuando se ha cumplido bien la obra de la vida*"
José Martí

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Índice

Abstract

Our knowledge about seed dispersal systems has been improved considerably in the last few years. An increasing number of studies has shown that the process of plant regeneration is often much more complex than we realize, including several different agents across subsequent steps of seed dispersal. For instance, ants may reshape the seed shadow after seeds fall to the ground, and this may influence the transition probabilities from seed to the seedling stage in plant recruitment. Here we report data showing that ants are important agents of secondary seed dispersal in the largest South American savanna, the cerrado. Ants interacted with fallen diaspores of many plants primarily dispersed by vertebrate frugivores. Ants often cleaned the seeds from fruit matter, what increased seed germination. The seeds of *Erythroxylum pelleterianum* (Erythroxylaceae), *Xylopia aromatica* (Annonaceae) and *Miconia rubiginosa* (Melastomataceae) are primarily dispersed by birds, but most fruits fall to the ground under the parent tree. Ants removed a considerable number of fallen diaspores of these plants, and provided directed dispersal to nutrient-enriched microsites where seedling survival was increased, as shown for *E. pelleterianum*. However, this benefit sometimes is attained at the cost of significant seed loss to granivorous ants, as in the case of *Xylopia aromatica*. Birds are likely responsible for long-distance dispersal and colonization of new patches, while ants reshape the seed shadow at a finer scale, delivering seeds to specific sites where seedling survival is more likely. Although most ant taxa were generalist in relation to diaspore traits, leaf-cutter ants showed a preference pattern for some diaspores, particularly carbohydrate-rich ones. We suggest that such preference may be driven by the chemically-protected plant leaves of the cerrado, which would constrain leaf-cutter ants to rely on fleshy fruits for fungus culturing inside their nests. Finally, we showed that ant-diaspore interactions are susceptible to edge effects, which decrease benefits obtained by plants secondarily dispersed by ants. This information is particularly relevant, since the cerrado is currently being converted to cropland at an alarming rate, and so far edge effects had not been recognized in the cerrado.

Resumo

Nosso conhecimento a respeito dos sistemas de dispersão de sementes tem aumentado consideravelmente nos últimos anos. Um número crescente de estudos tem mostrado que a regeneração de plantas é freqüentemente muito mais complexa do que pensávamos, incluindo vários agentes ao longo de etapas subseqüentes do processo de dispersão de sementes. Por exemplo, formigas podem rearranjar a sombra de sementes que cai ao solo, o que pode influenciar as probabilidades de transição do estágio de semente para plântula durante o recrutamento. Neste trabalho reportamos informações mostrando que formigas são importantes agentes de dispersão secundária de sementes na maior savana da América do Sul, o cerrado. Formigas interagiram com diásporos caídos de muitas plantas dispersas primariamente por vertebrados frugívoros. Formigas freqüentemente limparam as sementes da polpa dos frutos, o que aumentou sua germinação. As sementes de *Erythroxylum pelleterianum* (Erythroxylaceae), *Xylopia aromatica* (Annonaceae) e *Miconia rubiginosa* (Melastomataceae) são dispersas primariamente por aves, mas a maior parte de seus frutos cai ao solo sob a planta-mãe. Formigas removeram grande parte destes diásporos caídos, e promoveram dispersão direcionada a microsítios ricos em nutrientes onde houve maior sobrevivência de plântulas, como demonstrado para *E. pelleterianum*. Contudo, este benefício por vezes foi alcançado à custa de perdas significativas de sementes para formigas granívoras, como em *Xylopia aromatica*. Aves são responsáveis pela dispersão de sementes a longas distâncias e colonização de novos sítios, enquanto formigas rearranjam a sombra de sementes numa escala menor, depositando-as em sítios onde a sobrevivência das plântulas é aumentada. Embora a maioria das formigas foi generalista em relação às características dos diásporos, formigas cortadeiras mostraram algumas preferências, especialmente por diásporos ricos em carboidratos. Nós sugerimos que estas preferências podem ser devidas às defesas químicas da folhagem de plantas do cerrado, que forçariam as formigas a depender de frutos carnosos para o cultivo de fungo no interior dos ninhos. Finalmente, nós mostramos que as interações formiga-diásporo são suscetíveis a efeitos de borda, que diminuem os benefícios obtidos por plantas dispersas secundariamente por formigas. Esta informação é especialmente relevante, uma vez que o cerrado está sendo convertido para agricultura a taxas alarmantes e efeitos de borda não haviam sido reconhecidos para o cerrado até o momento.

Introdução geral

Identificar os agentes responsáveis pela dispersão de sementes e acompanhar o destino destes propágulos sempre foi uma questão fascinante para ecólogos, sendo também fundamental para compreender a diversidade e dinâmica de espécies vegetais (Janzen 1970, Connel 1971, Wang & Smith 2002, Jordano *et al*. 2007). Com o avanço dos estudos sobre dispersão de sementes, percebeu-se que os sistemas de dispersão são freqüentemente mais complexos do que imaginados até então, envolvendo vários agentes ao longo das etapas sucessivas de recrutamento de uma planta (Clifford & Monteith 1989, Kaufmann *et al*. 1991, Böhning-Gaese *et al*. 1999, Andresen 2002, Passos & Oliveira 2002). Até recentemente os estudos consideravam apenas a dispersão primária das sementes, ou seja, a remoção dos frutos da planta-mãe e sua deposição a alguma distância, sobre o solo. Esses estudos assumiam de maneira implícita que uma vez a semente tendo aterrissado sobre o solo, estaria determinado o lugar onde esta poderia germinar e recrutar um novo indivíduo, ou morrer ao longo de qualquer das etapas do recrutamento (Wang & Smith 2002, Figura 1). Contudo, uma vez sobre o solo, as sementes são freqüentemente transportadas para outras localidades pelos mais variados agentes, sejam abióticos, como enxurradas (Hampe 2004), ou bióticos, como animais que removem as fezes com sementes (Andresen 2002), ou removem as sementes das fezes e dos regurgitos de dispersores primários, ou os próprios frutos caídos sob a planta-mãe (Forget & Milleron 1991, Kaspari 1993, Passos & Oliveira 2002). Estudos recentes têm demonstrado que a dispersão secundária de sementes *(i.e.* o transporte das sementes uma vez que estas tenham chegado ao solo) pode alterar substancialmente a distribuição espacial da sombra de sementes, bem como as probabilidades de recrutamento de um novo indivíduo (Roberts & Heithaus 1986, Böhning-Gaese *et al*. 1999, Passos & Oliveira 2002, Vander Wall *et al*. 2005, Figura 1).

Boa parte das árvores e arbustos de florestas e savanas tropicais produz diásporos (i.e. frutos ou sementes que atuam como a unidade de dispersão) carnosos, que funcionam como uma recompensa atrativa para animais que atuam como dispersores de sementes. De fato, vertebrados, em especial as aves, são os principais agentes responsáveis pela remoção de frutos da copa das árvores e dispersão primária de sementes (Jordano 2000). Boa parte desses diásporos, no entanto, cai nas proximidades da planta-mãe naturalmente por abscisão do fruto, ou são derrubados durante o movimento dos animais frugívoros na copa, além de chegarem ao solo em regurgitos ou fezes de vertebrados (Howe 1980, Laman 1996). Uma vez sobre o solo esses diásporos ficam disponíveis a uma grande variedade de outros animais que utilizam parte ou todo o diásporo como alimento, podendo remover a polpa *in situ* ou transportá-lo para consumo posterior a alguma distância da planta-mãe. Roedores, lagartos, aves e peixes, além de besouros Scarabaeidae e formigas, comumente interagem com diásporos caídos (Goulding 1980, Forget & Milleron 1991, Figueira *et al*. 1994, Andresen 2002, Pizo & Oliveira 1998). Embora parte desses diásporos seja consumida por animais granívoros, freqüentemente essas interações beneficiam as plantas porque parte das sementes são depositadas intactas em locais adequados à germinação, a alguma distância do local de deposição original (Forget & Milleron 1991, Levey & Byrne 1993, Retana *et al*. 2004).

Graças à sua inegável biomassa e dominância numérica, formigas (Hymenoptera: Formicidae) estão entre os animais com maior probabilidade de interagirem com diásporos caídos. Por exemplo, formigas estão entre os insetos mais abundantes na maior parte dos ecossistemas terrestres, com exceção dos pólos e o cume de montanhas elevadas

(Hölldobler & Wilson 1990). Formigas, junto com cupins (Isoptera) compõem um terço de toda a biomassa animal terrestre na Floresta Amazônica (Fittkau & Klinge 1973), superando inclusive a contribuição de todos vertebrados terrestres. Formigas incluem ainda uma grande variedade de itens alimentares em sua dieta, inclusive produtos de origem vegetal como néctar, pólen, exsudatos, frutos e sementes (Hölldobler & Wilson 1990). Por essas razões, não chega a ser surpreendente constatar que formigas estão entre os animais que mais rapidamente descobrem e utilizam diásporos caídos ao solo (Hughes & Westoby 1990, Pizo & Oliveira 1998). Em algumas vegetações que crescem em regiões áridas e de solos pobres ao redor do mundo, como os “fynbos” sul-africanos e as formações vegetacionais esclerófilas da Austrália, além do sub-bosque de florestas temperadas do hemisfério norte, formigas são agentes comuns de dispersão primária de sementes, removendo-as diretamente da planta-mãe ou no solo (Beattie 1985). Muitas plantas nessas formações vegetais produzem diásporos mirmecocóricos, ou seja, com estruturas especializadas que atraem e estimulam o transporte por formigas (van der Pijl 1982). Uma característica típica desses diásporos é a presença de um elaiossomo, uma estrutura carnosa e freqüentemente rica em lipídeos aderida à testa da semente. Formigas utilizam o elaiossomo como um suporte para o transporte da semente até o ninho, onde o elaiossomo é removido para ser consumido por adultos e larvas da colônia, e a semente descartada, na maioria das vezes intacta (Beattie 1985). Já foi demonstrado que o consumo de elaiossomos aumenta a produção de indivíduos alados nas colônias de formigas e, portanto, seu valor adaptativo (e.g. Morales & Heithaus 1998). Assim, a interação formiga-planta mirmecocórica é tipicamente mutualística.

Do ponto de vista das plantas, a dispersão de sementes por formigas parece ter se constituído em um fator de seleção importante, já que a mirmecocoria evoluiu de maneira convergente em mais de 3.000 espécies de plantas distribuídas em cerca de 80 famílias (Berg 1975, Beattie 1985). Essas plantas podem se beneficiar da dispersão de sementes por formigas de várias maneiras (veja revisão em Giladi 2006): (a) escape de predadores, pois o transporte por formigas e o local aonde são depositadas as sementes, enterradas no solo, diminuem as chances de encontro das sementes por predadores (Heithaus 1981, Higashi *et al*. 1989); (b) escape do fogo, pois o descarte das sementes enterradas no solo pelas formigas aumenta a sobrevivência das sementes durante episódios de fogo porque o solo funciona como um isolante térmico (Christian & Stanton 2004); (c) distância de dispersão, pois ao remover sementes caídas sob a copa, formigas reduzem a competição da planta-mãe com as plântulas e entre plântulas aparentadas, geralmente encontradas em grande densidade sob a planta-mãe (Higashi *et al*. 1989, Retana *et al*. 2004); (d) dispersão direcionada, pois sementes dispersas por formigas são depositadas em micrositios onde a performance das sementes e plântulas é melhor do que em relação a sítios distribuídos ao acaso, provavelmente devido à melhor qualidade do solo nas proximidades do ninho em virtude da própria atividade das formigas que aí depositam o “lixo” das colônias (restos de presas, sementes, indivíduos mortos, etc.) (Hanzawa *et al*. 1988); (e) limitação de nutrientes, pois como elaiossomos não demandam quantidades relevantes de potássio para sua produção, estas estruturas são consideradas de custo relativamente baixo para plantas que crescem em solos pobres em potássio, um nutriente necessário em grande quantidade para a produção de frutos carnosos (Hughes *et al*. 1993). Embora o contexto determine uma importância relativa maior para algumas das hipóteses indicadas acima na evolução da dispersão de sementes por formigas, todas recebem algum tipo de suporte na literatura (revisões em Giladi 2006, Rico-Gray & Oliveira 2007).

Embora a mirmecocoria confira vantagens adaptativas a muitas espécies de plantas ao redor do mundo, em florestas e savanas neotropicais a ocorrência da mirmecocoria é bastante baixa, com exceção de algumas espécies de Maranthaceae e Gesneriaceae (Horvitz & Beattie 1980, Lu & Mesler 1981). No Neotrópico, elaiossomos estão presentes também em sementes de algumas plantas dispersas primariamente pela abertura explosiva dos frutos, que lançam as sementes a alguma distância da planta-mãe (por exemplo, Euphorbiaceae). Formigas então coletam as sementes com elaiossomos e as transportam até seus ninhos (Passos & Ferreira 1996). Sistemas de dispersão de sementes que envolvem mais de um agente ao longo de etapas subseqüentes de dispersão são conhecidos como diplocóricos (Vander Wall & Longland 2004). Embora a maior parte das árvores e arbustos de florestas tropicais não apresente adaptações aparentes à dispersão por formigas, estudos recentes mostram que formigas interagem com muitos desses diásporos não-mirmecocóricos caídos ao solo e podem potencialmente beneficiar as plantas (Farji Brener & Silva 1996, Leal & Oliveira 1998, Pizo & Oliveira 2000, Passos & Oliveira 2002, 2003) (Figura 2).

Pouco se sabe sobre a prevalência e a distribuição taxonômica da diplocoria, bem como as condições que propiciaram seu surgimento, além da conservação dessas interações (Vander Wall & Longland 2004). Formigas são organismos ideais para investigar estas questões porque a quantificação da contribuição de formigas na remoção de diásporos caídos é factível por meio de experimentos simples, além do acompanhamento do local exato de deposição das sementes (e.g. no ninho). Por outro lado, as formações savânicas que compõem o cerrado brasileiro possuem a maior parte das espécies de árvores e arbustos produzindo diásporos carnosos cuja dispersão primária é realizada por vertebrados (Gottsberger & Silberbauer-Gottsberger 1983) e crescem associadas a solos pobres em nutrientes, estando sujeitas a episódios recorrentes de fogo (Oliveira-Filho & Ratter 2002). Estas características fazem do cerrado um local onde a dispersão de sementes por formigas pode conferir algumas vantagens adaptativas. Assim, este estudo buscou investigar a existência de sistemas diplocóricos de dispersão de sementes no cerrado e a importância de uma eventual participação de formigas para a performance de sementes e plântulas nestes sistemas.

Objetivos gerais

(1) Identificar as espécies de formigas e de diásporos por elas explorados em três sítios de estudo no sudeste do Brasil.
(2) Identificar padrões de utilização de diásporos por formigas no cerrado.
(3) Investigar possíveis efeitos das formigas sobre a dispersão, germinação e estabelecimento de espécies vegetais no cerrado.
(4) Comparar os efeitos de vertebrados e formigas na regeneração e desempenho de plântulas de espécies vegetais selecionadas.
(5) Investigar os efeitos da fragmentação do cerrado sobre as interações formiga-diásporo.

Esta tese foi dividida em duas grandes frentes de investigação. Na primeira parte, procurei descrever e caracterizar as interações formiga-diásporo no cerrado e suas conseqüências imediatas para as plantas com as quais interagem. O **Capítulo 1** descreve as espécies de formigas e de diásporos observados em interação no cerrado. A existência de padrões nas interações entre formigas e plantas, com possíveis conseqüências para a regeneração de espécies vegetais, é investigada neste capítulo. O **Capítulo 2** compara o papel de formigas e vertebrados na remoção de diásporos caídos ao solo do cerrado e os possíveis benefícios para a germinação de sementes resultantes da interação com formigas.

A influência de características (morfológicas e químicas) dos diásporos na remoção e distância de dispersão por formigas é investigada neste capítulo. Na segunda parte da tese, investiguei em detalhe a contribuição de formigas para a regeneração de três espécies vegetais nativas, e como o resultado destas interações varia em ambientes sujeitos a perturbações decorrentes da fragmentação do cerrado. Os **Capítulos 3 e 4** comparam a contribuição de vertebrados e formigas para a regeneração de três espécies nativas do cerrado: *Xylopia aromatica* (Annonaceae), *Erythroxylum pelleterianum* (Erythroxylaceae) e *Miconia rubiginosa* (Melastomataceae). Nestes capítulos registro a produção de frutos e sementes por estas espécies e estimo as probabilidades de transição entre diferentes etapas consecutivas da regeneração destas plantas, identificando a contribuição da dispersão primária e secundária de sementes nestes processos. A hipótese de dispersão direcionada de sementes é de especial relevância no contexto destes capítulos. Aqui são sugeridos alguns dos fatores possivelmente relacionados ao surgimento de alguns sistemas complexos de dispersão de sementes de espécies vegetais tropicais. Finalmente, no **Capítulo 5** é abordada a susceptibilidade das interações formiga-diásporo a efeitos de borda decorrentes da fragmentação do cerrado. Uma vez que formigas e plantas respondem a variações de temperatura, luz e umidade, investigo neste capítulo como as interações formiga-diásporo variam nas bordas em relação ao interior de um fragmento de cerrado, e como estas variações influenciam no desempenho das plântulas de *Erythroxylum pelleterianum*.

Os capítulos que compõem a tese foram redigidos em inglês a fim de agilizar sua publicação.

Área de estudo

Este estudo foi realizado entre maio de 2003 e agosto de 2006 nos cerrados da Estação Ecológica (ECO) e da Estação Experimental (EXP) de Itirapina, no município de Itirapina, centro-leste do estado de São Paulo (22º12'S, 47º51'O) (Figura 3). Ambas as áreas estão a aproximadamente 730 m de altitude (a.n.m.), com temperatura média anual de 21,8º C e sujeitas a geadas ocasionais durante o inverno. A pluviosidade média anual é de 1.360 mm, com uma estação quente e úmida de outubro a março e uma estação fria e seca de abril a setembro (Figura 4). O clima é do tipo Cwa, segundo a classificação de Köppen, mesotérmico de inverno seco (Giannotti 1988). O solo é classificado como neossolo quartzarênico, caracterizado pela baixa fertilidade e pelo alto teor de areia e alumínio (F. R. Martins, comunicação pessoal).

Embora as áreas de estudo sejam contíguas, elas estão cobertas por diferentes fisionomias de cerrado (*sensu* Coutinho 1978). A ECO ocupa uma área de 2.300 ha onde predominam os campos sujos e campos cerrados, com pequena parcela da área ocupada por matas de galeria que acompanham a drenagem do terreno, em faixas de até 50 m de largura. Há ainda uma pequena área de cerrado *sensu stricto*, no limite da estação com uma área de propriedade da Universidade de São Paulo (USP, Figura 3). A ECO é entrecortada por várias estradas e aceiros e está sujeita a incêndios ocasionais de origem antrópica a cada dois ou três anos. A EXP ocupa cerca de 1.200 ha, a maior parte recoberta por plantações de espécies exóticas como *Pinus* spp. e *Eucalyptus* spp., voltadas para exploração de resina e madeira pelo Instituto Florestal de São Paulo. A implantação desses cultivos remonta aos anos de 1960-1970, quando a vegetação de cerrado deu lugar ao reflorestamento com espécies exóticas (Instituto Florestal de São Paulo 1994). No interior da EXP, porém, permaneceram pequenas áreas de cerrado remanescentes, e o maior deles, conhecido como Valério, possui cerca de 140 ha. O Valério é composto por cerrado *sensu stricto*, possuindo diversas bordas com aceiros cuja manutenção anual com o uso de arado restringe muito o desenvolvimento da vegetação. A área de estudo no Valério está protegida do fogo há pelo menos 20 anos (D. Zanchetta, com. pess.), e possui um grande adensamento de árvores e arbustos, muitos deles cobertos por líquens e musgos, além de uma grossa camada de serrapilheira sobre o solo, características de áreas de cerrado livres de incêndios recentes (Mistry 1998).

Foram selecionadas para estudo uma área de campo cerrado e uma área de mata de galeria na ECO, e uma área de cerrado *sensu stricto* (Valério) na EXP (Figura 5). As áreas de cerrado *sensu stricto* e de campo cerrado estudadas são floristicamente similares (A. V. Christianini obs. pess.), mas no campo cerrado as árvores apresentam menor porte (1 a 3 m), típicos dessa fisionomia (Oliveira-Filho & Ratter 2002). No cerrado *sensu stricto* as famílias dominantes no estrato arbustivo-arbóreo são Leguminosae (*sensu lato*) (14 espécies), Myrtaceae (12), Rubiaceae (8) e Melastomataceae (8) (Giannotti 1988), com árvores atingindo 4 a 6 m de altura, e emergentes de *Dalbergia miscolobium* Benth. (Fabaceae) chegando a 10 m. No estrato herbáceo predominam gramíneas, cyperáceas e bromélias terrestres, como *Bromelia balansae* Mez. Detalhes da florística e fitossociologia dessa área são encontrados em Giannotti (1988). Boa parte da mata de galeria estudada é alagável após chuvas mais intensas (Figura 5), possuindo árvores de 6 a 12 m de altura, com grandes populações de *Calophyllum brasiliense* Cambess. (Clusiaceae). Arecaceae, Melastomataceae e Myrtaceae são famílias comuns entre as árvores da mata de galeria. Lianas, epífitas e briófitas também são abundantes (A. V. Christianini obs. pess.).

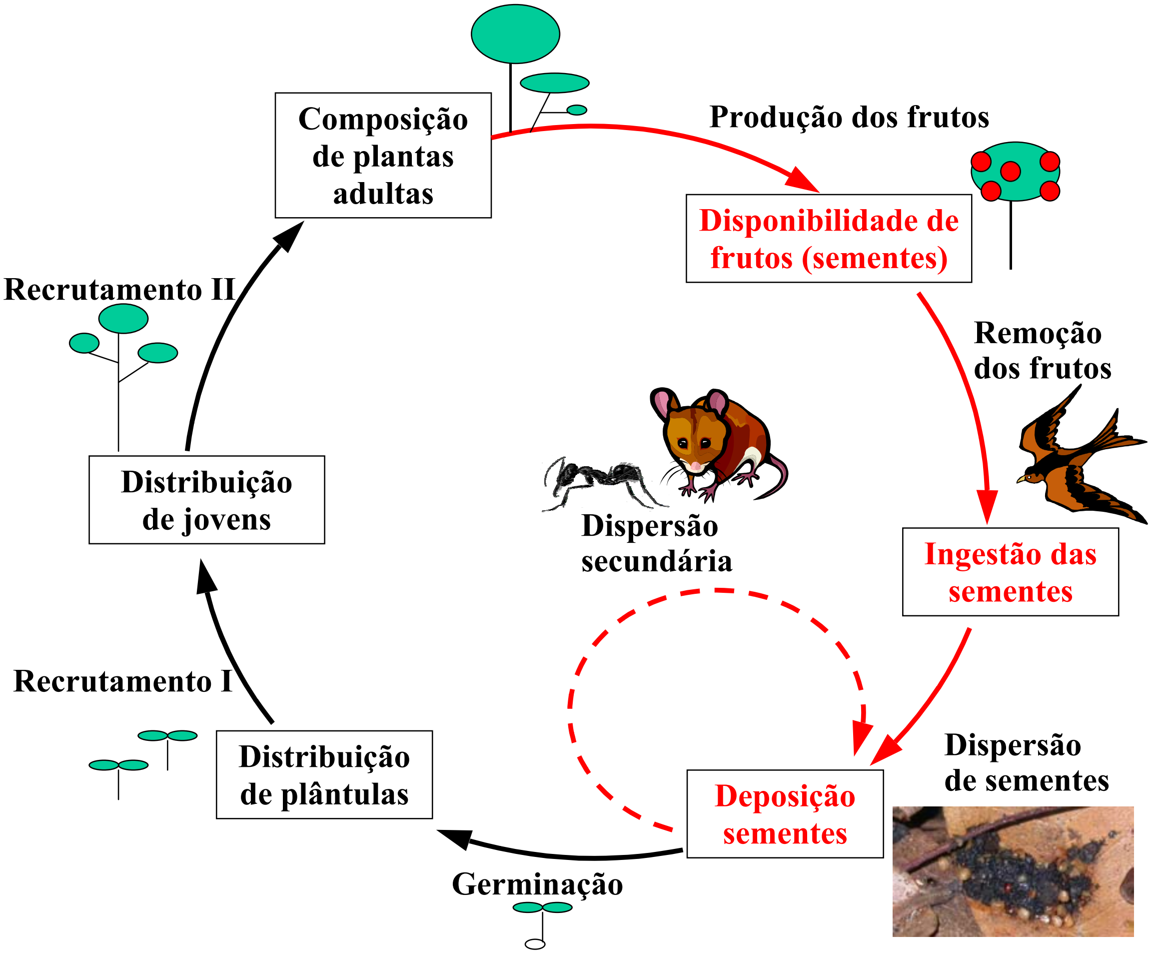

Figura 1: Diagrama representando as etapas consecutivas de um ciclo de dispersão de sementes e sua relação com o recrutamento de uma espécie vegetal zoocórica. Estudos sobre dispersão de sementes estiveram historicamente concentrados na dispersão primária, (setas vermelhas contínuas), enquanto que estudos de fitossociologia e demografia de plantas estiveram concentrados nos estágios subseqüentes da dispersão (setas pretas). Estudos recentes têm demonstrado que a dispersão secundária (seta pontilhada) pode influenciar significativamente as probabilidades de transição entre os estágios subseqüentes, enfatizando a necessidade de relacionar os processos seqüenciais envolvendo a dispersão de sementes ao padrão de recrutamento de novos indivíduos na população (modificado de Wang & Smith 2002).

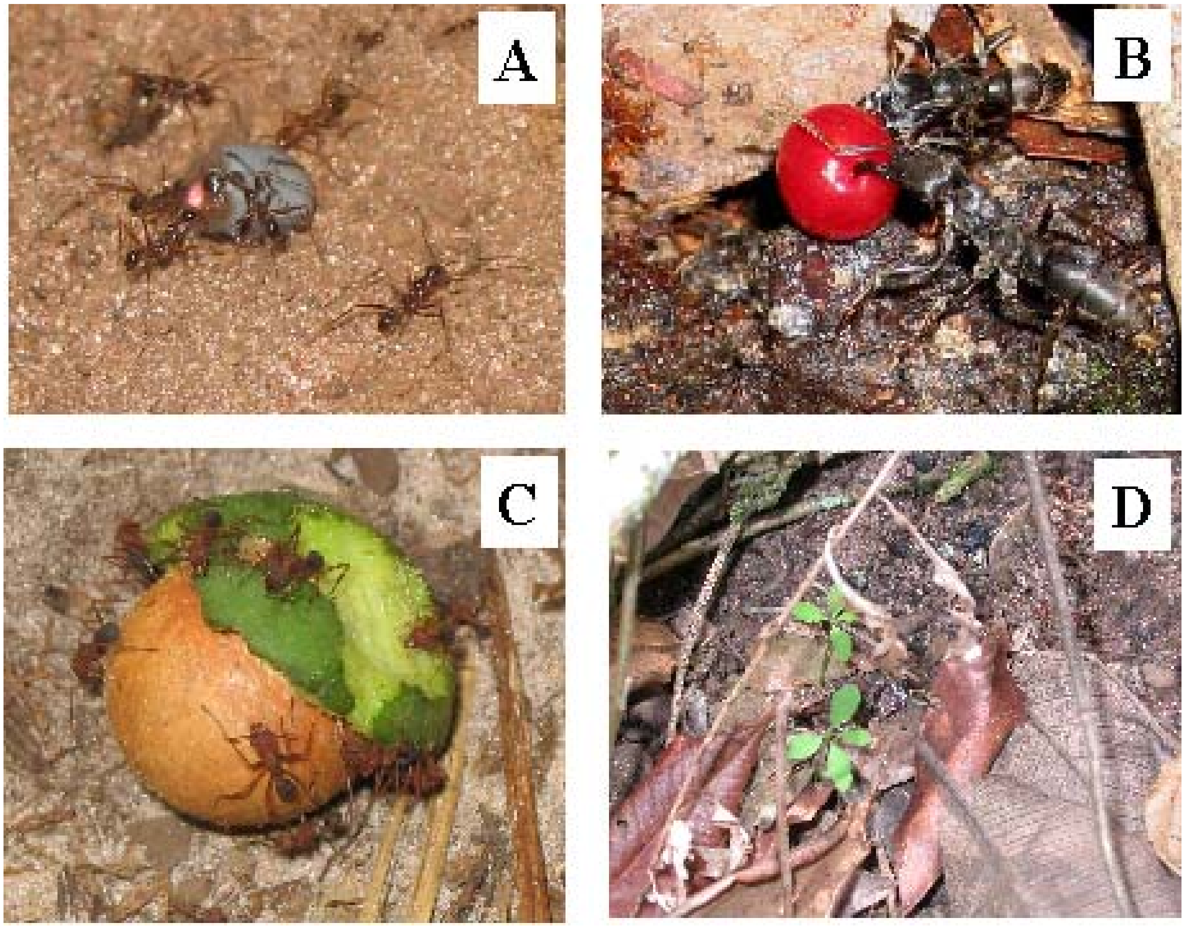

Figura 2: Formigas (Formicidae) freqüentemente interagem com diásporos não-mirmecocóricos caídos ao solo no cerrado e podem potencialmente influenciar o destino das sementes. (A) *Pheidole* sp. removendo semente arilada de *Xylopia aromatica* (Annonaceae); (B) *Pachycondyla striata* removendo fruto de *Erythroxylum pelleterianum* (Erythroxylaceae); (C) *Acromyrmex coronatus* removendo *in situ* a polpa de *Calophyllum brasiliense* (Clusiaceae); (D) Plântulas de *E. pelleterianum* na lixeira de um ninho de *Odontomachus chelifer* (veja Capítulo 1 para uma lista das interações formiga-diásporo observadas no cerrado).

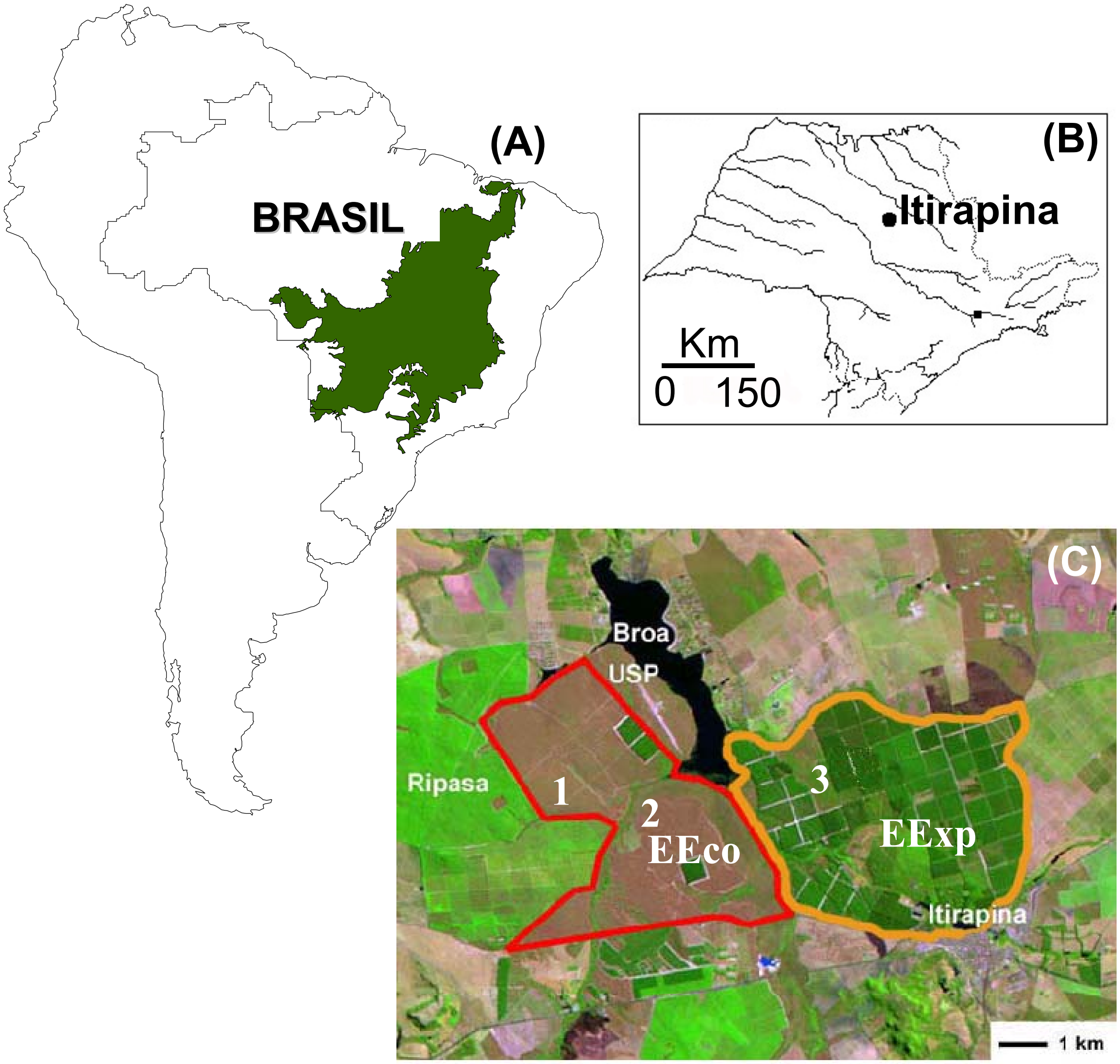

Figura 3: Localização da área de estudo. (A) Distribuição do cerrado (em verde) na América do Sul (omitindo as manchas disjuntas de cerrado encontradas na Amazônia); (B) Localização do município de Itirapina no estado de São Paulo; (C) Uma perspectiva regional da localização da Estação Ecológica (EEco, delimitada em vermelho), da Estação Experimental de Itirapina (EExp, delimitada em laranja) e dos sítios de estudo dispostos nestas áreas: (1) campo cerrado; (2) mata de galeria; (3) cerrado *sensu stricto*.

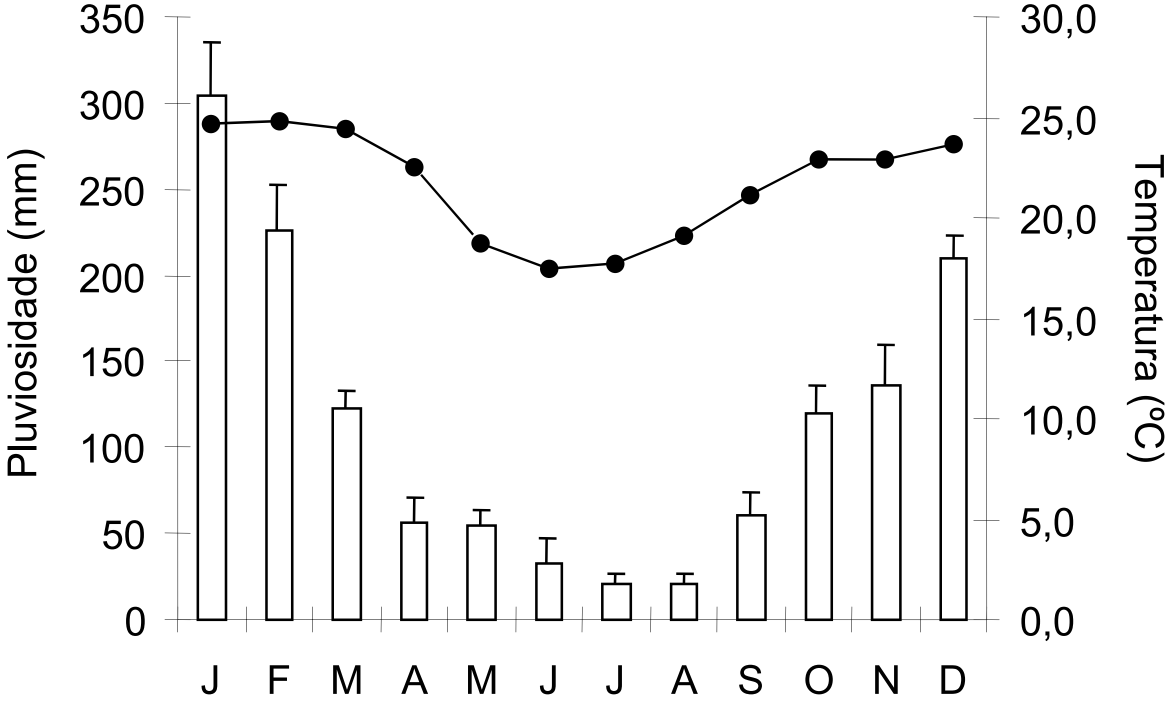

Figura 4: Distribuição da pluviosidade (colunas) e temperatura (linha) no período de janeiro de 1994 a dezembro de 2004 na Fazenda Siriema, propriedade da Cia. Ripasa de Papel e Celulose, vizinha a Estação Ecológica de Itirapina, Itirapina, SP (veja Figura 3). Os valores correspondem às médias mensais de cada variável, com o respectivo erro padrão no caso da pluviosidade. Como a variação ao redor da média mensal de temperatura é muito pequena, o erro padrão foi omitido da figura.

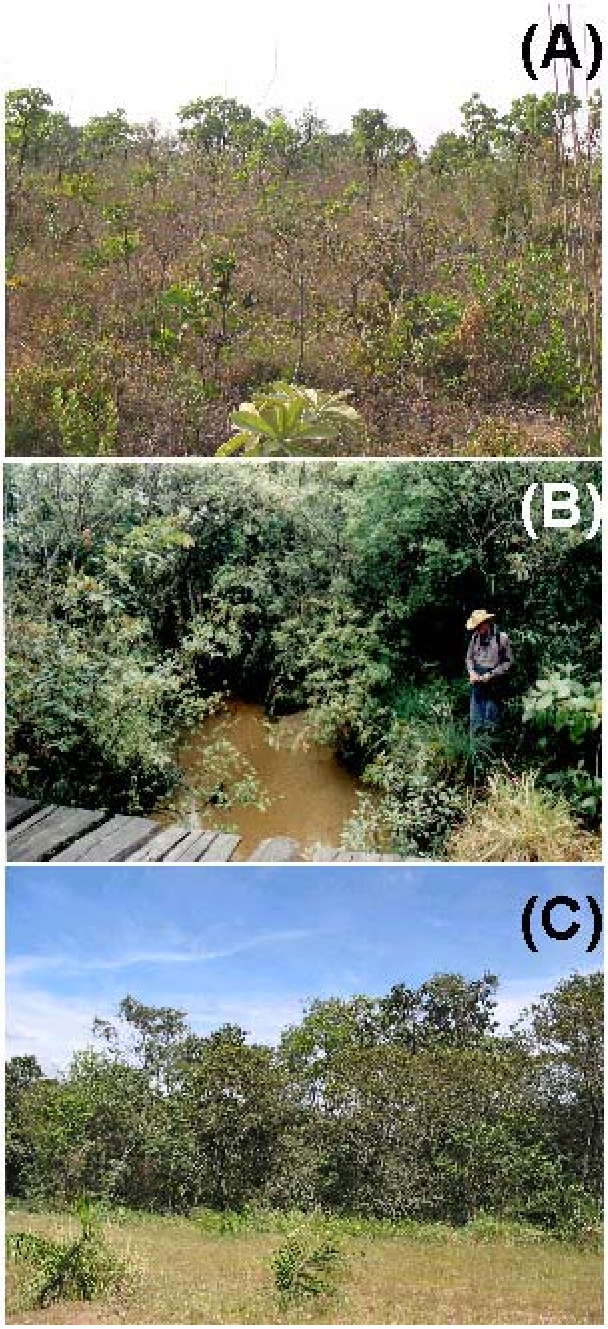

Figura 5: Fisionomias de vegetação selecionadas para o estudo das interações entre formigas e diásporos caídos ao solo em Itirapina (SP): (A) campo cerrado; (B) mata de galeria; (C) cerrado *sensu stricto*.

Capítulo 1

Exploitation of fallen diaspores by ants in a neotropical savanna: are there ant-plant partner choices?

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Running-title: Ant-diaspore interactions in cerrado

Key-words: ant-fruit/seed interaction, ant-plant interaction, attine ants, *Atta*, Cerrado, secondary seed dispersal, seed germination

ABSTRACT

The exploitation of diaspores (seeds or fruits) by ants was investigated in three sites of cerrado (savanna-like vegetation) in Southeast Brazil. During 2 years ant-diaspore interactions were recorded at each site through various methods, yielding a total of 521 records of ant-diaspore interactions, including 71 ant species and 38 plant species. Exploitation of fallen diaspores occurred across several ant foraging guilds, including carnivorous, omnivorous, and fungivorous ants. Fallen fruits may play a relevant role as food sources for the ground-dwelling ant community, similar to that of plant and insect exudates for ants that forage in the canopy of tropical rainforests. The temporal and spatial unpredictability of fallen diaspores in cerrado, however, make this food source less prone to monopolization by aggressive ants and likely explains the widespread and opportunistic use of fallen fruits by the ant community. Ant body size, plant life form, and diaspore size did not segregate species in interaction. However, a subset of plant diaspores exploited mainly by fungus-growing ants (Attini) was recognized. These included some fleshy carbohydrate-rich, or dry diaspores. We suggest that attines may prefer carbohydrate-rich diaspores because these would be a better substrate for fungus-growing than the often tough, nutrient-poor and toxic leaves of cerrado plants. The frequent association between certain ant and plant taxa may create a geographical mosaic of interactions across the shared range distribution of such groups, with possible consequences for seed fate and ant community structure.

INTRODUCTION

Although ants have long been considered mainly as predators and scavengers of animal matter, recent evidence indicates that they rely heavily on plant-derived food sources such as extrafloral nectar and honeydew of phloem-sucking hemipterans in the plant canopy (Tobin 1994, Blüthgen *et al.* 2003, Davidson *et al.* 2003). Despite the huge amount of fruit fall in tropical forests and savannas (Denslow & Gomez-Dias 1990, Galetti 1996, Leal & Oliveira 1998), their role in providing food resources and in structuring ant communities has received considerable less attention (Kaspari 1996). Indeed numerous ant species exploit fallen fleshy diaspores (fruits or seeds) in tropical vegetation (Leal & Oliveira 1998, Pizo & Oliveira 2000, Christianini *et al.* 2007, Leal *et al.* 2007), and may influence the fate of seeds primarily dispersed by vertebrates (Passos & Oliveira 2002, 2003, 2004). If fruits and seeds comprise an important food source for the general ground-dwelling ant community, they should be exploited by ants irrespective of the foraging guild. For instance, the consumption of plant and insect exudates is spread out across many foliage-dwelling ant groups, with the exception of the fungus-growing Attini (Davidson *et al.* 2003).

Recent studies on the use of fallen fleshy diaspores by ants in the Atlantic forest of Brazil have suggested that two main groups of ants interact preferably with either of two broad categories of diaspores: large predatory ants (Ponerinae) are attracted mainly to lipid-rich diaspores, while fungus-growing attines interact more frequently with lipid-poor, carbohydrate-rich diaspores (Figure 1; see Pizo & Oliveira 2001, Pizo *et al.* 2005). Carnivorous ants are more likely to interact with lipid-rich diaspores probably because these act as chemical analogues of dead insects (Carrol & Janzen 1973, Hughes *et al.* 1994). In vegetation growing on poor soils, such as sandy forests, carnivorous ants tend to predominate in interactions with fallen fruits irrespective of the chemical composition of the fleshy portion (Passos & Oliveira 2003). This is possibly due to bottom-up effects from nutrient-poor soils on vegetation, which indirectly limits the availability of litter-dwelling arthropod prey for carnivorous ants (Pizo *et al.* 2005). Why attine ants prefer lipid-poor, carbohydrate-rich diaspores is still an open question. The recognition of such preferences is important because the outcome of ant-fruit/seed interactions can vary with the identity of the participants (Hughes & Westoby 1992a, Passos & Oliveira 2002, 2004), providing a pathway for the evolution of partner choices among ants and plants (Giladi 2006). Although case studies on seed dispersal of single plant species are enlightening (e.g. Pizo *et al.* 2005), the recognition of patterns of interactions among ants and plants would be better recognized at a broader, community level. If patterns of ant-fruit/seed interactions exist, certain groups of ant and plant species in interaction might thus be distinguished in an analysis of ordination.

The cerrado savanna grows on nutrient-poor soils in central Brazil (Oliveira-Filho & Ratter 2002), and nearly 65% of its shrubs and trees produce fleshy diaspores adapted for dispersal by vertebrates, especially birds and mammals (Gottsberger & Silberbauer-Gottsberger 1983). However, most fruit production in cerrado falls beneath parent trees and thus become available to ground-dwelling ants that opportunistically exploit these diaspores and may influence seed fate (Leal & Oliveira 1998, Christianini *et al.* 2007). In the present study we investigate how pervasive is fruit and seed use as food by the general ant community in the cerrado savanna. We provide quantitative data on the exploitation of plant diaspores by ants at three nearby sites in Southeast Brazil. We searched for patterns of interactions between ant and plant taxa, and examined how ant and fruit/seed traits can mediate such interactions.

STUDY SITE

The study was carried out in the cerrado reserve of Itirapina (22^{o}12'S, 47^{o}51'W; altitude 730 m a.s.l.), Southeast Brazil. Average annual1 rainfall is 1360 mm, concentrated mostly in the warm/wet season (October to March). A dry/cold season occurs from April to September. Mean annual temperature is 21.8ºC (data from 1994 to 2004 from the reserve's climatological station). We selected three vegetation physiognomies for the study: gallery forests, cerrado *sensu stricto* (dense savanna) and campo cerrado (open savanna) (Figure 2). Study sites are 1.5 to ca. 5 km apart, and comprise a gradient of tree cover and soil fertility. Gallery forests occur along the drainage in a narrow strip (10 to 25 m) on both sides of water courses, and are prone to flooding during heavy rains. They grow on alluvial organic-rich soils, and have > 80% of tree cover, with herbaceous vegetation virtually restricted to the forest edge and gaps. *Calophyllum brasiliense* Cambess. (Clusiaceae) stands dominate the local plant community, but vines, epiphytes, and mushrooms are also abundant (A. V. Christianini, pers. obs.). Tree canopy is ca. 6 to 12 m in height (Figure 2). Cerrado *sensu stricto* is the typical physiognomy growing on sandy soils, which is characterized by an intermediate density and cover of trees and bushes (ca. 50 to 80%), interspaced by grasses (Oliveira-Filho & Ratter 2002). A detailed account of the flora of this site can be found in Giannotti (1988). Campo cerrado is a relatively opened vegetation growing on sandy nutrient-poor soils, with a dominant grass layer comprising ca. 30% of ground cover, and interspaced by palms, bushes, and small trees reaching 1.5 to 3 m in height (Oliveira-Filho & Ratter 2002). Virtually all plant species found in campo cerrado are also found in cerrado *sensu stricto*, but in the latter trees can reach 4 to 6 m, with emergent *Dalbergia miscolobium* Benth. (Fabaceae) reaching up to 10 m (Figure 2).

METHODS

Surveys of ant-diaspore interactions

Transect samplings -- We established one transect at each physiognomy to search for ant-diaspore interactions. Transects at campo cerrado and cerrado *sensu stricto* were 1 km long, whereas the gallery forest transect was 0.5 km in length due to the small size of the patch with this vegetation. We walked the transects monthly from October 2003 to November 2004, and in January 2005 (total of 15 monthly samples) looking for ants at fallen diaspores at ca. 2 m off both sides of the transect. Each time an ant was observed interacting with a diaspore (i.e., in contact with the surface of the diaspore apparently collecting liquids, or removing portions of it), an interaction was recorded and the ants were collected for identification. We also recorded the number of ants present and the portion of the diaspore mediating the interaction (e.g., fruit pulp, seed appendage). Ant-diaspore interactions were surveyed between 0730 and 1300 h, encompassing the overall peak of ant activity at our study site (A. V. Christianini, pers. obs.).

Using diaspores as baits for ants -- To increase the number of records of ant-diaspore interactions, we placed diaspores of six selected plant species on the floor of the cerrado *sensu stricto* site and recorded the ants attracted to them in diurnal and nocturnal samplings. Diurnal samplings began at 0800 h, and nocturnal ones at 1900 h. The following plants were selected for these surveys: *Xylopia aromatica* (Annonaceae), *Erythroxylum pelleterianum* (Erythroxylaceae), *Ocotea pulchella* (Lauraceae), *Miconia albicans* and *M. rubiginosa* (Melastomataceae), and *Amaioua guianensis* (Rubiaceae). The number of diaspores used to attract ants varied according to their availability (from 30 to 100 diaspores), and samplings were carried out during the fruiting period of each species. Fresh diaspores of each species were placed at sampling stations 10 m apart, 1-2 m off the transect. At each sampling station, two diaspores of a single species were placed on a small piece of white filter paper (4 x 4 cm) to facilitate visualization. The filter paper apparently does not influence ant behavior (Pizo & Oliveira 2000, Passos & Oliveira 2003). At each sampling we walked slowly along the transect during 2 hours and recorded all ant-diaspore interactions following the same procedure described above. Sampling of ant-diaspore interactions was also done during diaspore removal experiments (Christianini *et al.* 2007), and through opportunistic observations until September 2005. Voucher specimens of ants and plants are deposited in the entomological collection of the Universidade Federal Rural do Rio de Janeiro (CECL) and at the herbarium of the Universidade Estadual de Campinas (UEC), respectively.

Classification of diaspores and ant foraging guilds

Because diaspore size can influence ant behavior (e.g. removal of entire diaspore *versus* removal of fleshy part on spot, Figure 1) and constrain the subset of the ant community that may remove it to the nest (Kaspari 1996, Pizo *et al.* 2005), diaspores recorded in interaction with ants were classified as large (> 1 g) or small (< 1 g). Ants recorded in interaction with plant diaspores were classified into foraging guilds following the classification proposed for ant genera found in Brown (2000), which was corroborated by observations about the feeding habits of ants in the cerrado (Silvestre *et al.* 2003, Oliveira & Freitas 2004, Christianini & Oliveira pers. obs). Foraging habits were grouped into broad categories for the analyses, and ant genera were classified as carnivores, omnivores or fungivores. For instance, army ants, hypogeic, generalized or specialized predators were grouped in a single category of carnivorous ants, which included Ecitoninae, Ectatomminae, and Ponerinae. Fungus-growing ants (Myrmicinae: Attini) were classified as fungivorous. Ants with generalized feeding habits, seed harvesters, pollen or plant exudate-feeders were classified as omnivores, including non-Attini Myrmicinae, Dolichoderinae, Formicinae, and Pseudomyrmecinae. Ants were also classified according to total body size (large ants > 1 cm, small ants < 1cm), which is linked with several aspects of ant behavior, including dominance status, seed size selection, and distance of seed dispersal provided (Kaspari 1996, Brown 2000, Ness *et al.* 2004).

Statistical analysis

The total number of species found in each habitat during the transect sampling were compared using sample-based rarefaction curves (Gotelli & Colwell 2001). We estimated the number of ant species that could be found in interaction with diaspores by pooling all ant species observed in interaction with a given diaspore, and treating each diaspore species as a sample. We estimated ant species richness based on randomized species accumulation curves of ant at samples (incidence-based of ants in interaction with a given diaspore species) using EstimateS (Colwell 2001; "Chao2" estimator; 50 randomizations). We used Sorensen's similarity index to estimate the similarity of ant and plant species recorded in interaction among the vegetation types (Krebs 1989).

The frequency of ant foraging guilds recorded in interaction with diaspores was compared with the frequency of foraging guilds of the whole ant community through a goodness-of-fit test. A lack of detailed data on the ant community composition from Itirapina precluded our comparisons with the local ant community. We compared our results with the classification of foraging guilds for ant communities from two nearby sites of cerrado from where more complete surveys of the ant community are available, Luiz Antônio and Cajuru, 72 km and 108 km from Itirapina, respectively (Silvestre *et al.* 2003).

Carebara, *Myrmelachista*, and *Pyramica* ants were removed from the analysis due to a lack of detailed information about their feeding habits.

Patterns in ant-diaspore interactions were investigated through correspondence analysis (CA) performed in STATISTICA software, version 5 (©1996, STATSOFT, INC.). Since species with only one record could bias the analysis (Manly 1997), we lumped records by plant and ant genus, and removed those genera with only one record. This procedure may also reduce pseudoreplication due to phylogenetic autocorrelation that generates non-independence among morphological and physiological traits within common taxonomic affiliates (i.e. species within genus) that can influence preference patterns, allowing a more conservative analysis (Jordano 1995). CA was performed on the reduced contingency table of interactions between ants and plants (16 ant x 18 plant genera) across all study sites. Coordinates for the first two dimensions extracted by CA were used for testing differences in plant preferences between a priori defined groups of ants (ant foraging guild, ant body size) and of ant visitor spectra between plant groups (plant life form, diaspore size), performing one-factorial multiple analysis of variance (MANOVA) for each comparison. Significance levels were adjusted by Bonferroni corrections (Rice 1989).

RESULTS

Spatial and seasonal patterns

We recorded a total of 521 ant-diaspore interactions, 129 of which during transect samplings. The number of interactions recorded per km of transect decreased from cerrado *sensu stricto* to the campo cerrado and gallery forest (Figure 2). Overall, ant species richness was almost twice plant species richness, with 71 ant species recorded interacting with 38 species of plants along the whole study (Appendices 1-2). However, only 12 ant species had a high frequency (> 10 records) of interaction with diaspores (Appendix 1). Species accumulation curves did not reach saturation indicating that the samples represent a fraction of a richer species pool of ants and plants (Figure 3). The curves were more saturated for gallery forest and cerrado *sensu stricto* plants. Estimated ant species richness (± SD) pooled for all sites is 116 ± 20 ant species (EstimateS; Chao2 estimator). Species turnover (beta diversity) of ants recorded in interaction was high, especially between gallery forest and the other physiognomies (Table 1, Appendix 1). Only three plant species were recorded in interaction with ants in more than one physiognomy (Table 1, Appendix 2).

There was great seasonal variation in the number of interactions recorded within sites. The gallery forest site could not be surveyed all year round due to floods following heavy rains. The number of interactions observed was not regularly distributed through time for any site (Kolmogorov-Smirnov tests: $D_{max} \geq 0.58$; $p \leq 0.05$). Records were concentrated mostly in the wet season (October to March). The number of ant-diaspore interactions recorded at a given site along the year was not correlated with records at the other sites (Spearman rank correlations: $r_s \leq 0.48$; $n = 12$; $p \geq 0.11$, for all comparisons), and probably followed distinctive patterns of plant phenology and ant activity of each site.

Ant behaviour towards diaspores, and patterns of diaspore exploitation

Ants exploited diaspores of 18 species of trees, 11 shrubs, three palms, three lianas, and three herbs. Ants interacted with diaspores up to 26 g in weight, such as the fruits of *Dyospyros hispida* (Ebenaceae). Most diaspores had a fleshy portion (either pulp or aril) mediating the interaction, but dry diaspores primarily adapted to wind dispersion (e.g. Poaceae, Asteraceae) were also exploited by fungus-growing ants (Attini) (Appendix 2). *Atta sexdens* was observed removing seeds of *Solanum lycocarpum* (Solanaceae) and *Miconia rubiginosa* (Melastomataceae) from vertebrate faeces, indicating that these ants can reshape the seed shadow generated by primary seed dispersers. Workers of *Atta* spp. were also frequently observed carrying unripe fruits of *Miconia* spp., which were collected on the floor or directly from the plants. With the exception of Attini ants, all other ants were observed interacting with ripe fruits. Fleshy fruits that were partially eaten and dropped to the ground by vertebrate frugivores (e.g. *Hancornia speciosa*, Apocynaceae) attracted many ants that collected liquids from the remains of the juicy pulp.

Myrmicine ants (except Attini) were well represented in interactions with diaspores at all sites (Figure 2), accounting for nearly 47% of all interactions recorded (Appendix 1). *Pheidole* ants were observed at all sites either cleaning seeds from pulp, removing fruit parts, or carrying diaspores to their nests. Although common in all cerrado physiognomies, accounting for ca. 33% of all interactions recorded, Attini ants were best represented in cerrado *sensu stricto* (Figure 2). *Atta sexdens* was the most important species in number of interactions, and the only species recorded at all sites (Appendix 1). Ponerine ants accounted for ca. 13% of all observed interactions; these ants were never observed in the gallery forest, but were common in campo cerrado and cerrado *sensu stricto* (Figure 2). Ants in the subfamilies Formicinae, Dolichoderinae, and Pseudomyrmecinae accounted together for ca. 7% of the interactions recorded, and only collected liquids from fallen fruits.

In general, ant behavior toward diaspores can be classified into four categories: (1) large Ponerinae (*Dinoponera*, *Pachycondyla*, and *Odontomachus*; Figure 1a) and Attini

(*Atta*) ants individually remove diaspores of up to 1 g to their nests. Large fruits ants (e.g. *Calophyllum brasiliense*; Figure 1b) have their pulp sequentially removed in small pieces by Attini; (2) small Myrmicinae (e.g. *Pheidole*) recruit nestmates to consume the diaspore on the spot (as occurs with *Ocotea pulchella*, Lauraceae), or to remove the diaspore to the nest (as occurs with *Xylopia aromatica*, Annonaceae and *Erythroxylum pelleterianum*, Erythroxylaceae; both < 0.2 g); (3) *Solenopsis* spp. and *Pheidole* spp. frequently cover the diaspore with soil before removing the pulp (as occurs with *O. pulchella*); (4) species of Formicinae, Dolichoderinae, and other arboreal ants (*Cephalotes*, *Crematogaster*, *Pseudomyrmex*) collect liquids from fallen flesh fruits, or from fruits still attached to the plant (as occurs with *X. aromatica*), but never remove them.

Omnivorous ant genera predominate in interactions with fallen diaspores, followed by carnivorous and fungivorous ant genera (Figure 4). There is no difference in fruit exploitation by different ant guilds in Itirapina in relation to the abundance of each guild in the whole ant community from nearby cerrado sites at Luiz Antônio ($\chi^2 = 0.94$; d.f. = 2; P = 0.62) or Cajuru ($\chi^2 = 0.28$; d.f. = 2; P = 0.87) (Figure 4). Thus, use of plant diaspores for food is widespread among ant foraging guilds in the cerrado. However, some patterns of association between ant and plant taxa are detectable in the ordination analysis. Attini (fungivorous) ants were significantly segregated from other ants, while carnivorous and omnivorous appeared together in the ordination space (Table 2, Figure 5). Plant preferences were not affected by ant body size; neither plant life form or diaspore size segregate the ant species in interaction (Table 2). Plant genera associated with attine ants included *Amaioua*, *Copaifera*, *Miconia*, *Myrcia*, *Brachyaria*, *and Stryphnodendron* (Figure 5), the latter two with dry fruits.

DISCUSSION

Fallen plant diaspores constitute a considerable food source for ants in the cerrado. Indeed recent data on fruit production and seed fate in three fleshy-fruited plants in cerrado indicate that 11-53% of the ripe fruits fall beneath parent trees in each fruiting season (Christianini & Oliveira unpubl. data). Results from the current study and from previous experiments in cerrado demonstrate that ants effectively remove the fleshy portion of fallen fruits, or transport entire diaspores to their nests (Leal & Oliveira 1998, Christianini *et al.* 2007). Indeed, even ants considered as primarily carnivorous and scavengers (e.g. Ponerinae) make extensive use of fruit (see also Horvitz & Beattie 1980, Horvitz 1981, Fourcassié & Oliveira 2002, Passos & Oliveira 2002, 2004, Pizo *et al.* 2005), which resembles the use of plant and insect exudates by canopy-dwelling ants in tropical forests. Comparisons of the ratio of N isotopes ($^{15}N/^{14}N$, formulated as $\delta^{15}N$) across tropical ant taxa have shown that a number of exudate-feeding ant species obtain their nitrogen lower in the trophic chain than do other predominantly predacious arthorpods in the same habitat (Davidson *et al.* 2003, Blüthgen *et al.* 2003). This finding supports Tobin's (1994) hypothesis that most canopy-dwelling ants act chiefly as herbivores rather than predators or scavengers, and rely mostly on plant- and insect-derived exudates for nutrition. Widespread exploitation of fallen fleshy diaspores by ground-dwelling carnivorous and omnivorous ants (*sensu* Brown 2000) in tropical habitats may perhaps be explained by a similar mechanism. As suggested for canopy ants feeding on liquid food (Davidson *et al.* 2003), it is possible that nutrient-rich fleshy diaspores may as well constitute a reliable food source and fuel prey-hunting activities by ground-dwelling ants. Moreover, cerrado foliage also contains abundant sources of plant and insect exudates that are readily exploited by part of the ground-dwelling ant community that climbs on plants to search for these liquid foods (Del-Claro & Oliveira 1999, Ribas & Schoereder 2004, Oliveira & Freitas 2004).

Small myrmicines (e.g. *Pheidole*) were the most common ants interacting with diaspores in cerrado (Figure 1, Appendix 1) as well as in rainforests (Pizo & Oliveira 2000, Passos & Oliveira 2003, Pizo 2007) and in the semi-arid caatinga of Brazil (Leal *et al.* 2007). While ponerine ants prominently interact with diaspores in the Atlantic forests (Pizo *et al.* 2005), Attini ants (e.g. *Atta*) were much more common at diaspores in the cerrado (see also Leal & Oliveira 1998). Although the ant assemblage interacting with diaspores on cerrado floor is somewhat distinct from that in Atlantic forest and caatinga sites, categories of ant behavior toward fruits are remarkably similar (see Pizo & Oliveira 2000, Passos & Oliveira 2003, Leal *et al.* 2007). A common feature among all these studies is that ants exploit many plant diaspores that possess no apparent adaptation for ant dispersal. These diaspores can comprise a relevant part of the diet of ground-dwelling omnivorous ants in the Neotropics (Byrne 1994, Pizo *et al.* 2005), which in turn may influence seed fate and plant recruitment (Horvitz & Beattie 1980, Horvitz 1981, Levey & Byrne 1993, Passos & Oliveira 2002).

A subset of the plant community from the cerrado interacted mainly with Attini ants, as shown by our CA analysis (see Table 2, Figure 5). This subset includes several plant genera that produce lipid-poor, carbohydrate-rich diaspores such as *Myrcia* (75% water and 75% carbohydrates in dry mass of fleshy portion), *Copaifera* (75% and 87%), and *Miconia* (76% and 86%) (Galetti 1996, Christianini & Oliveira unpubl. data). It is possible that such fruits provide a better substrate for fungus-culturing by Attini ants than lipid-rich fruits. It is well known that leaf-cutters choose among the available plant material for fungus-culturing, and that the chemical composition of plant matter may play a role in this choice (Howard *et al.* 1988, Farji-Brener 2001, Wirth *et al.* 2003). Since lipids are hydrophobic, there is often a trade-off between lipid and water/carbohydrate content in fruit pulp (Jordano 1995). Lipid-rich fruits may constrain the water available for optimal fungus growth inside nests. Leaf-cutters may also prefer certain fleshy fruits rather than leaves because of the toxic compounds, high toughness, and/or poor nutrient content of leaves of many plants in the cerrado (Marquis *et al.* 2002). For instance, terpenoids, which are toxic to leaf-cutters and their fungus (Howard *et al.* 1988), are common in some plant clades from the cerrado (Gottlieb *et al.* 1975). Indeed, fruits and seeds comprise a large portion of the plant material collected by Attini ants in another cerrado locality (Leal & Oliveira 2000), and their fleshy part (pulp or aril) can be highly susceptible to fungus growth during seed germination experiments (Oliveira *et al.* 1995, Leal & Oliveira 1998, Christianini *et al.* 2007). Although known as insect pests and seed predators, leaf-cutter ants may benefit plants through positive effects on seed germination, dispersal, and establishment (Oliveira *et al.* 1995, Farji Brener & Silva 1996, Dalling & Wirth 1998, Leal & Oliveira 1998, Wirth *et al.* 2003). Recent studies have proposed that a coevolutionary mosaic may be established across the shared range of distribution of interacting species (Thompson 2005). Records of melastome fruit exploitation by attine ants occur across the range of distribution of both taxa (Dalling & Wirth 1998, Leal & Oliveira 1998, this study). Thus, patterns of diaspore exploitation by ants found for the cerrado may be common to other tropical sites.

Neither plant life form nor ant body size segregate species in ant-plant interactions. Plant life form would have a lower influence on diaspore exploitation by ground-dwelling ants compared to ants that forage in the plant canopy (Blüthgen *et al.* 2004), because fruits are collected mainly when fallen to the ground. Fallen fruits may also constitute a more scattered and temporally unpredictable resource (Jordano 2000) compared to nectar or honeydew sources in plant canopy (e.g. Blüthgen *et al.* 2004), what should make fallen fruit less prone to monopolisation by more aggressive ants. Although aggressive encounters can be observed while ants exploit fallen diaspores in cerrado (e.g. large *Pachycondyla* and *Dinoponera* displacing small *Pheidole* and *Solenopsis*), such interactions are usually restricted to the nest vicinity of the aggressive species, and are unlikely to determine the whole interaction pattern. Large ant body size does not preclude exploitation of fallen diaspores by small ants, because small Myrmicinae, for instance, frequently recruit nestmates to exploit the food source. Large fruits are also cleaned at the spot rather than transported to the ant nest (Leal & Oliveira 1998, Pizo & Oliveira 2000, Passos & Oliveira 2003) (Figure 4b). These traits may contribute for the widespread use of fallen fruits by the ant community, and for the absence of segregation of interacting species in relation to diaspore size.

In conclusion, we showed that a large subset of the local ant community in the cerrado exploits fallen plant diaspores, irrespective of the ant foraging guild. Fallen diaspores may play a relevant role as food sources for ground-dwelling ants in a manner similar to plant exudates for canopy ants in tropical forests (Davidson *et al.* 2003, Blüthgen *et al.* 2003). However, ants do not interact randomly with fallen diaspores. For instance, a subset of the plant community in the cerrado interacts mainly with Attini ants. Such interactions may be guided by the ants' preference patterns associated with the chemical composition of diaspores that improve fungus-culturing by the colonies. It would be interesting to investigate how seed fate vary between different subsets of species in interaction, and the influence of such ant-plant partner choices to the evolution of those interactions (see Giladi 2006).

ACKNOWLEDGEMENTS

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Table 1. Sorensen's similarity index among ants (below diagonal, N = 71) and plants (above diagonal, N = 38) recorded in interaction in three cerrado physiognomies in Southeast Brazil employing several sampling methods.

	Campo cerrado	Cerrado *sensu stricto*	Gallery forest
Campo cerrado	--	15.8	0
Cerrado *sensu stricto*	45.8	--	0
Gallery forest	5.6	9.0	--

Table 2. Multiple one-way analyses of variance (MANOVA) for effect factors selected *a priori* on first two dimensions from correspondence analysis (explanatory power: 28.8% and 26.4%, respectively); (a) differences among plants in ant visitation spectra, and (b) among ants in plant preferences (see also Figure 5). Significant effects after Bonferroni correction in boldface.

Effect	Wilks	d.f. effect	d.f. error	P
(a) ants				
foraging guild	0.349	4	22	**0.0169**
body size[1]	0.857	2	12	0.397
(b) plants				
life form[2]	0.845	2	14	0.308
diaspore size	0.920	2	15	0.536

[1] excluding *Camponotus*, which had extensive variation in body size among species

[2] excluding the herb *Brachyaria*

Appendix 1. Ant species observed in interaction with fallen diaspores in cerrado in Southeast Brazil. Data pooled from monthly samples of ant-diaspore interactions recorded along transects at three sites, observations at diaspore removal experiments (Christianini *et al.* 2007), and opportunistic records. Key to habitat types: CSS = cerrado *sensu stricto*; CC = campo cerrado; GF = gallery forest. Ant behavior: C = clean seeds through aril or pulp removal on the spot, no displacement; E = inspect or collect liquids, no removal; R = remove diaspores > 5 cm; T = try to remove diaspores, < 5cm displacement.

Ant subfamily and species	Ant behavior	Habitat	No. of diaspore species used (no. of records)
Ectatomminae			
1. *Ectatomma opaciventre* Roger	R	CC, CSS	2 (10)
2. *Ectatomma planidens* Borgmeier	T	CSS	1 (1)
Ponerinae			
3. *Dinoponera australis* Emery	R, T	CSS	4 (10)
4. *Odontomachus chelifer* (Latreille)	R, E	CSS	5 (15)
5. *Pachycondyla striata* Fr. Smith	R	CSS	4 (24)
6. *Pachycondyla villosa* (Fabricius)	R	CSS	4 (6)
Myrmicinae			
7. *Cephalotes depressus* (Klug)	E	CSS	1 (1)
8. *Cephalotes pusillus* (Klug)	E	CSS	2 (2)
9. *Crematogaster* prox. *evallans* Forel	E	CC, CSS	2 (3)
10. *Crematogaster curvispinosa* Mayr	E	CSS	1 (1)

continued

11. *Crematogaster* sp.	E	CC, CSS	4 (6)
12. *Megalomyrmex* sp.	C, E	CSS	1 (2)
13. *Carebara sp.*	C, E	CSS	2 (5)
14. *Pheidole fracticeps* Wilson	C	CSS	1 (1)
15. *Pheidole* gr. *tristis* prox. *allarmata*	C	CSS	2 (2)
16. *Pheidole* sp. 1	C, R	CC, CSS	9 (24)
17. *Pheidole* sp. 2	C, R	CSS	8 (15)
18. *Pheidole* sp. 3	C	CSS	1 (2)
19. *Pheidole* sp. 4	C	CC, CSS	6 (19)
20. *Pheidole* sp. 5	C, E, R	CC, CSS	14 (58)
21. *Pheidole* sp. 6	C	CC, CSS	4 (5)
22. *Pheidole* sp. 7	C	CSS	4 (11)
23. *Pheidole* sp. 8	C, R	CSS	2 (7)
24. *Pheidole* sp. 9	C, R	CC, CSS	2 (2)
25. *Pheidole* sp. 10	C, E	GF	1 (2)
26. *Pheidole* sp. 11	C, E	CC	1 (1)
27. *Pheidole* sp. 12	C	GF	1 (1)
28. *Pheidole* sp. 13	C	CSS	1 (1)
29. *Pheidole* sp. 14	C	CSS	2 (9)
30. *Pheidole* sp. 15	C	CC, CSS	2 (3)
31. *Pheidole* sp. 16	C	CSS	1 (2)
32. *Pheidole* sp. 17	C, R	CSS	4 (7)

continued

33. *Solenopsis* sp. 1	C, E	CSS, GF	2 (2)
34. *Solenopsis* sp. 2	C, R	CC, CSS	4 (4)
35. *Solenopsis* sp. 3	C	CC, CSS	2 (2)
36. *Solenopsis* sp. 4	E	GF	1 (1)
37. *Solenopsis* sp. 5	C	CSS	1 (1)
38. *Solenopsis* sp. 6	C	CSS	1 (1)
39. *Solenopsis* sp. 7	C, E	GF	1 (3)
40. *Solenopsis* sp. 8	C	CSS	1 (1)
41. *Solenopsis* sp. 9	C, E	CC	1 (3)
42. *Wasmannia auropunctata* (Roger)	C, T	CC, CSS	5 (34)
Myrmicinae (Attini)			
43. *Acromyrmex coronatus* (Fabricius)	C	GF	1 (1)
44. *Acromyrmex* sp.	C	CC, CSS	2 (2)
45. *Atta laevigata* (Fr. Smith)	C, R	CSS	3 (23)
46. *Atta sexdens rubropilosa* Forel	C, R	CC, CSS, GF	18 (128)
47. *Cyphomyrmex minutus* Mayr	R	CSS	1 (1)
48. *Cyphomyrmex rimosus* (Spinola)	R	CSS	1 (1)
49. *Cyphomyrmex lectus* Forel	R	CSS	1 (1)
50. *Mycocepurus goeldii* Forel	R	CC, CSS	3 (8)
51. *Mycocepurus* sp.	C, E	CSS	1 (2)
52. *Trachymyrmex oetkeri* Forel	C, E	CSS	1 (3)

continued

53. *Trachymyrmex ruthae* Weber	E	CC	1 (1)
54. *Trachymyrmex* sp.	T	CC	1 (1)
Dolichoderinae			
55. *Azteca* sp. 1	C, E	CC	1 (1)
56. *Azteca* sp. 2	C, E	CSS	1 (2)
57. *Azteca* sp. 3	E	CSS	1 (1)
58. *Linepithema* sp.	E	GF	1 (3)
59. *Dorymyrmex* sp. 1	E	CC, CSS	1 (2)
60. *Dorymyrmex* sp. 2	E	CSS	1 (2)
Formicinae			
61. *Brachymyrmex* sp. 1	E	CC	1 (2)
62. *Brachymyrmex* sp. 2	E	CSS	1 (1)
63. *Brachymyrmex* sp. 3	E	CC, CSS	2 (3)
64. *Camponotus renggeri* (Emery)	E	CSS	1 (2)
65. *Camponotus rufipes* (Fabricius)	E	CC, CSS	2 (6)
66. *Camponotus* sp. 1	E	CC	1 (2)
67. *Camponotus* sp. 2	E	CC, CSS	2 (2)
68. *Camponotus* sp. 3	E	CSS	1 (1)
69. *Camponotus* sp. 4	E	CSS	1 (7)
70. *Paratrechina* sp.	E	GF	1 (1)
Pseudomyrmecinae			
71. *Pseudomyrmex* sp.	E	CSS	1 (1)

continued

Total number of diaspore species exploited	38
Total number of ant-diaspore interactions	521

Appendix 2. Plant diaspores exploited by ants in cerrado in Southeast Brazil. Data pooled from monthly samples of ant-diaspore interactions recorded along transects at three sites, observations at diaspore removal experiments (Christianini *et al.* 2007), and opportunistic records. Key to habitat types: CSS = cerrado *sensu stricto*; CC = campo cerrado; GF = gallery forest. Growth forms: T = tree, S = shrub, H = herb, L = liana, P = palm. Diaspore types were broadly classified as fleshy (with pulp or aril) or dry (no presence of fleshy portion) according to diaspore morphology. Ant species numbers as in Appendix 1.

Plant family and species	Habitat	Growth form	Diaspore type	Ant species
Annonaceae				
Annona crassiflora Mart.	CC	T	fleshy	26
Xylopia aromatica (Lam.) Mart.	CSS	T	fleshy	2, 3-6, 7, 9-14, 16, 17, 19, 20, 22, 23, 28, 29, 32, 34, 37, 38, 40, 42, 46, 60, 68, 71
Apocynaceae				
Forsteronia glabrescens Müll. Arg.	CSS	L	dry	46
Hancornia speciosa Gomes	CC	T	fleshy	1, 11, 16, 19, 20, 34, 44, 53, 59, 61, 63, 65-67
Araliaceae				
Didymopanax vinosus (Cham. & Schltdl.) Marchal	CSS	T	fleshy	20, 46
Arecaceae				
Attalea geraensis Barb. Rodr.	CSS	P	fleshy	44

continued

Syagrus romanzoffiana (Cham.) Glassman	GF	P	fleshy	11, 25, 27, 33, 36, 39, 58, 70
Syagrus petraea (Mart.) Becc.	CSS	P	fleshy	20, 46
Asteraceae				
Chromolaena pedunculosa (Hook. & Arn.) R.M. King & H. Rob.	CC	H	dry	50
Bromeliaceae				
Bromelia balansae Mez	CC	H	fleshy	46
Caryocaraceae				
Caryocar brasiliense Cambess.	CC	S	fleshy	21, 35
Clusiaceae				
Calophyllum brasiliense Cambess.	GF	T	fleshy	43
Fabaceae				
Copaifera langsdorffii Desf.	GF	T	fleshy	16, 17, 46
Dilleniaceae				
Davilla elliptica A. St.-Hil.	CC	S	fleshy	9, 20, 24, 46, 54
Ebenaceae				
Diospyros hispida A. DC.	CSS	T	fleshy	21
Erythroxylaceae				
Erythroxylum pelleterianum A. St.-Hil	CSS	S	fleshy	3-6, 8, 16, 17, 19, 20, 22, 30, 32-34, 42, 46, 51, 56, 57, 64, 65

continued

Euphorbiaceae				
Sapium glandulatum (Vell.) Pax	CC	S	fleshy	46
Lauraceae				
Ocotea pulchella Mart.	CSS	T	fleshy	3, 15, 20, 23, 31, 35, 46
Malpighiaceae				
Banisteriopsis sp.	CC	L	dry	55
Byrsonima verbascifolia (L.) DC.	CC	T	fleshy	41
Melastomataceae				
Miconia albicans (Sw.) Triana	CSS	S	fleshy	4, 5, 8, 11, 16, 17, 19, 20, 22, 32, 34, 46, 47, 49, 52, 62, 63
Miconia fallax DC.	CSS	S	fleshy	17, 46
Miconia rubiginosa (Bonpl.) DC.	CSS	T	fleshy	1, 3-6, 15, 16, 20, 21, 45-47, 50
Mimosaceae				
Stryphnodendron adstringens (Mart.) Coville	CSS	T	dry	17, 46
Myrtaceae				
Campomanesia pubescens (DC.) O. Berg	CC, CSS	S	fleshy	16, 17, 20
Eugenia punicifolia (Kunth.) DC.	CSS	T	fleshy	29, 48
Myrcia lingua (O. Berg.) Mattos & D. Legrand	CSS	T	fleshy	17, 20, 46, 47

continued

Psidium cinereum (Mart.) ex DC.	CC	S	fleshy	16
Nyctaginaceae				
Guapira noxia (Netto) Lundell	CSS	T	fleshy	46
Ochnaceae				
Ouratea spectabilis (Mart. ex Engl.) Engl.	CSS	T	fleshy	13, 18, 21, 22
Poaceae				
Brachyaria sp.	CC, CSS	H	dry	20, 50
Rubiaceae				
Amaioua guianensis Aubl.	CSS	T	fleshy	4, 19, 20, 24, 32, 45-47, 69
Tocoyena formosa (Cham. & Schltdl.) K. Schum.	CC, CSS	S	fleshy	6, 67
Sapindaceae				
Serjania sp.	CSS	L	dry	46
Sapotaceae				
Pouteria torta (Mart.) Radlk.	CSS	T	fleshy	30
Solanaceae				
Solanum lycocarpum St. Hil.	CC	S	fleshy	46
Styracaceae				
Styrax ferrugineus Nees & Mart.	CC	T	fleshy	19

continued

Verbenaceae				
Aegiphila lhotskiana Cham.	CC	S	fleshy	16, 42

LEGENDS TO FIGURES

Figure 1. An illustration of how ant foraging guild and diaspore size may influence ant-diaspore interactions in the cerrado. (A) Worker of carnivorous *Odontomachus chelifer* (size ca. 1.8 cm) transporting a diaspore of *Xylopia aromatica* (0.06 g) (Annonaceae) on the leaf litter of the Cerrado. These large ponerine ants regularly carry diaspores to the nest, where the fleshy portion is consumed by workers and larvae, and the seeds are discarded. (B) Workers of fungivorous *Acromyrmex coronatus* (size ca. 0.5 cm) removing the fleshy portion of a *Calophyllum brasiliense* (Clusiaceae) fruit on the floor of a gallery forest in Brazil. Such large seeds (> 1 g) are not removed, but pieces of fleshy portion are sequentially transported by Attini ants that use the material for fungus culturing inside the nest.

Figure 2. A representation of the three vegetation physiognomies at Itirapina, Southeast Brazil, in which ant-diaspore interactions were surveyed, with the general ant and plant data obtained in each site. See also Appendices 1-2.

Figure 3. Ant (A) and plant (B) species accumulation curves as a function of the number of records of ants interacting with diaspores in transects at each of three cerrado physiognomies, and at all transects combined.

Figure 4. Proportion of ant foraging guilds at three cerrado areas in Southeast Brazil, based on the trophic generic classification of Brown (2000). Fungivorous are fungus-growing ants (Attini), while omnivorous and carnivores are mainly generalist and predatory ants,

respectively. Numbers above bars indicate quantity of genera in each category for each site. Data for the cerrado areas of Cajuru and Luiz Antônio are from Silvestre *et al.* (2003).

Figure 5: Reciprocal ordination of ants and diaspore's genera found in interaction in the cerrado of Itirapina. Squares indicate plants (see name of each genus) while collored circles indicate ants according to foraging guild: green circles = fungivorous ants (Myrmicinae: Attini); black circles = omnivorous ants (non-Attini Myrmicinae, Formicinae, and Dolichoderinae); yellow circles = carnivorous ants (Ectatomminae, Ponerinae, and Pseudomyrmecinae). Note the segregation among fungivorous ants and a subset of plants from other ants and plants in interaction (see also Table 2). Key for ant genera: (1) *Mycocepurus*; (2) *Atta*; (3) *Trachymyrmex*; (4) *Ectatomma*; (5) *Dinoponera*; (6) *Odontomachus*; (7) *Pachycondyla*; (8) *Brachymyrmex*; (9) *Camponotus*; (10) *Cephalotes*; (11) *Crematogaster*; (12) *Dorymyrmex*; (13) *Pheidole*; (14) *Solenopsis*; (15) *Wasmannia*.

Figure 1:

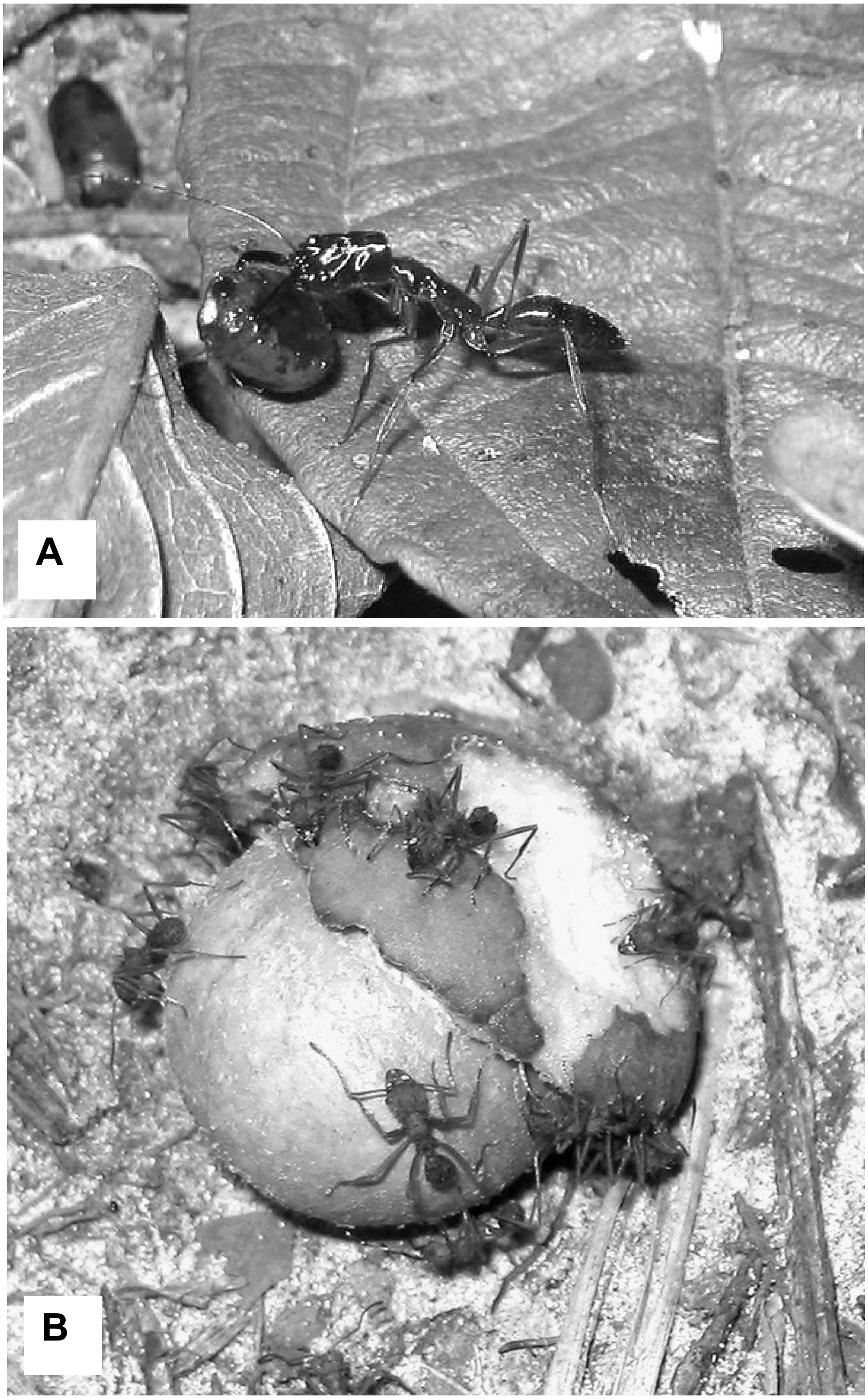
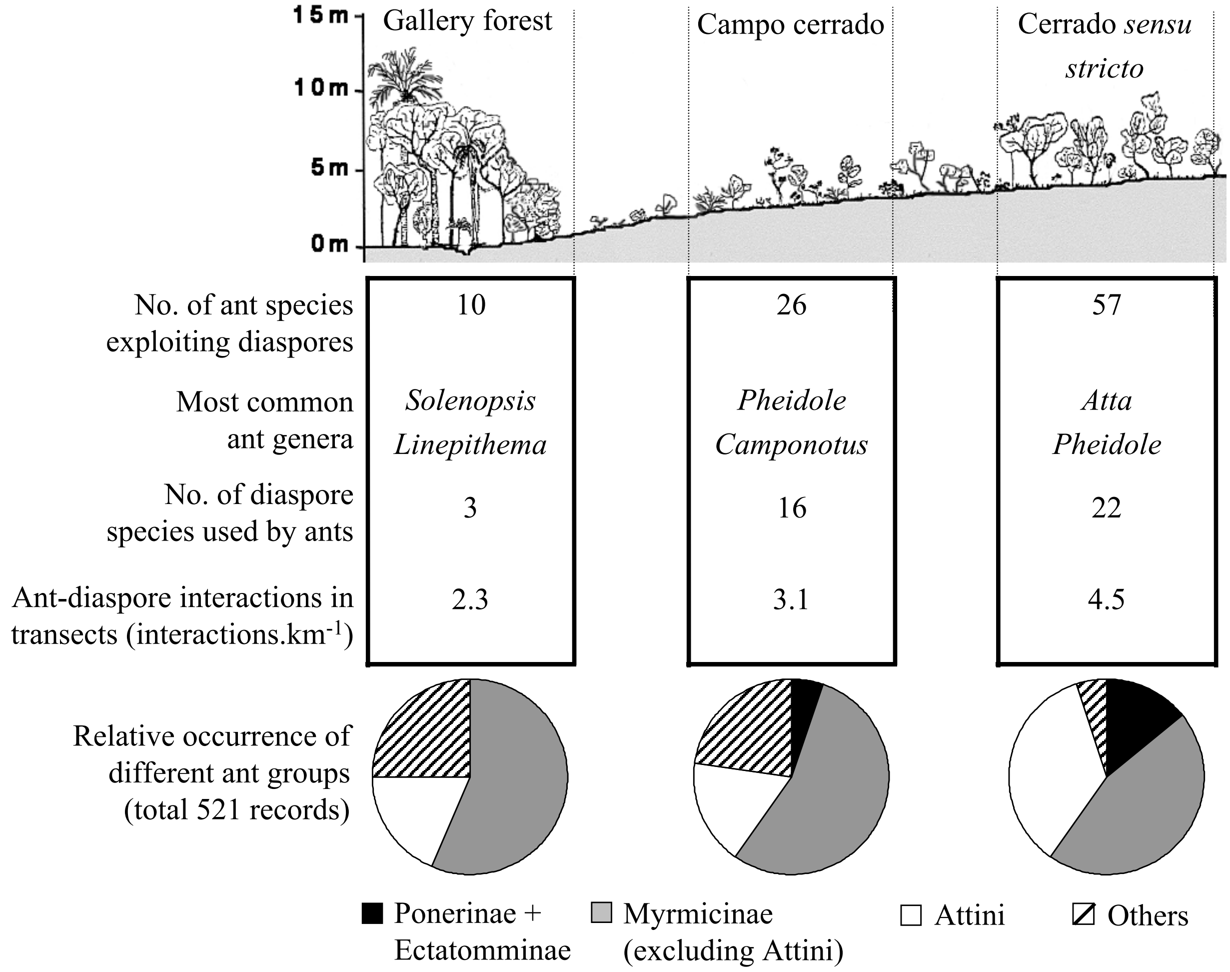


Figure 2:



	Gallery forest	Campo cerrado	Cerrado *sensu stricto*
No. of ant species exploiting diaspores	10	26	57
Most common ant genera	*Solenopsis* *Linepithema*	*Pheidole* *Camponotus*	*Atta* *Pheidole*
No. of diaspore species used by ants	3	16	22
Ant-diaspore interactions in transects (interactions.km^{-1})	2.3	3.1	4.5

Figure 3:

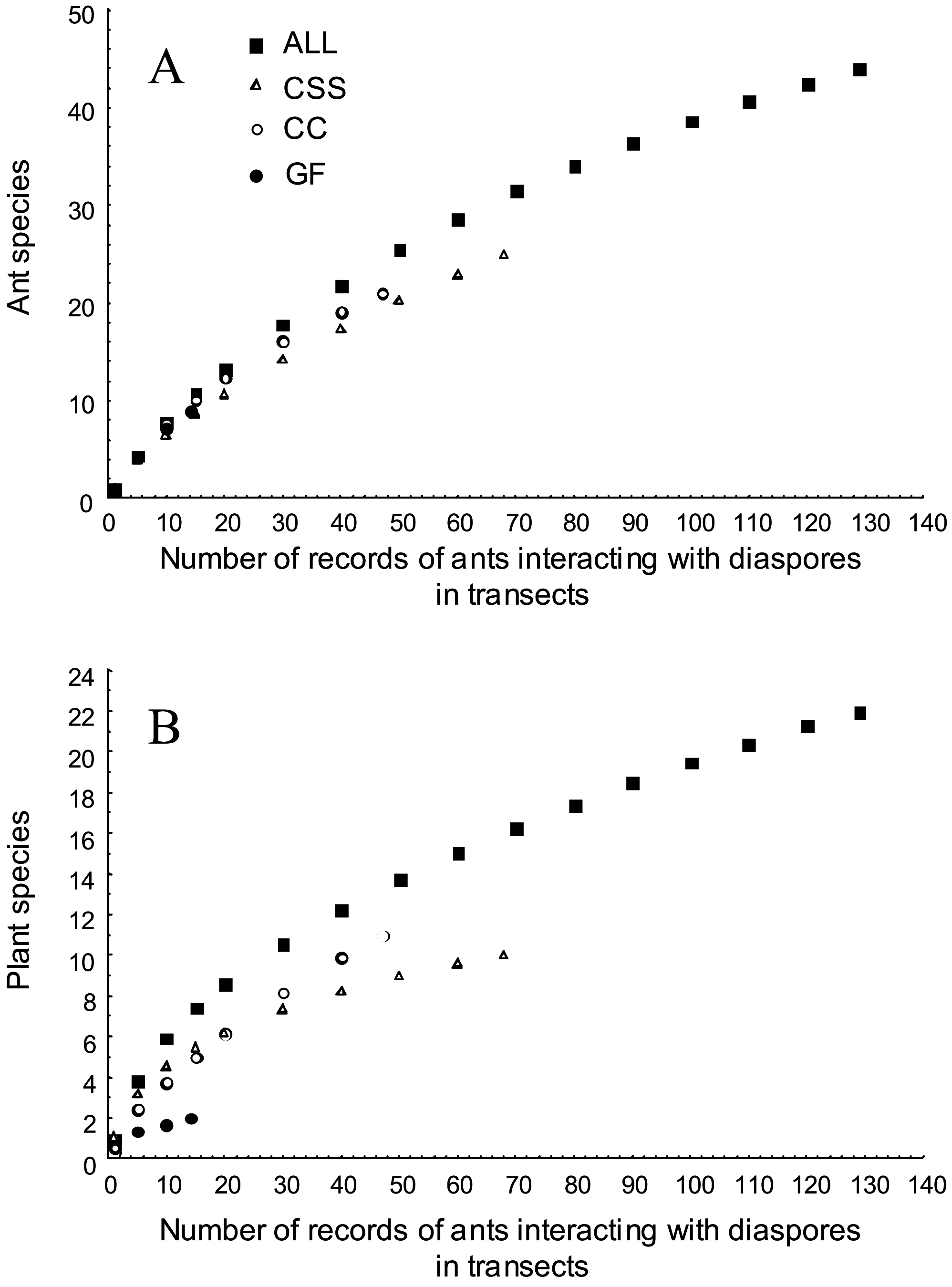

A
ALL
CSS
CC
GF
Ant species
0
10
20
30
40
50
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140
Number of records of ants interacting with diaspores in transects
B
Plant species
0
2
4
6
8
10
12
14
16
18
20
22
24
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140
Number of records of ants interacting with diaspores in transects

Figure 4:

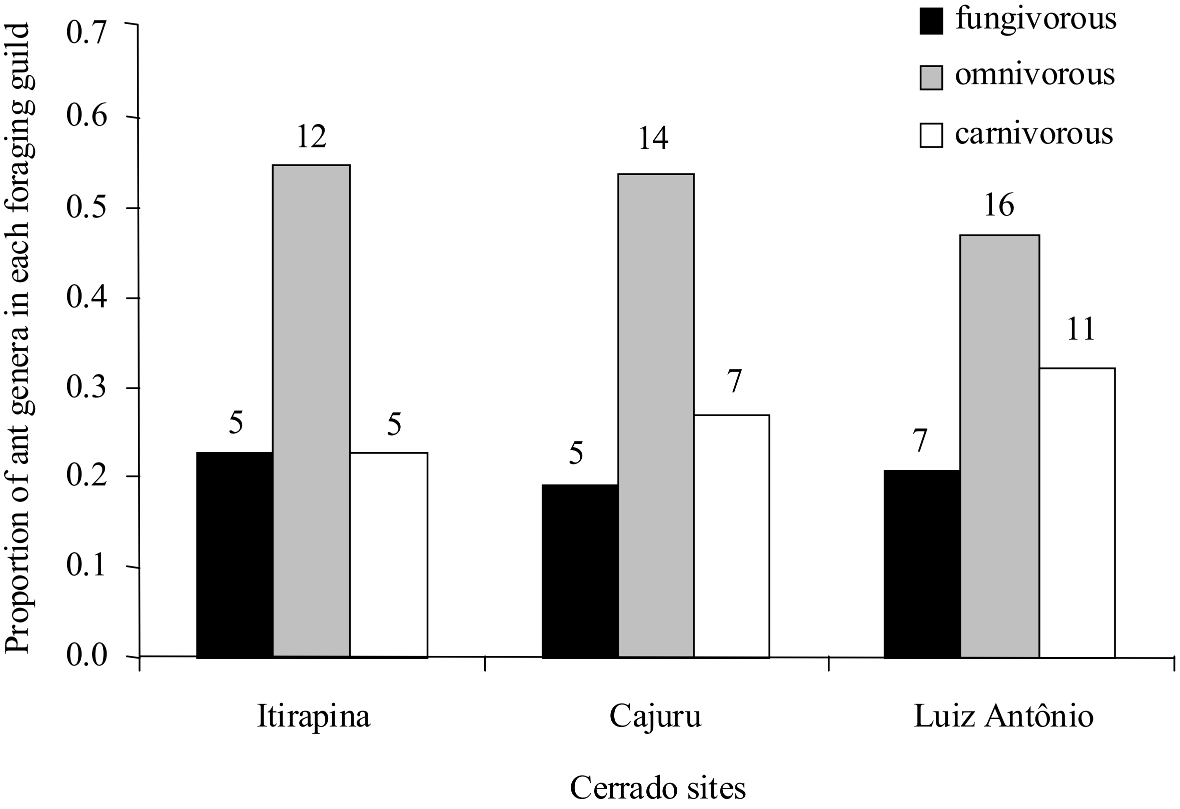
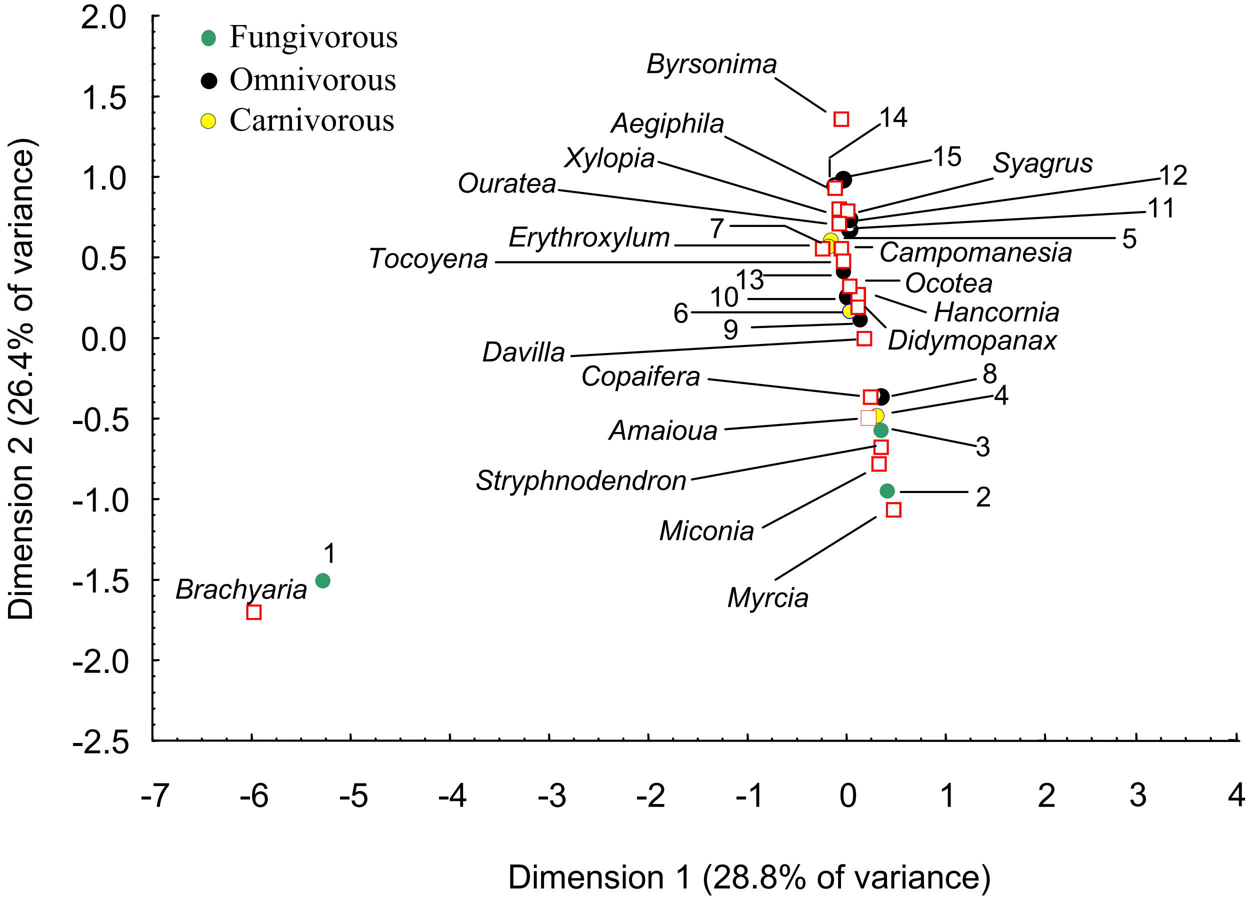

■ fungivorous
▨ omnivorous
☐ carnivorous
Proportion of ant genera in each foraging guild
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
5
12
5
5
14
7
7
16
11
Itirapina
Cajuru
Luiz Antônio
Cerrado sites

Figura 5:


Fungivorous
Omnivorous
Carnivorous
Byrsonima
Aegiphila
Xylopia
Ouratea
Erythroxylum
Tocoyena
Syagrus
Campomanesia
Ocotea
Hancornia
Didymopanax
Davilla
Copaifera
Amaioua
Stryphnodendron
Miconia
Myrcia
Brachyaria
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Dimension 2 (26.4% of variance)
Dimension 1 (28.8% of variance)
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-7
-6
-5
-4
-3
-2
-1
0
1
2
3
4

Capítulo 2

The role of ants in the removal of non-myrmecochorous diaspores and seed germination in a Neotropical savanna *

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Key-words: ant-plant interaction, cerrado, myrmecochory, secondary seed dispersal, seed predation

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ABSTRACT

Ants frequently interact with non-myrmecochorous diaspores on the ground in neotropical savannas. Our objective was to quantify the removal rate of these diaspores by ants and vertebrates in order to test the predator avoidance hypothesis, and to test how diaspore traits influence removal by ants and dispersal distance. We also investigated whether seed cleaning (removal of fruit matter simulating ant activity) can influence seed germination. We performed removal experiments with nine diaspore species in a reserve of cerrado savanna in south-east Brazil. Considerable differences in removal rates were found among the nine species. We found a positive linear relationship between lipid content and removal rates for five diaspore species. Vertebrate predation pressure was low for most species, limiting the benefits that ants can provide to plants to escape predators. Ants displace diaspores up to 25 m, which may increase the chance of a seed hitting a safe site. Smaller diaspores attain longer distances of dispersal than large ones. Seed cleaning increased the germination rate for five out of six species tested in greenhouse experiments. Ant activity can have relevant and possibly lasting effects on seed fate of plants adapted for vertebrate dispersal in the cerrado savanna.

INTRODUCTION

Although myrmecochory (i.e. primary seed dispersal by ants) is much more frequent in plants of arid zones of Australia and South Africa (Beattie 1985), recent work in other tropical areas has shown that ants can positively affect seed fate of diaspores (i.e. fruits or seeds that acts as the unit of plant dispersal) adapted for vertebrate dispersal (Farji Brener & Silva 1996, Levey & Byrne 1993, Passos & Oliveira 2003). The exploitation of such non-myrmecochorous diaspores by ants usually involve the removal of the diaspore to the ant nest, where the fleshy portion (seed aril or fruit pulp) is fed to larvae and the seed discarded unharmed (Pizo & Oliveira 2001). Because ants quickly remove seeds from predation-prone zones such as the ground surface beneath the plant crown, they reduce the chance of seed encounter by predators (Beattie 1985). This predator-avoidance hypothesis, i.e. a presumed selective advantage of the increased escape from seed predators as seeds are carried back to ant nests, has been frequently supported in studies with true myrmecochorous diaspores (Giladi 2006). However, the role of ants in providing an escape from post-dispersal seed predators of non-myrmecochorous diaspores has received considerably less attention (Levey & Byrne 1993, Pizo & Oliveira 1998).

Ant behaviour may have been an important selective force in the evolution and maintenance of diaspore traits, since ants show differential response to variation in the degree of seed clumping and morphology of elaiosome-bearing seeds (Hughes & Westoby 1992b). Approaches to explain differences in seed preferences by ants include the foraging strategy hypothesis, under which ants would use diaspore characteristics to increase foraging efficiency and net energy intake (Hughes & Westoby 1992b, Pyke *et al.* 1977). The size of the reward, either absolute or relative to seed size, has been shown to be especially important. Relationships between diaspore traits and removal rates can indicate specific foraging strategies of ants (Hughes & Westoby 1992b). However, adaptations to dispersal by ants may bring significant costs to the plants. Fallen diaspores are also attractive to seed predators, usually rodents (Fedriani *et al.* 2004), and intact fruits are especially susceptible to pathogen attack that may kill the seed (Ohkawara & Akino 2005). If removal of the seed's fleshy coat (aril or fleshy pulp) enhances the probability of seed survival and germination, ants may influence seed fate as they clean the seed from fleshy matter. If this is true, seeds deprived of fleshy matter should present a higher germination success than intact diaspores.

The role of ants in diaspore removal and seed fate has largely been neglected in the largest South American savanna known as cerrado (Oliveira-Filho & Ratter 2002). Although the vast majority of plants from this savanna has no visible adaptation for seed dispersal by ants (Gottsberger & Silberbauer-Gottsberger 1983), these insects may influence the fate of large numbers of seeds that fall to the ground spontaneously or are dropped by vertebrate frugivores (Leal & Oliveira 1998). Specific benefits derived from the interaction between ants and diaspores primarily adapted for vertebrate dispersal are still poorly understood in savannas compared to other formations (Vander Wall & Longland 2004). In this study we collected evidence to examine three hypotheses that might account for the role of ants while interacting with non-myrmecochorous diaspores in the cerrado savanna. To test the predator-avoidance hypothesis we compared the relative contribution of ants and vertebrates for the removal of nine non-myrmecochorous diaspore species, and also recorded the dispersal distance provided by ants. In order to test the foraging strategy hypothesis, we measured several morphological parameters of plant diaspores and evaluated their relationship with removal rates by ants. Finally, to evaluate the effect of seed cleaning (i.e. removal of fleshy matter) we compared the percentage of germination of seeds coated in fleshy pulp with that of seeds from which the fleshy portion was removed simulating ant behaviour.

STUDY SITE

Diaspore removal experiments were carried out in the reserve of the Estação Experimental de Itirapina (22°12'S, 47°51'W), south-east Brazil. Average annual rainfall is 1360 mm, concentrated mostly in the warm/wet season (December to March). A dry/cold season occurs from April to November. Mean annual temperature is 21.8ºC (data from 1994 to 2004 from the reserve's climatological station). The vegetation at the study site is the cerrado *sensu stricto*, the typical savanna-like vegetation that grows on sandy, nutrient-poor soils (Oliveira-Filho & Ratter 2002). The vegetation is characterized by 50% to 80% of ground cover by small palms (*Syagrus petraea* (Mart.) Becc., *Attalea geraensis* Barb. Rodr.), shrubs (*Miconia albicans* (Sw.) Triana, *Campomanesia pubescens* (DC.) O. Berg), and 4-6-m-tall trees (*Miconia rubiginosa* (Bonpl.) DC., *Xylopia aromatica* (Lam.) Mart., *Pouteria torta* (Mart.) Radlk). A fair amount of herbaceous vegetation covers the soil (*Echinolaena inflexa* (Poir.) Chase, *Paspalum* spp., *Bromelia balansae* Mez). Further floristic details of the study site are given by Giannotti (1988).

METHODS

Diaspore removal experiments: ants versus vertebrates

To examine the contribution of ants to diaspore removal on the savanna floor we performed field experiments with fruits collected from nine plant species at the study site: *Erythroxylum pelleterianum* A. St.-Hil (Erythroxylaceae), *Miconia albicans* (Sw.) Triana, *M. fallax* DC., *M. rubiginosa* (Bonpl.) DC. (Melastomataceae), *Myrcia lingua* (O. Berg.)

Mattos & D. Legrand (Myrtaceae), *Ocotea pulchella* Mart. (Lauraceae), *Ouratea spectabilis* (Mart. ex Engl.) Engl. (Ochnaceae), *Psychotria tricholoba* Müll Arg. (Rubiaceae), and *Stryphnodendron adstringens* (Mart.) Coville (Mimosaceae). Large numbers of ripe fruits or seeds frequently fall from these trees, either naturally or dropped by vertebrate frugivores. During the fruiting period of each species, diaspore removal by ants only (exclosure treatment) was compared to combined removal by ants and vertebrates (open control) through a paired experiment on the savanna floor. We assume that removal of diaspores by vertebrates is equal to predation. This is based on data showing that small seeds are indeed eaten rather than dispersed by small rodents (Vieira *et al.* 2003). Tinamous and doves, the birds most likely to remove such diaspores on the floor, are also recognized as seed predators by cracking the seeds in their gizzards (Schubbart *et al.* 1965). Vertebrates were excluded from treated diaspores with the aid of a wire cage (17 × 17 × 8 cm), fenced on top and sides with mesh (1.5 cm) and staked to the ground (see Roberts & Heithaus 1986 for a similar method). Control diaspores were placed outside the cage, 15 cm away. At each station, treatment and control categories each received ten diaspores of a single plant species, and were exposed for 24 h. After this period we recorded the ant species interacting with diaspores, and the number of diaspores missing. The diaspores were distributed under fruiting plants of the same species to simulate natural conditions under which they are found by ants and vertebrates. Experimental diaspores were marked with a small dot of a permanent ink marker (Testors, USA) to distinguish them from naturally fallen ones. The ink mark has no detectable effect on ant behaviour (Passos & Oliveira 2002). We kept a minimum distance of 10 m between replicates to provide independent discoveries by different ant colonies (Levey & Byrne 1993, Pizo & Oliveira 2000). The number of replicates varied between species according to diaspore availability.

To compare diaspore removal of treated (accessible to ants only) and control (accessible to vertebrates and ants) diaspores of each plant species we used Wilcoxon paired sample tests, except for *O. spectabilis* and *P. tricholoba* for which we used Mann-Whitney U-tests due to small sample sizes.

The ant assemblage removing diaspores

To increase the number of records of ants interacting with diaspores and to measure the distance of displacement we also conducted systematic samplings. We placed diaspores of four selected plant species on the floor of the cerrado, and recorded the ants removing them in diurnal and nocturnal samplings. The following plants were selected for these surveys: *Erythroxylum pelleterianum* (Erythroxylaceae), *Ocotea pulchella* (Lauraceae), *Miconia albicans* and *M. rubiginosa* (Melastomataceae). The number of diaspores used to attract ants varied according to their availability (from 40 to 100 diaspores), and samplings were carried out during the fruiting period of each species. Fresh diaspores of each species were placed at sampling stations 10 m apart, 1-2 m off a transect that cross the study site. At each sampling station, two diaspores of a single species were placed on a small piece of white filter paper (4 × 4 cm) to facilitate visualization. The filter paper apparently did not influence ant behaviour (Pizo & Oliveira 2000). Diurnal samplings began at 08h00, and nocturnal ones at 19h00. At each period we walked slowly along the transect over 2 h and recorded the ants removing diaspores. We followed ants carrying diaspores until they reached the nest or disappeared in the leaf litter. The distance of displacement was then measured. At any time during the study, opportunistic observations of ants removing diaspores were also recorded. Voucher specimens of ants and plants are deposited in the entomological collection of the Universidade Federal Rural do Rio de Janeiro (CECL), and at the herbarium of the Universidade Estadual de Campinas (UEC), respectively.

Diaspore traits affecting removal by ants

To investigate the influence of diaspore traits on removal by ants we measured several morphological and chemical traits of plant diaspores and evaluated their relationship with removal rates by ants. Diaspore morphology was characterized by fresh mass, fresh mass of seeds, and pulp/seed ratio. Measurements were taken on fruits of 1-5 plants per species, and from 1-10 fruits per individual according to diaspore availability. Ripe fruits were frozen and brought to the laboratory for measurements. For the chemical analyses, diaspores were selected based solely on their availability. We limited the chemical analyses to lipid content because lipids are among the main elicitants of diaspore removal by ants (Hughes *et al.* 1994, Pizo & Oliveira 2001). Fruit pulp was obtained from ripe fruits collected at the plant crown, or from recently fallen ones. Lipids were analysed according to the methods described in AOAC (2000). Mean diaspore removal inside exclosure cages (access only to ants) was related to per cent lipid content of the fruit pulp, fruit fresh mass, and pulp/seed ratio (all log-transformed) with linear regression. For seven species for which we had records of the displacement distance, we performed the same analysis using the mean or the maximum dispersal distance (log-transformed) as the dependent variable to evaluate the effect of diaspore traits on the distance of dispersal by ants.

Seed germination tests

We evaluated the effect of ant-diaspore interactions on seed germination through greenhouse experiments. Six plant species observed interacting with ants in the field were used in the experiments: *Copaifera langsdorfii* Desf. (Fabaceae), *Erythroxylum pelleterianum* (Erythroxylaceae), *Guapira noxia* (Netto) Lundell (Nyctaginaceae), *Miconia albicans*, *M. rubiginosa* (Melastomataceae) and *Ouratea spectabilis* (Ochnaceae). Diaspores from at least three individuals of each species were collected in the field and mixed to form a bulk sample. Diaspores were then sorted into two groups and subjected to one of the following treatments: removal of aril/pulp (to simulate removal by ants), or control (manipulated by us but without removal of fleshy part). Both groups were sowed in plastic trays (37 × 28 cm) containing regularly moistened vermiculite. Fruits of both treatments were sown interspersed with each other in a systematic design type in the greenhouse of the Universidade Estadual de Campinas. Fruits were buried 1 cm in the substrate, 2 cm apart from each other, and checked at 7-10-d intervals. Fruits of *Miconia* spp. were sown on the surface of the vermiculite, because these species require light to germinate (Hoffmann 1996). Since the number of seeds varies with fruit size in *Miconia* (from 5 to 25 seeds), we first separated fruits by size to control for the number of seeds used in each treatment. We then assigned an equal number of diaspores from each size category to both treatment groups. Seeds were removed from single fruits and sown in a cluster about the same diameter as the fruit. A fruit of the same size was sown as control. When a seed germinated, the whole cluster (or fruit) was removed from the experiments. Germination experiments ran until seeds presented signs of decay or until no new germination was recorded for 2 mo. We used G-tests to compare the final percentage of germination between treatments.

RESULTS

The ant assemblage removing diaspores

We recorded 27 ant species interacting with diaspores in the field (Appendix 1). The number of ant species recorded per diaspore species varied from zero (for *Psycothria tricholoba*) to 19 (for *Erythroxylum pelleterianum*). Most species (18 out of 27) did not remove diaspores, and just cleaned the seed (i.e. removed the fleshy part) or collected liquids from the diaspore on the spot. This was especially true for *Camponotus* spp. and myrmicine ants in the genera *Pheidole*, *Solenopsis* and *Wasmannia*. Diaspore removal was recorded among ants in the genera *Atta*, *Pheidole*, *Dinoponera*, *Ectatomma*, *Odontomachus* and *Pachycondyla*, the last four usually regarded in the literature as primarily carnivorous.

Diaspore traits and their removal by ants and vertebrates

Diaspore traits differed greatly among species used in the removal experiments (Table 1). For instance, diaspore size varied from 0.07 g in *Miconia albicans* to 0.52 g in *Ouratea spectabilis*. Due to limited diaspore availability, per cent lipid content data was obtained for five species only. The amount of lipids in the fleshy portion of diaspores varied from 0.78% fresh mass in *Miconia rubiginosa* to 31.6% fresh mass in *Ocotea pulchella*, a 40-fold difference.

Diaspore removal differed between caged and open treatments only for two species (Table 2), and ants were likely to be the main source of removal for most diaspores in both treatments because no other invertebrates were observed removing diaspores. Removal at open controls was significantly correlated with removal at caged treatments across the plant species studied ($r = 0.75$; $N = 9$; $P = 0.02$).

To test the foraging strategy hypothesis we related the removal rates of diaspores inside exclosure cages with the following seed parameters (Table 1): per cent lipid content of the fleshy portion, fruit mass and pulp/seed ratio. We applied stepwise regression analysis using the backward selection procedure from STATISTICA software, version 5 (©1996, STATSOFT, INC.). The final model included only lipid content of the fleshy portion which explained most of the variation in the removal rates ($y = 2.6x + 2.5$; $N = 5$; $t = 13.3$; $P < 0.001$; $r^2 = 0.98$) (Figure 1). Additional variables (fruit mass and pulp/seed ratio) when included in the model were not significant. Stepwise or univariate regression analysis without lipid data gave similar results. A negative, but non-significant, correlation was found between lipid content of the fleshy portion and pulp/seed ratio ($r = -0.85$; $N = 5$; $P = 0.07$).

Ants displace diaspores to considerable distances (up to 25.4 m), but there is also great variation among the distances achieved per diaspore species (Table 3). No relationship was detected between the mean dispersal distance and diaspore traits. However, the maximum distance of dispersal recorded was negatively influenced by diaspore mass ($y = -11.7x + 1.7$; $N = 7$; $t = -5.04$; $P = 0.004$; $r^2 = 0.80$).

Seed germination tests

The removal of fruit pulp simulating ant activity increased seed germination of five out of six plant species tested (Table 4). Greenhouse conditions apparently were not adequate for the germination of *Ouratea spectabilis*. Pulp removal increased germination success by 35% to 77% compared to controls. For species with germination success > 40% in the control group, we also compared the germination rate. In such cases seed cleaning increased germination speed, as recorded for *Miconia albicans* (Kolmogorov-Smirnov test: $D_{max} = 0.90$; $P < 0.001$) and *Copaifera langsdorfii* ($D_{max} = 0.55$; $P < 0.01$).

DISCUSSION

We found evidence that ants are an important source of diaspore removal on the floor of the cerrado savanna, and that this activity is influenced by the lipid content of the fleshy portion. Diaspores are transported to the nests up to 25 m, and seeds deprived of the fleshy part showed higher germination rates than intact seeds. Taken together these sources of evidence indicate that ants can influence the fate of non-myrmecochorous diaspores in the cerrado.

Fast removal of vulnerable seeds by non-granivorous ants supports the predator-avoidance hypothesis (Manzaneda *et al.* 2005, Pizo & Oliveira 1998, Roberts & Heithaus 1986). However, the interpretation of these results is more difficult when seed predation pressures are weaker (Giladi 2006). Differences between the mean diaspore removal in exclosure treatments and open controls indicated that predation levels (removal of diaspores by rodents and granivorous birds) are small to moderate for most species, with differences ranging from 0% (as in the case of *M. fallax*) to 38% (for *M. albicans*), most of which are not significant. Exceptions were *O. spectabilis* and *S. adstringens*, with removal rates at open controls 100% and 250% higher than caged treatments, respectively. However, the large variance found in removal rates indicates great spatial variation within the study site and in the potential benefits ants may render to plants, as found by other authors working at larger spatial scales (Fedriani *et al.* 2004, Manzaneda *et al.* 2005). In addition, not all ants recorded interacting with diaspores are recognized as good dispersers. *Atta* and *Pheidole* are considered as potentially important seed consumers, although they can also disperse some seeds (Dalling & Wirth 1998, Farji Brener & Silva 1996, Hughes & Westoby 1992a, Leal & Oliveira 1998, Levey & Byrne 1993). However, we believe that most diaspores have a higher probability of survival when removed by ants for three reasons. First, ants can remove seeds to considerable distances (up to 25 m), far from the parent-offspring conflict zone under the parent crown (Retana *et al.* 2004). Second, seed cleaning behaviour can increase the germination percentage and speed of some plant species, minimizing the time seeds are exposed to predators or pathogens (Oliveira *et al.* 1995). Third, seedlings of several plants investigated in this study are found mainly in refuse piles of ant nests (A. V. Christianini & P. S. Oliveira unpubl. data). Some of this evidence, however, cannot be exclusively linked to the predator-avoidance hypothesis. Indeed, in a review of the evidence for the evolution of myrmecochory, Giladi (2006) found several examples of studies showing simultaneous support to non-competing hypotheses such as the predator-avoidance and the directed-dispersal hypothesis. Our findings could reflect weak selection pressure from vertebrate predators, which can also be affected by spatial or temporal heterogeneity (Manzaneda *et al.* 2005, and references therein). Our experimental design does not allow us to examine the historical selective forces that shaped ant dispersal of non-myrmecochorous diaspores, but give some insights on seed preference by ants in cerrado.

At least for the five species for which data on lipid content of fruit pulp was available, our data suggests that ants collect diaspores on the basis of high lipid content, i.e. maximizing the amount of lipids ingested per unit of diaspore mass handled. This finding supports the foraging strategy hypothesis. Animals are thought to adjust their foraging strategies to optimise their net rate of energy intake (Pyke *et al.* 1977). Lipids are an important food resource for ants, serving a variety of purposes that include nutrition, physiological constituents, and behavioural releasers (Beattie 1985, and references therein). The fatty acid composition of lipid-rich diaspores and typical myrmecochorous seeds is closely similar (Hughes *et al.* 1994, Pizo & Oliveira 2001), and therefore it is not surprising that ants responded positively to lipid-rich diaspores in the present study. The fleshy portion of lipid-rich diaspores should provide a higher caloric reward to ants, since average gross-energy equivalents of lipids (38.9 kJ g^{-1}) are much larger than equivalents for protein (17.2 kJ g^{-1}) and carbohydrates (17.2 kJ g^{-1}) (see Izhaki 2002, and references therein). Water and carbohydrates are often the predominant reward in fruit pulp (Jordano 1995). Preference of ants for diaspores with a high absolute reward has been shown in other studies (Mark & Olesen 1996, Peters *et al.* 2003), but diaspore size (Gorb & Gorb 1995, Pizo & Oliveira 2001) and pulp/seed ratio (Hughes & Westoby 1992b) may also play a role.

We found that the maximum dispersal distances provided by ants in the cerrado were constrained by diaspore size, which suggests a disproportionate increase in the costs of transport of larger diaspores as travel distances of a forager ant increase. It is recognized that forager ants become more selective as the distance of a resource patch from the nest increases. Selectivity is reduced when resources are scarce (see Traniello 1989, and references therein). Larger diaspores should demand more time and energy to be brought to the nest, exposing the ants to higher risks of predation and/or to superior competitors. In resource-rich habitats large and low-rewarding diaspores would be removed at lower rates when found far from nests, thus achieving shorter distances of dispersal by ants. Future studies should investigate the role of resource availability and net energy gain on the distance of seed dispersal by ants.

The distances of displacement of diaspores found in this study (mean for all events 5.04 m) were larger than the mean 0.96 m global estimate of myrmecochorous dispersal distances (Gómez & Espadaler 1998), and larger than most distances recorded in other studies on seed dispersal by ants in the Neotropics (Ness *et al.* 2004). The larger distances recorded in the cerrado likely result from the many records of Attini (mainly *Atta* spp.)

carrying diaspores. Dispersal distances by *Atta* were larger than those found for all other species (A. V. Christianini, unpubl. data). Similar displacement distances recorded for Attini ants in another cerrado locality reinforce this hypothesis (Leal & Oliveira 1998). *Atta* workers are among the largest ants in the local ant assemblage. Ant body size bears a positive linear relationship with dispersal distances (Ness *et al.* 2004), which is possibly linked with the density and distribution of the ant nests (Gómez & Espadaler 1998). Nevertheless all studies report great variance around the distances of dispersal. Many diaspores are also dropped and not recovered during transport to the nest (Dalling & Wirth 1998, Leal & Oliveira 1998). From a plant's perspective, it means that seeds can be spread out at variable distances from the parent plant, and at variable densities (Dalling & Wirth 1998, Passos & Oliveira 2002), which can be important for a plant that needs to reach a particular microsite for regeneration. *Miconia* species, for instance, need particular conditions for germination and establishment (Hoffmann 1996), and dispersal to long distances should increase the probability of a seed hitting a safe site (Green 1983).

Even large-seeded diaspores may benefit from the interaction with ants. For instance seed cleaning by ants may reduce fungal attack on fallen fruits (Oliveira *et al.* 1995, Ohkawara & Akino 2005), and pulp removal increased germination success of most species tested in this study. For those species dispersed near the end of the wet season and with no seed dormancy (as in the case of most species tested in this study) seed cleaning may also be advantageous because early emergence maximizes the length of the first growing season and allows time for the growing of a larger and deeper root system. This might ensure survival during the first dry season when seedlings are in general more susceptible to death during periods of water shortage (Moles & Westoby 2004, Passos & Oliveira 2004), which is particularly severe in the study site.

In conclusion secondary seed dispersal by ants can have relevant and possibly lasting effects on the fate of non-myrmecochorous diaspores in the cerrado savanna (see also Passos & Oliveira 2002, 2004). Ant activity can enhance seed germination, reduce seed predation by increasing dispersal distance from the parent plant, and produce a fine tuning of directed dispersal following longer primary dispersal by vertebrates (Giladi 2006, Horvitz & Le Corff 1993). At the moment we cannot discuss if ants are a selective force towards a particular suite of traits of non-myrmecochorous diaspores in cerrado. A large assemblage of vertebrate frugivores primarily disperses the seeds in this savanna (Gottsberger & Silberbauer-Gottsberger 1983), and fruit and seed traits are strongly constrained by phylogenetic inertia (Jordano 1995). Ignoring secondary dispersal, however, may produce a misleading picture of the seed dispersal loop of cerrado plants (Vander Wall & Longland 2004).

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Appendix 1. Ants recorded exploiting diaspores on the floor of cerrado. Plant species abbreviations: *Erythroxylum pelleterianum* (Eryp); *Miconia albicans (*Mica*)*; *M. fallax (*Micf*)*; *M. rubiginosa (*Micr*)*; *Myrcia lingua* (Myrl); *Ocotea pulchella* (Ocop); *Ouratea spectabilis* (Ours); *Psychotria tricholoba* (Psyt); *Stryphnodendron adstringens* (Stra). Ant species are in alphabetical order, following a local species list (A. V. Christianini unpubl. data). Values inside cells indicate the number of records each ant species interacted with each diaspore species. Key to ant behaviour: C clean seeds through pulp removal on the spot, no displacement; I = inspect or collect liquids, no displacement; R = remove diaspores ≥ 5 cm; T = try to remove diaspores, displacement < 5 cm.

Ant species	Behaviour	Eryp	Mica	Micf	Micr	Myrl	Ocop	Ours	Psyt	Stra
Atta laevigata (Fr. Smith)	R	-	-	-	11	-	-	-	-	-
Atta sexdens rubropilosa Forel	I, R	3	21	3	29	8	2	-	-	3
Azteca sp. 2	C	2	-	-	-	-	-	-	-	-
Azteca sp. 3	I	1	-	-	-	-	-	-	-	-
Camponotus renggeri (Emery)	I	2	-	-	-	-	-	-	-	-
Camponotus rufipes (Fabricius)	I	1	-	-	-	-	-	-	-	-
Dinoponera australis Emery	R	6	-	-	1	-	1	-	-	-
Ectatomma opaciventre Roger	R	-	-	-	7	-	-	-	-	-
Mycocepurus sp.	C, I	2	-	-	-	-	-	-	-	-

Continued

Odontomachus chelifer (Latreille)	I, R	5	1	-	3	-	-	-	-	-
Oligomyrmex sp.	C	-	-	-	-	-	-	1	-	-
Pachycondyla striata Fr. Smith	R	19	1	-	2	-	-	-	-	-
Pachycondyla villosa (Fabricius)	R	1	-	-	1	-	-	-	-	-
Pheidole gr *tristis* prox *allarmata*	C	-	-	-	1	-	1	-	-	-
Pheidole sp. 2	C, R	6	-	1	-	1	-	-	-	1
Pheidole sp. 4	C	5	-	-	-	-	-	-	-	-
Pheidole sp. 5	C, T, R	13	1	-	-	1	1	-	-	-
Pheidole sp. 6	C	-	-	-	1	-	-	1	-	-
Pheidole sp. 7	C	8	2	-	3	-	-	2	-	-
Pheidole sp. 8	I, R	-	-	-	-	-	3	-	-	-
Pheidole sp. 15	C	2	-	-	-	-	-	-	-	-
Pheidole sp. 16	C	-	-	-	-	-	2	-	-	-
Pheidole sp. 17	C, R	2	-	-	-	-	-	-	-	-
Solenopsis sp. 1	C	1	-	-	-	-	-	-	-	-
Solenopsis sp. 2	I	1	-	-	-	-	-	-	-	-

Continued

Solenopsis sp. 3	C	-	-	-	-	-	1	-	-	-
Wasmannia auropunctata (Roger)	C	7	1	-	-	-	-	-	-	-
Total number of records at diaspores		87	27	4	59	10	11	4	0	4

Table 1. Growth form, primary dispersal agent, mean masses of fresh diaspores, seeds, and pulp/seed ratio of the plant species studied (N = 1-10 diaspores from 1-5 plants). Seeds of *S. adstringens* have no fleshy portion, thus pulp/seed ratio was set to be zero.

Plant species	Growth form	Primary dispersal	Diaspore mass (g)	Seed mass (g)	Pulp/seed ratio
Erythroxylum pelleterianum	Shrub	Bird	0.15	0.041	2.63
Miconia albicans	Shrub	Bird	0.07	0.009	6.78
M. fallax	Shrub	Bird	0.25	0.015	16.5
M. rubiginosa	Tree	Bird	0.12	0.012	11.7
Myrcia lingua	Tree	Bird	0.23	0.082	2.04
Ocotea pulchella	Tree	Bird	0.16	0.100	0.693
Ouratea spectabilis	Tree	Bird	0.52	0.338	0.525
Psychotria tricholoba	Shrub	Bird	0.11	0.033	2.44
Stryphnodendron adstringens	Tree	Abiotic	0.09	0.090	0

Table 2. Results from diaspore removal experiments on the savanna floor. Ten diaspores of each species were set out under an exclosure cage (access to ants; vertebrates excluded) paired with an open control (access to all animals). The number of diaspores removed was recorded after 24 h. N refers to the number of replicates; ns indicates no significance difference (P > 0.05).

Plant species	N	Number of diaspores removed (mean ± SD)		P
		Exclosure treatment	Open control	
Erythroxylum pelleterianum	5	5.6 ± 3.9	6.8 ± 4.0	ns
Miconia albicans	16	3.1 ± 3.4	4.3 ± 4.0	ns
M. fallax	5	5.6 ± 4.1	5.6 ± 4.2	ns
M. rubiginosa	33	3.2 ± 4.4	4.0 ± 4.5	0.02
Myrcia lingua	5	3.8 ± 4.8	4.2 ± 5.3	ns
Ocotea pulchella	5	6.2 ± 3.0	4.8 ± 4.8	ns
Ouratea spectabilis	3	1.0 ± 1.7	2.3 ± 0.6	ns
Psychotria tricholoba	3	0.0 ± 0.0	0.3 ± 0.6	ns
Stryphnodendron adstringens	7	1.6 ± 1.9	5.7 ± 4.2	0.05

Table 3. The mean distance (m) that ants disperse diaspores in the cerrado savanna, range and sample size.

Plant species	Distance of displacement (m)		
	Mean	Range	N
Erythroxylum pelleterianum	1.46	0.1-7.1	22
Miconia albicans	5.91	2.0-13.9	12
Miconia fallax	1.90	-	1
Miconia rubiginosa	6.54	0.2-14.6	25
Myrcia lingua	3.20	2.3-4.6	3
Ocotea pulchella	4.52	0.1-9.0	2
Stryphnodendron adstringens	17.7	10.0-25.4	3

Table 4. Results of G-tests on the germination of selected plant species in the cerrado. Treatment refers to seeds from which the fleshy part (aril or pulp) was manually removed to simulate ant activity; control seeds were just hand manipulated, but without removal of fleshy part. Per cent differences between treatment and control groups in final germination are indicated. Positive differences indicate increased seed germination in treatment compared to control group.

Plant species	Number of seeds sown		Number of germinated seeds		% difference	G-value
	Treatment	Control	Treatment	Control		
Copaifera langsdorfii	21	12	18	5	+ 43%	7.0**
Erythroxylum pelleterianum	60	60	31	0	+ 52%	54.0***
Guapira noxia	16	13	11	0	+ 69%	18.6***
Miconia albicans	60	60	60	39	+ 35%	33.6***
Miconia rubiginosa	30	30	24	1	+ 77%	42.7***
Ouratea spectabilis	20	20	4	0	-	-

** P < 0.01; *** P < 0.001

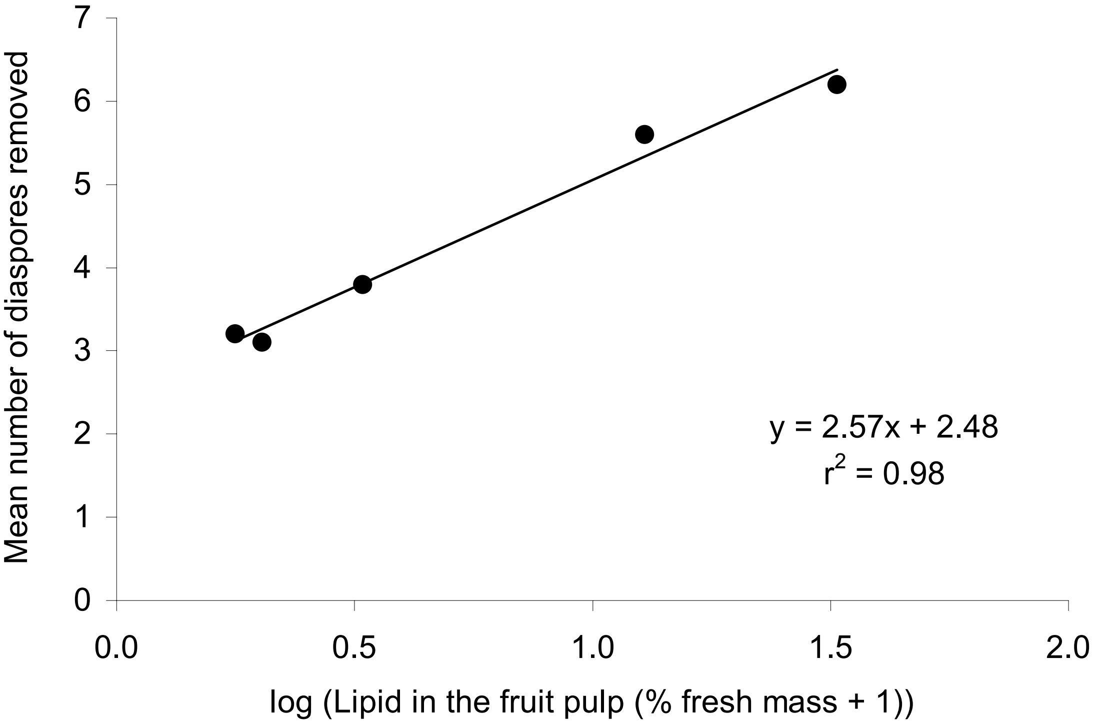

Figure 1. Effect of lipids on diaspore removal by ants in the cerrado savanna. Mean number of diaspores removed by ants as a function of per cent lipid in the fleshy portion (amount of lipid relative to total fresh mass) for five plants. Each point represents one plant species.

Capítulo 3

Contribution of primary and secondary seed dispersal to early seedling regeneration in a Neotropical savanna

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Running-title: Primary and secondary seed dispersal in savanna

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Abstract

1. We investigated the consequences of a possible diplochorous seed dispersal system for the regeneration of two fleshy-fruited plants in the Cerrado, a neotropical savanna, in south-east Brazil: *Erythroxylum pelleterianum* (Erythroxylaceae) and *Xylopia aromatica* (Annonaceae). An observational/experimental approach was adopted to estimate the relative contribution of birds (phase one of dispersal) and ants (phase two) for the regeneration of these plants until seedling stage.
2. Mean crop size varied within and between plant species, but birds (up to 11 species) invariably removed 7 to 32% of the diaspore production of the trees. From 26 to 53% of the diaspores produced reached the ground under tree crown as ripe, undamaged fruits or seeds. Seventeen ant genera exploited these fallen diaspores, removing most of them (up to 82%) to their nests in 24 hours. The average distance of diaspore displacement by ants was short (0.7 m to 1.5 m for a given plant species), while birds removed seeds at average distances 40-fold farther.
3. Although some seeds were lost to granivorous ants, plants benefited from this interaction due to directed dispersal to the ant nest refuse piles. Seedlings of *Erythroxylum* and *Xylopia* were more commonly found growing in ant nests than in random spots. Seedlings of *Erythroxylum* growing close to ant nests were more likely to survive after one year than seedlings growing away from nests. Better soil quality close to ant nests should be responsible for this effect.
4. The contribution of birds and ants in the regeneration cycle differed between plant species, but birds were more likely to provide effective long-distance dispersal allowing plant colonization of new sites for recruitment. Ants, on the other hand, reshaped the seed shadow at a finer scale, delivering seeds to specific sites where seedling survival is more likely.
5. We propose that evolution of diplochorous seed dispersal systems involving sequencial dispersal by birds and ants in Cerrado are likely due to correlated selective pressures exerted by birds and ants upon diaspore traits such as high lipid content.

Introduction

In recent years, the complexity of seed dispersal systems has been revealed by an increasing number of studies that track seed fate over time. These studies often reveal a multi-step process of plant regeneration (termed diplochory), where different guilds of animals taking part at subsequent stages of the seed dispersal process (Clifford & Monteith 1989, Kaufmann *et al.*1991, Böhning-Gaese *et al.*1999, Passos & Oliveira 2002, Vander Wall *et al.* 2005). In diplochory, primary dispersers (usually birds) move seeds away from the parent plant (phase one of dispersal), and secondary dispersers take part after the seeds reach the ground naturally or embedded in faeces (phase two of dispersal), moving seeds to specific sites, such as below litter (Vander Wall *et al.* 2005). For instance, rodents, beetles, or ants may cache seeds from mammal and bird faeces across the forest, reshaping the seed shadow provided by primary dispersers, and influencing the spatial distribution and performance of seedlings (Vander Wall *et al.* 2005, Passos & Oliveira 2002, Andresen 2002). Ants are among the animals with greater likelihood to participate in phase two of dispersal because they are very abundant across virtually all ecosystems, and they are often attracted to fallen, fleshy diaspores (i.e. the unit of dispersal of plants such as fruits and seeds), and to seeds embedded in faeces (Roberts & Heithaus 1986, Kaspari 1993, Pizo & Oliveira 1999). Recent studies have emphasized the role of ants in determining the fate of seeds of non-myrmecochorous plants, i.e, whose diaspores bear no visible adaptation for ant dispersal (Kaufmann *et al.* 1991, Pizo & Oliveira 1998, Passos & Oliveira 2002, 2003, 2004). Recognition of diplochory challenges the role played by primary seed dispersal in plant ecology and evolution (Forget & Milleron 1991, Kaufmann *et al.*1991, Aronne & Wilcock 1994, Wang & Smith 2002, Vander Wall & Longland 2004). It is suspected that many more plant species can take advantage of such multi-phase dispersal, but the few studies available are biased to tropical forests. To fully understand of the spread and importance of diplochory, we need to gather data from other geographical regions and biomes, evaluate the prevalence of diplochory relative to other dispersal modes, and determine which plant taxa are dispersed in multi-phasic ways (Vander Wall & Longland 2004).

Seed dispersal may benefit plants in at least three ways (Howe & Smallwood 1982): (1) escape from density- or distance-dependent seed and seedling mortality near the parent plant, (2) colonization of suitable sites unpredictable in either space or time, and (3) seed delivery to particular sites with a comparatively higher probability of survival (i.e. directed dispersal). Support for these benefits is abundant in the literature (e. g. Howe *et al.* 1984, Wenny 2001). However, the hypotheses above were formulated when diplochory was virtually unrecognized, and the role of phase two of dispersal in providing benefits to plants was poorly documented. Diplochory may have evolved because combining two dispersal mechanisms can increase benefits of seed dispersal over a single means of dispersal. Studies that partition seed mortality and plant fitness between phase one and two of dispersal are rare, and present conflicting results regarding the relative importance of each phase for plant recruitment (Bohning-Gaese *et al.* 1999, Passos & Oliveira 2002).

In this study we compared the relative contribution of phase one and two of seed dispersal for the regeneration of two non-myrmecochorous and un-related plants in the Cerrado, a neotropical savanna. Current evidence suggests that ants potentially influence seed fate of many plants with non-myrmecochorous diaspores in this savanna (Leal & Oliveira 1998, Christianini *et al.* 2007). We investigated simultaneously the possible effects of a bird and ant species-rich assemblage for the regeneration of plants up to the seedling stage. We specifically addressed the relative roles of phases one and two of dispersal in the escape hypothesis, and in the directed dispersal hypothesis. We show that phase two of dispersal plays a key role in the regeneration strategy of both plant species studied.

Study site

Field observations and experiments were carried out from February 2004 to March 2006 in the reserve of the Estação Experimental de Itirapina (22°12'S, 47°51'W), a 200 ha fragment of cerrado savanna in Southeast Brazil. Average annual rainfall is 1,190 mm, concentrated in the warm and wet season (December to March). A dry and cold season occurs from April to November. Mean annual temperature is 19.7ºC. The vegetation at the study site is the cerrado *sensu stricto*, the typical fire-prone savanna that grows on sandy, nutrient poor soils of the Cerrado domain, characterized by 50-80% ground cover by small palms, bushes and trees (Oliveira-Filho & Ratter 2002). A fair amount of leaf litter and herbaceous vegetation still covers the soil. Trees can reach 4-6 m, with emergent *Dalbergia miscolobium* Benth. (Fabaceae) reaching up to 8 m. The flora of the study site is detailed by Giannotti (1988).

The plant species

Two plant species that bear fleshy fruits were chosen for this study based on their great abundance at the study site, and on their widespread occurrence in the cerrado: *Erythroxylum pelleterianum* A. St.-Hil (Erythroxylaceae) and *Xylopia aromatica* (Lam.) Mart. (Annonaceae) (hereafter referred to by genus only). A summary of the traits of both species are show in Table 1. *Erythroxylum* grows in shaded portions of the cerrado, while *Xylopia* grows in patches exposed to full sun. Both species present great interannual variation in seed output (A. V. Christianini, pers. obs.). *Erythroxylum* produces red, single-seeded fruits (drupes, ca. 8 mm long). *Xylopia* fruits are multiple capsules (ca. 8 cm in diameter) that dehisce to expose ca. 40 diaspores per fruit. Each diaspore is a 0.06 g seed coated by a thin blueish fleshy layer, and an aril-like fleshy portion that covers a quarter of the seed. The inner portion of the capsule is redish when opened, which produces a contrasting display with the blueish diaspores likely to attract avian frugivores. Both plants are dispersed primarily by birds. *Erythroxylum* presents no morphological trait with secondary seed dispersal by ants or other animals. *Xylopia* has an aril that may be used as an appendage by ants to transport the seeds to their nests. Seeds of both plant species remain viable for up to 2 months (Lorenzi 1992, A. V. Christianini, pers. obs.).

Methods

Diaspore production and fate

In order to examine diaspore production by representative focal individuals of each species, we selected nine trees of *Xylopia* (seven in 2004, and other two in 2005) and 10 of *Erythroxylum* (in 2004), dispersed within a 4 ha plot in the savanna reserve. Each tree was isolated from the nearest reproductive conspecific by 5 to 30 m. Fruit traps were used to evaluate diaspore dispersal rates by primary dispersers, and diaspore fall to the ground. Traps (0.14 m^2 plastic trays lined with 0.2-mm nylon mesh) were placed below the crown of focal trees. Traps were kept 20 cm above ground by four stakes, each coated by a sticky resin (Tanglefoot®) to prevent ants from reaching the diaspores. We placed 2-10 traps under each focal tree, covering up to 80% of the area beneath the tree crowns to catch fallen diaspores, and seeds embedded in bird faeces. We removed fruit debris in the traps every 2-4 weeks throughout the fruiting season. Diaspores were then counted and classified as: (1) ripe (mature fruit with viable seed), (2) unripe (mostly aborted undeveloped fruits), (3) preyed on (seeds with visible signs of damage by predators), or (4) manipulated by primary dispersers (seeds embedded in faeces or regurgitated/dropped by birds with fleshy matter still attached). *Erythroxylum* fruit production was obtained from direct counts of fruits on the crown of each plant at the beginning of the fruiting season. For *Xylopia* we counted the number of open valves and then the number of diaspores that were available for bird dispersers. If birds remove the seeds in the canopy, the capsules are left and reach the fruit traps intact, allowing us to estimate the total number of capsules and diaspores produced by the tree (see Passos & Oliveira 2002).

Fruit traps allowed us to estimate how much of the crop was removed by birds, as well as the number of diaspores that reached the cerrado floor. We determined the number of non-dispersed seeds for each tree by dividing the sum of the sampled material in traps (within the four categories specified above) by the fraction of canopy area sampled (see Passos & Oliveira 2002). To estimate the number of seeds dispersed by birds, we subtracted the total material in traps from the total crop size estimated by the visual counts. For the purpose of this paper, seed dispersal efficiency will estimate the percentage of seeds that is dispersed away from the parent plant (see Jordano & Schupp 2000).

Observation of frugivorous visitors

To obtain information about frugivorous visitors we monitored fruiting trees of each species in the study plot. Observations were conducted along the day, between 05:50 h and 18:30 h for a total of 14 h for *Erythroxylum* and 59 h for *Xylopia*. For each visitor we recorded the duration of the visit, seed handling behaviour, and counted the number of seeds, either dropped under the plant, or dispersed away from the tree crown. Seeds swallowed by birds that left the tree afterwards were considered dispersed away. We also recorded postfeeding flight distances of birds departuring from the focal tree until the first landing perch as an estimate of dispersal distance (Jordano & Schupp 2000). We used the following distance intervals: 0-1.9 m, 2-4.9 m, 5-9.9 m, 10-19.9 m, 20-39.9 m, and > 40 m. Birds could not be followed to longer distances due to vegetation obstruction, and thus our distances of bird dispersal were probably underestimated at some degree.

Ant attendance to fallen diaspores, and secondary seed dispersal

To determine which ants interact with fallen diaspores of the focal plant species we recorded all ant-diaspore interactions observed throughout the fruiting seasons of 2004 and 2005. Systematic sampling was also carried out by placing marked diaspores at ground stations 10 m apart, 1-2 m off transects that crossed the study site. For each species, two diaspores per sampling station were placed on white filter paper (4 x 4 cm) to facilitate visualization on the leaf litter. The filter paper has no detectable effect on ant behaviour (Byrne & Levey 1993, Passos & Oliveira 2003). Diaspores were set at 08:00 and 18:00 h and checked through regular intervals through two hours during the fruiting season of each species. During observations we recorded the ant species attracted and their behaviour toward diaspores. Ant behaviour was classified as follows: (1) remove whole diaspore to nest (> 5 cm), (2) clean fleshy portion at the spot, no removal, (3) inspect or manipulate diaspore, but without removal (< 5cm). We followed ants carrying diaspores until they entered their nests or disappeared in the leaf litter. The distance of diaspore displacement was then measured. Voucher specimens of the ants are deposited in the collection of the Universidade Federal Rural do Rio de Janeiro (CECL).

To examine how patterns of seed shadow are altered by secondary dispersal and predation on the soil surface, we measured fruit and seed removal rates below the crown of the focal trees. The relative contribution of ants and vertebrates was assessed by performing an exclosure experiment during the fruiting period of each species. Vertebrates were excluded from diaspores by a wire cage (17 x 17 x 8 cm), fenced on the top and sides with mesh (1.5 cm) and staked to the ground (see Roberts & Heithaus 1986). Pairs of diaspores were set out at about 08:00 h at five stations placed radially beneath fruiting trees (n = 30 for each plant species). Diaspores were marked with a small dot of enamel paint (Testors, Rockford, USA) to distinghish them from naturally fallen ones. Each pair consisted of a diaspore placed directly on the floor under a wire cage and an exposed diaspore (control) placed outside the cage, 15 cm away. After 24 h, we recorded the ant species interacting with diaspores, and the number of diaspores missing in each group. A diaspore was considered removed if not found within a 30 cm radius from its original location. Seed removal trials were performed a few weeks later, and followed the same procedure for fruits, using the same individual trees. We kept a minimum distance of 20 m between replicates to increase the probability of independent discoveries by different ant colonies (see Levey & Byrne 1993). Experiments were performed in two fruiting seasons for *Xylopia* (2004 and 2005), but only in 2004 for *Erythroxylum* due to the small number of fruits produced by this species in 2005. Data on diaspore removal were analysed using factorial analysis of variance. The dependent variable was the number of diaspores removed per tree, while the independent variables were plant location, year, exclusion type (caged versus uncaged), and presence/absence of a reward (fruit pulp or aril). A separate analysis was done for each species. For *Erythroxylum* (2004) and *Xylopia* (2005), pulp reward (presence or absence) was used as an independent fixed factor, and tree location as a block, random effect factor. For *Xylopia*, fruiting season (2004 or 2005) was entered as a fixed factor in a separate analysis to investigate temporal variation in the removal of fallen diaspores. Data were square root (x + 1) transformed before analyses to improve normality and homocedasticity.

Seed germination

Previous greenhouse germination experiments indicated that removal of pulp material (simulating seed cleaning by ants) increased germination of *Erythroxylum* up to 52% compared to intact fruits (Christianini *et al.* 2007). To examine the effect of seed handling by ants on germination of *Xylopia* we sowed 60 seeds whose aril had been removed by us simulating ant behavior (treatment), as well as 60 manipulated intact seeds coated by an aril (control). Seeds were sowed in plastic trays (37 x 28 cm) containing regularly moistened vermiculite. Trays were kept in the greenhouse of the Universidade Estadual de Campinas. Seeds were sowed 1 cm buried into the substrate and 2 cm apart from each other. The experiments were checked at 7 to 10-day intervals until seeds germinated or showed signs of decay.

To evaluate the effects of seed cleaning on seed germination under natural conditions, germination tests with *Erythroxylum* were run in the field. Fruits were obtained from 10 plants and mixed to form a bulk sample. Fruits were stored in a cool, dry place until the end of the fruiting season (January 2005), when they were sowed in experimental seed banks. We arranged experimental seed banks in six blocks, 50 x 50 cm in size, scattered through the study plot. In each block, we randomly assigned two treatments: (1) seeds cleaned from fleshy pulp, (2) intact fruits. Before planting the seeds in experimental seed banks, we sieved the soil in two adjacent 15 x 15 x 15 cm areas to remove any *Erythroxylum* seed present before sowing. An open-topped basket made of plastic mesh (12 x 12 x 14 cm, 1 mm mesh size) was placed into each pit and then filled with the sieved soil (see Christian & Stanton 2004). Each basket was buried at 12 cm deep leaving 2 cm of the rim above the surface to aid relocation. Tanglefoot resin was applied to the exposed rim to prevent ants access to the seeds. In each pair of baskets we sowed 20 fruits or cleaned seeds (i.e. with fruit pulp removed), 1 cm deep and 2 cm apart. A cage (20 x 20 x 10 cm) covered on the top and sides with mesh (1.5 cm) was staked to the ground covering each seed basket to prevent vertebrate access. These paired germination tests (replicate blocks) were performed at six cerrado locations (n = 240 seeds in total) where *Erythroxylum* seedlings occurred naturally. Seedling emergence was monitored monthly for one year. We assessed variation in seed germination between treatments using two-way analysis of variance (ANOVA) with block, and pulp removal (yes or no) as grouping factors. The number of germinated seeds plus one was square-root transformed for the analysis. To determine the fate of seeds that did not emerge as seedlings, we sieved the soil from experimental seed banks to recover any seed remaining after one year (January 2006), and inspected them visually for viability.

Seedling distribution and performance

To investigate if ants affected the spatial distribution and perfomance of seedlings we compared the number of seedlings of the two focal plant species growing on quadrats (0.5 x 0.5 m) established in ant nests, and in paired controls without nests (random direction, 2 m from each nest). Nests were located by following laden ant workers that had been attracted to fruits of each plant species (see above), or attracted to tuna baits placed on the ground by day and night (Horvitz 1981). Nests were tagged, and the sampling quadrat was established centered on nest entrance, or on the external refuse pile, if present (usually 5-15 cm from nest entrance). Nest and control plots were surveyed in February-March

2005. Seedlings of the focal species inside nest and control quadrats were individually marked with a numbered flag, and monitored for survival monthly until July 2005, and then every two months until February 2006. The 2005 seedling cohort of *Erythroxylum* was easily distinguished from juveniles (older than one year) because young seedlings had a single green stem while juveniles had a brown stem, frequently ramified. For *Xylopia*, individuals up to 10 cm tall and non-ramified were considered as seedlings. The abundance of seedlings growing on ant nests and in control plots was compared with Wilcoxon paired-sample sign rank tests. Seedling survivorship was compared with Peto and Peto´s logrank test (Pyke & Thompson 1986).

Since soil physical and chemical properties may be modified locally by ant nests (Beattie 1985), and hence affect seedling establishment and growth, we compared soil variables between ant nests and control plots. Soils samples were collected from nests of *Odontomachus chelifer* and from random control plots (n = 10 for each), air-dried and analysed for total N, macronutrients, micronutrients, pH, and organic matter (Sparks *et al.*1996). Prior to collecting the soil, we evaluated soil penetrability in refuse piles of ant nests and in control plots. At each location we released a sharpened wire stake (30 cm long) from the inside top of a 1.5 m high PVC pipe. The depth reached by the stake into the ground was the estimate of soil penetrability for that location (Passos & Oliveira 2004). Soil variables were compared between nest and control plots with paired T-tests.

Results

Diaspore production and fate

There was a large variation in seed output within- and among-species for 2004 and 2005 (Table 2). In both years and for both species, most diaspores reached the ground beneath the parent tree rather than away from the tree crown. In each year, a mean of 7 to 32% of the diaspores produced per tree were removed by birds, while 68 to 93% of the remaining fruit crop fell beneath the tree spontaneously or dropped by birds (Table 2). On average, 26-53% of the diaspores produced fall under crown as intact ripe fruits. Abortion and pre-dispersal seed predation accounted for the single largest category of fate, with ca. 40% of the total diaspores produced. These numbers indicated that many diaspores are available to secondary dispersers under trees at each fruiting season.

Frugivorous visitors

We observed from 5 to 11 species of birds feeding on fruits of each of the focal plant species (Table 1 shows the most common bird visitor genera). Several birds acted as legitimate dispersers by ingesting the whole diaspore, and afterwards defecating or regurgitating intact seeds (e.g. *Turdus leucomelas*, *Elaenia flavogaster*). Many diaspores, however, were also dropped beneath the parent plant by birds that act as pulp consumers, and provide no dispersal away from the parent plant (e.g. *Coryphospingus cucullatus*, *Conirostrum speciosum*). Some of the latter species, however, may also disperse the seeds when they remove the fruit and fly to another tree before feeding, hence acting as both pulp consumers and seed dispersers. Mean flight distances from fruiting trees were 5.3 ± 4.0 m for *Erytroxylum* and 16.3 ± 11.8 m for *Xylopia* (Figure 1).

Ant attendance to fallen diaspores, and secondary seed dispersal

Ants belonging to 37 species and 17 genera were attracted to fallen diaspores, but ant species that acted as main seed vectors varied between plant species (Figure 2). Details about the ant fauna exploiting diaspores can be found elsewhere (Chapter 1). Results from surveys indicated that large ponerines (*Dinoponera*, *Odontomachus*, and *Pachycondyla*) and ectatommines (*Ectatomma*) accounted for 36% of the ant-diaspore interactions recorded for *Erythroxylum*. For *Xylopia*, small Myrmicinae ants (mainly *Pheidole* spp. and *Wasmannia auropunctata*) accounted for 68% of the records, while large ponerines and ectatommines were responsible for 10% of the records. Ants usually displaced diaspores to short distances, but this varied between plant and ant species (Figure 1). Mean (± SD) distance of displacement by ants was 1.46 ± 1.42 m for *Erythroxylum*, and 0.78 ± 0.88 m for *Xylopia* (Figure 1). Most ants attending the diaspores (e.g., *Camponotus*, *Crematogaster*, *Pheidole*, *Solenopsis*, and *Wasmannia*) typically recruited nestmates to collect liquids or remove the fleshy reward on spot, without displacing the seed (Figure 2).

A summary of the results of diaspore removal experiments can be found in Table 3 and Figure 3. For *Erythroxylum*, more diaspores were removed from open than caged treatments ($F = 5.32$, $P = 0.02$), indicating that vertebrates play a role in the removal of fallen diaspores of this species. Diaspore removal however, varied significantly with plant location (block effect). Fruit pulp increased the removal of caged diaspores two-fold compared to cleaned seeds (Figure 3), indicating a role of fruit pulp in ant attraction. Although the interaction between exclusion treatment and presence of reward (fruit pulp) was non-significant, an analysis of covariance with data from the paired-exclusion treatments indicated that ants are an important source of removal of fallen fruits from open controls, but not of cleaned seeds (ANCOVA: $F = 26.27$; $df = 1,56$; $P < 0.001$) (Figure 4). For *Xylopia*, a positive effect of the presence of a reward (aril) covering the seed on the removal of diaspores was also apparent in 2005, causing a two-fold increase in removal compared to cleaned seeds ($F = 108.7$, $P < 0.001$) (Figure 3). Plant location influenced removal (block effect), but there was no effect of fruiting season (2004-2005). No difference was detected in the removal of diaspores between cage and open treatments across two fruiting seasons, indicating that ants are the main agents of diaspore removal on the ground. No interaction between exclusion treatment and presence of reward was found, a trend reinforced by analysis of covariance (ANCOVA: F = 0.31, df = 1,56, P = 0.58) (Figure 4). This result suggests that ants are interested in the seed itself and thus may act mainly as seed predators rather than dispersers of *Xylopia*.

Seed germination

Greenhouse conditions seemed to be unsuitable for the germination of *Xylopia*, with only one germination record from 120 seeds. In the field, pulp removal simulating ant behavior had no significant effect on germination of *Erythroxylum*. We recorded 3.8 ± 1.7 (mean ± SD) emerging seedlings from cleaned seed treatments and 5.3 ± 1.9 emerging seedlings from intact fruit treatments, a non-significant difference ($F_{1,5} = 4.19$, $P = 0.096$). All seeds recovered one year after sowing were either killed by predators or rotten, and none was viable, suggesting that *Erythroxylum* does not produce persistent seed banks.

Seedling distribution and performance

Seedlings of *Erythroxylum* (Wilcoxon paired-test: Z = 2.94; p = 0.003) and *Xylopia* (Z = 2.2; p = 0.028) were more commonly found growing on plots laid out in ant nests than in plots outside nests (Figure 5). After one year, seedlings of *Erythroxylum* showed greater survival in ant nests (59%) than in control plots (33%) (Peto & Peto logrank-test: $\chi^2 = 5.18$, $p < 0.025$) (Figure 5a). Furthermore, soils from *O. chelifer* nest refuse piles were softer and richer in several essential nutrients compared to controls, including total nitrogen, phosphorus and potassium (Table 4).

Discussion

For the two plant species examined in this study, the seed shadow provided by primary dispersal is not a good predictor of where seeds will rest on the ground and ultimately germinate. Results demonstrate that ants reshape the seed shadow by depositing fallen seeds in disproportionate numbers in particular locations on the cerrado floor (i.e. in nest refuse piles) where seedling performance may be increased, as shown for *Erythroxylum*. This study also shows that diplochory may be widespread in plants from the cerrado, involving complex interactions between the plant, its guild of primary dispersers, and its many ant secondary dispersers. Causes and consequences of these findings are discussed below.

The high concentration of diaspores beneath fruiting trees enhanced the potential role of ants in seed dispersal and fate for the plants investigated. Seed shadows are usually leptokurtic, showing large seed densities under or near the parent plant, and declining abruptly with distance (Willson 1993), as shown for *Ficus* spp. trees in Bornean rainforests. In all instances 50 to 100% of the copious seed production of these trees fall to the ground, or are dropped by vertebrate frugivores under parent plants (Laman 1996). Such huge amount of diaspores may be secondarily distributed by animals that exploit them, with possible changes in the probability of seed survival and plant recruitment (Roberts & Heithaus 1986). Indeed, our diaspore removal experiments indicated that ants play a major role in the fate of small diaspores fallen to the floor of the cerrado savanna, as they do elsewhere (Leal & Oliveira 1998, Böhning-Gaese *et al.*1999, Passos & Oliveira 2002, 2004). Estimates of seed displacement indicate that ants can be true dispersers since many diaspores are transported beyond the parent tree's crown area, where density-dependent seedling mortality is increased (e.g. Retana *et al.* 2004). However, there are great differences in the scale of seed displacement between birds and ants (Figure 1). Frugivorous birds potentially deliver seeds to distances over 40-fold greater than do ants, and therefore are far more effective in removing seeds from the predation-prone zone near the parent tree and in providing new sites for plant colonization and recruitment (Figure 1). Because ants quickly remove seeds from beneath the plant crown, they may reduce seed predation in this zone as well (Beattie 1985). The local rodents and granivorous birds are likely to destroy rather than secondarily disperse the fallen seeds of the plants investigated in this study (Vieira *et al.* 2003, Schubart *et al.* 1965). Consequences of the scale of seed dispersal by birds and ants may lead to differences in the spatial patterns of plant recruitment and dispersion (Horvitz & Le Corff 1993), and in the genetic structure of plant populations (Hanzawa *et al.* 1988).

We found great spatial variance in removal rates of fallen diaspores among trees, as also detected in other studies (Pizo & Oliveira 1998, Passos & Oliveira 2002, Fedriani *et al.* 2004). Large ponerine ants were involved in many interactions with fallen diaspores, and usually removed fallen fruits to their nests, but ignored cleaned seeds. Feeding trials with captive ant colonies indicate that these ants discard viable seeds on refuse piles after consumption of the fruit pulp (A. V. Christianini & P. S. Oliveira, unpubl. data), thus acting as legitimate dispersers. However, *Pheidole* ants accounted for most of the removal of seeds of *Xylopia*. These ants are recognized as granivores and opportunistic feeders, and should provide few benefits to the seeds (but see Levey & Byrne 1993). Curiously, *Xylopia* is the only plant in this study perhaps adapted for ant dispersal, an aril used as appendage for seed transport by ants. Seed predation can be a step towards the evolution of seed dispersal by ants (Levey & Byrne 1993), and variation in the outcome of interspecific interactions should drive the interaction to specialization among partners (Schemske & Horvitz 1984). It is possible that predator satiation or a high nest turnover in *Pheidole* can play a role in the survival of *Xylopia* seeds harvested by ants (see Levey & Byrne 1993). Moreover, the aril that partially covers the seed may discourage seed consumption, or even stimulate other ant taxa to remove the seeds (Hughes & Westoby 1992b). This latter hypothesis seems more likely, since *Xylopia* seedlings were more frequently found in refuse piles of large ponerines than in nests of other ants (A. V. Christianini & P. S. Oliveira unpubl. data).

Seed cleaning by ants has been demonstrated to reduce fungal attack to fallen fruits and facilitate germination in some species (Horvitz 1981, Oliveira *et al.* 1995, Ohkawara & Akino 2005). For *Erythroxylum*, however, such benefits were detected in greenhouse tests only (Christianini *et al.* 2007), with no significant effect of seed cleaning by ants on seed germination in the field. Because abiotic factors, such as light and water availability, are usually ideal under greenhouse compared to natural conditions, conflicting results on germination performance often occur (Traveset & Verdu 2002). Unless seeds are removed to ant nests (see below), seed cleaning by ants should provide few benefits to *Erythroxylum* in the field. Despite *Xylopia* seedlings were found in the field no germination was obtained in greenhouse tests. *Xylopia* present morphophysiological seed dormancy and special conditions are apparently required for full embryonic development and germination (Baskin & Baskin 1998). Seed handling by ants and/or particular conditions near ant nests may facilitate germination in *Xylopia,* since seedlings are found in nest refuse piles.

Seedlings of *Erythroxylum* and *Xylopia* were found in higher numbers in ant nests than random spots, but almost always in low densities (< 1 seedling/0.25 m^2). Seedlings of non-myrmecochorous plants growing in clumps at ant nests are often reported (e.g. Farji

Brener & Silva 1996, Böhning-Gaese *et al.*1999, Passos & Oliveira 2003, 2004), but most studies do not follow seedling survival over time (but see Passos & Oliveira 2002). It would be possible that density-related mortality factors compensate over time the benefits ants provide to plants during the early phases of seedling establishment (see Augspurger & Kelly 1984, Hanzawa *et al.*1988). In the present study, however, seedling performance was increased near ant nests. The low density of seedlings and the better soil quality in ant nests likely account for the absence of widespread mortality of seedlings in ant nests. Ant nests are frequently spots of improved soil quality in nature (see Giladi 2006 for examples worldwide), and the increased penetrability of soils from ant nests enhances soil aeration and root growth (Farji-Brener & Medina 2000, Passos & Oliveira 2004). Ant nests can also provide additional benefits to seedling performance through protection against insect herbivores (Passos & Oliveira 2004), and water retention in soil (Farji-Brener & Ghermandi 2004). By frequently removing seeds from bird and mammal faeces in tropical forests (Kaspari 1993, Byrne & Levey 1993, Passos & Oliveira 2002) and savannas (Leal & Oliveira 1998, and the current study) ants rearrange the seed shadow provided by primary dispersal. Thus, the role of ants in diplochorous seed dispersal systems in the cerrado savanna is mainly to reshape the seed shadow at a finer scale, providing a fine tunning of seed delivery to ant nests after long distance dispersal by vertebrates (Wenny 2001).

In conclusion, our study suggests that ant-seed interactions may markedly affect patterns of recruitment in species primarily dispersed by vertebrates in the cerrado. Although there is substancial phylogenetic inertia in fruit traits (Jordano 1995), there is ample evidence to suggest that plants have evolved various mechanisms to attract high quality ant dispersers (Cuautle *et al.* 2005, Giladi 2006). Subtle traits of diaspores linked with dispersal by ants may be easily overlooked (Aronne & Wilcock 1994, Kaufmann *et al.*

1991). Indeed, selection by primary dispersers upon diaspore traits may be a step towards secondary dispersal by ants of fallen diaspores. It is already known that lipid-rich fruits are prefered by avian dispersers (e.g. Stiles 1993, Izhaki 2002), and that such fruits are highly attractive to ants, especially those providing high quality dispersal services, such as large ponerines (Hughes *et al.* 1994, Pizo & Oliveira 2001, Passos & Oliveira 2002). Thus, there may be correlated selective pressures exerted by birds and ants upon diaspore traits. This can be one way towards the evolution of diplochorous seed dispersal systems involving birds and ants.

Acknowledgements

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Table 1: Summary characteristics of the two focal plant species studied in the cerrado reserve of Itirapina, Southeast Brazil (Lorenzi 1992, Giannotti 1988, A.V. Christianini unpublished data).

Characteristic	*Erythroxylum pelleterianum*	*Xylopia aromatica*
Growth form	shrub-treelet	tree
Crown radius (m)	1.1 ± 0.5	1.7 ± 0.6
Density (ind./ha)	125	123
Fruit type	drupe	capsule
Seeds per fruit	1	15-50
Diaspore colour (display)	red	red and blue
Diaspore size (g fresh mass)	0.15	0.06
Seed size (mg fresh mass)	41	52
Fleshy part	pulp	seed aril
Fruiting period	Oct-Dec	Mar-Jul
Number of bird visitor species	5	11
Most common bird visitor genera	*Coryphospingus*, *Elaenia*, *Zonotrichia*	*Elaenia*, *Turdus*, *Conirostrum*
Chemistry composition of the fleshy part of the diaspore (% dry mass)		
Lipids	68.2	32.9
Carbohydrates	22.0	50.3
Protein	7.9	16.4
Ash	1.9	2.1

Table 2: Diaspore production and fate for *Erythroxylum pelleterianum* and *Xylopia aromatica* across 2004-2005 fruiting seasons in a cerrado savanna in Southeastern Brazil. Values express the relative importance of each fate category in relation to total fruit production of 2 to 10 plants per species in each year.

Fate category		*Erythroxylum*	*Xylopia*	
		2004	2004	2005
Dropped under crown	Mature	53.4%	26.0%	42.1%
	Immature, preyed on, or rotten	39.5%	42.2%	43.0%
Dispersed away from crown		7.1%	31.8%	14.9%
Total diaspore production (mean ± SD)		1162 ± 1346	253 ± 180	171 ± 187
Range		252 - 4828	100 - 627	39 - 303

Table 3: Results from the analysis of variance of the diaspore removal experiments in a cerrado savanna in southeast Brazil. Factors included in the analyses were: year (2004 or 2005), exclusion (access to ants only, or open to all animals), and reward (seed coated by fruit pulp or aril against cleaned seed). Comparisons between years were performed in a separate analysis for *Xylopia* only. The effect of the reward was investigated for *Eryhroxylum*, and separately for *Xylopia* in the 2005 fruiting season. Significant differences are in bold ($p < 0.05$).

Effect	df	MS	F	P
Erythroxylum				
Block	29	0.258	2.09	**0.005**
Exclusion	1	0.657	5.32	**0.02**
Reward	1	4.614	37.41	**< 0.001**
Exclusion x Reward	1	0.024	0.20	0.66
Error	118	0.200		
Xylopia (2005)				
Block	29	3.361	3.26	**<0.001**
Exclusion	1	2.133	2.07	0.15
Reward	1	112.13	108.72	**<0.001**
Exclusion x reward	1	0.000	0.000	1.00
Error	87	1.031		

continued

Xylopia (both years)				
Year	1	0.003	0.033	0.86
Exclusion	1	0.141	1.480	0.23
Year x Exclusion	1	0.001	0.009	0.92
Error	116	0.115		

Table 4: Comparison of soil variables between nests of *Odontomachus chelifer* and those of random spots on the cerrado floor (n = 10 for each group). Mean values of soil penetrability are given in mm; K, Ca, Mg, H+Al, base saturation and C.E.C. (caption exchange capacity) are given in mmol dm^{-3}; P, B, Cu, Fe, Mn, Zn in mg dm^{-3}; organic matter in g dm^{-3}; N in g Kg^{-1}. Significant differences are in bold ($p < 0,05$).

Soil variable	*O. chelifer*	Random	P
Penetrability	122.4	94.6	**<0.01**
Organic matter	73.4	56.7	**0.02**
pH	4.13	4.10	0.39
Total nitrogen	1.64	1.06	**0.03**
Base saturation	8.72	4.47	**<0.01**
C.E.C.	100.2	87.5	0.24
H+Al	91.5	82.9	0.40
Calcium	5.20	2.70	**<0.01**
Potassium	2.23	1.31	**<0.01**
Magnesium	2.10	1.40	**0.02**
Boron	0.43	0.34	**0.02**
Cooper	0.92	0.80	0.09
Iron	165.8	178.2	0.39
Manganese	7.57	3.52	**0.02**
Phosphorus	10.0	6.40	**0.02**
Zinc	1.22	0.75	**<0.01**

Legend to figures

Figure 1: Comparative distances of seed displacement by ants and birds feeding on *Erythroxylum pelleterianum*, and *Xylopia aromatica* in a cerrado savanna, southeast Brazil. Distribution of distances of diaspores carried by ants, and carried by birds in their first flight from focal feeding trees. n = number of independent records of seed displacement by ants, or number of flights observed for birds. The figure does not include data of seeds that were not dispersed (i.e. seeds cleaned on spot by ants with no displacement, or those dropped under the plant crown by birds). See text for further details.

Figure 2: Genera of ground-dwelling ants recorded interacting with fallen diaspores in a cerrado savanna in southeast Brazil. Frequency of interactions differs among ant genera and plant species. Key to main ant behaviour towards diaspores: I = inspect or collect liquids, no removal; C = clean seeds by pulp removal on the spot, no displacement; R = remove diaspores > 5 cm; C/R = clean and/or remove diaspores > 5 cm.

Figure 3: Removal rates of fallen diaspores in a cerrado savanna in southeast Brazil, in caged treatments (accessible only by ants) and the paired open controls (accessible by ants and vertebrates). Mean (± SE) removal rates of fruits and seeds of *Erythroxylum pelleterianum* in 2004 fruiting season, and of fruits (2004) and fruits and seeds (2005) for *Xylopia aromatica*.

Figure 4: The relationship between the number of diaspores removed from caged treatments (accessible only by ants) and paired open controls (accessible by ants and vertebrates). Code to treatments: fruit (solid circles, solid lines), and cleaned seeds (open

circles, dashed lines). Number of samples for each species and treatment combination is 30. Not all points are seen due to overlap.

Figure 5: Spatial distribution and survival over one year of seedlings of (a) *Erythroxylum* (N = 57 nests) and (b) *Xylopia* (N = 81 nests) growing in plots (50 x 50 cm) laid out in ant nests, and in control areas without ant nests. Note difference in scales between plant species. Data are means ± SE.

Figure 1

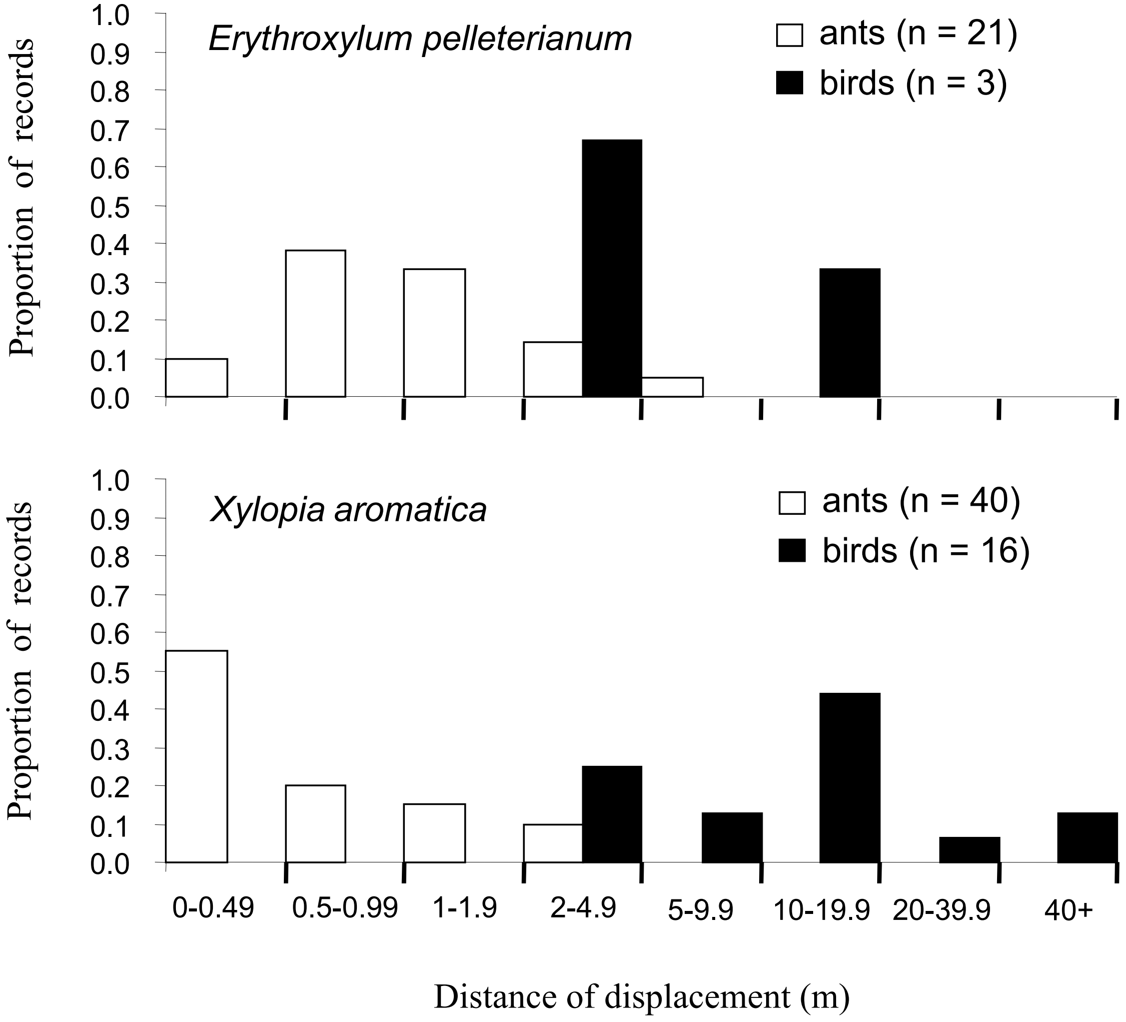

Erythroxylum pelleterianum
☐ ants (n = 21)
■ birds (n = 3)
Xylopia aromatica
☐ ants (n = 40)
■ birds (n = 16)
Proportion of records
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0-0.49
0.5-0.99
1-1.9
2-4.9
5-9.9
10-19.9
20-39.9
40+
Distance of displacement (m)

Figure 2

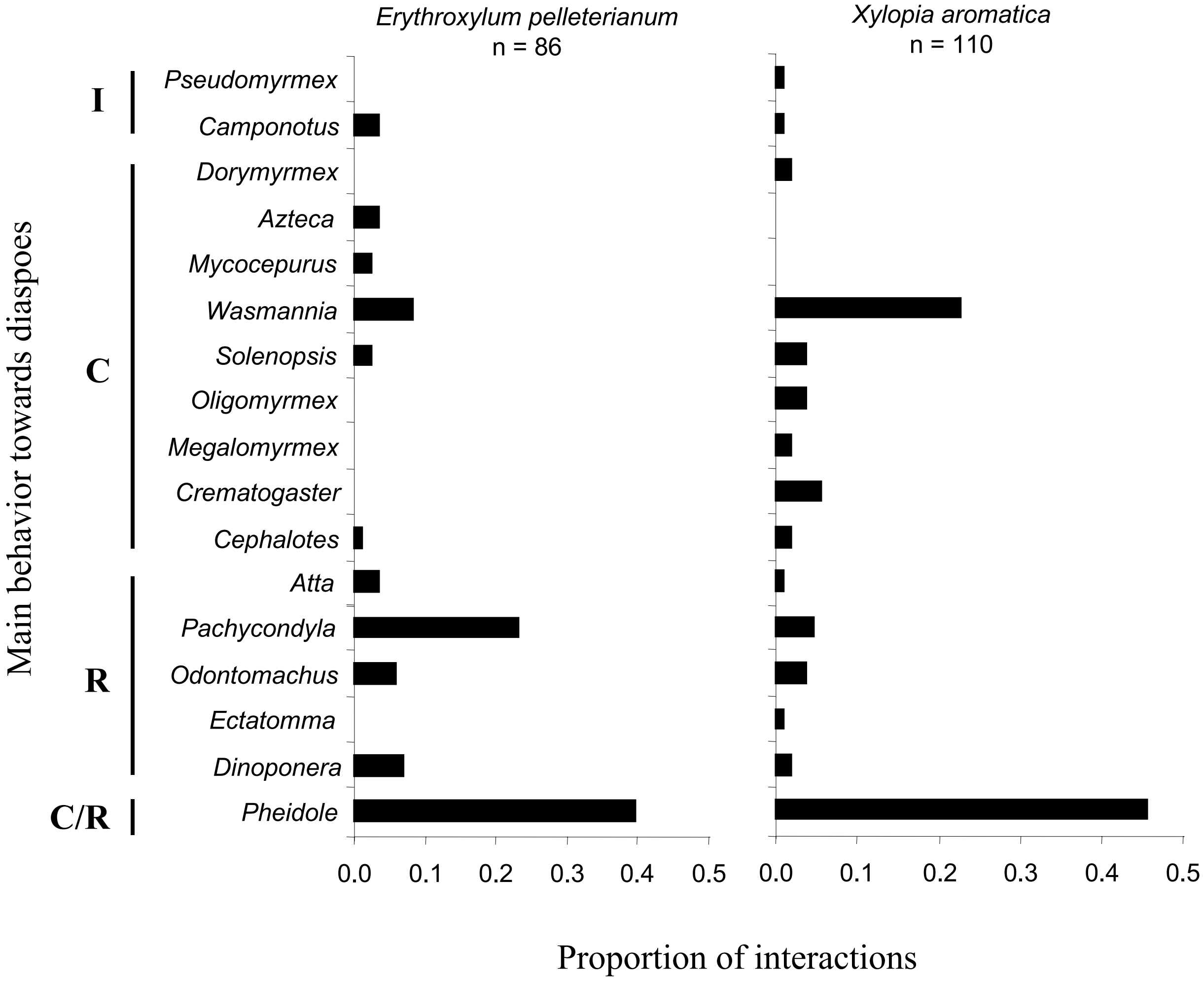

Erythroxylum pelleterianum
n = 86
Xylopia aromatica
n = 110
Main behavior towards diaspoes
I
C
R
C/R
Pseudomyrmex
Camponotus
Dorymyrmex
Azteca
Mycocepurus
Wasmannia
Solenopsis
Oligomyrmex
Megalomyrmex
Crematogaster
Cephalotes
Atta
Pachycondyla
Odontomachus
Ectatomma
Dinoponera
Pheidole
0.0
0.1
0.2
0.3
0.4
0.5
Proportion of interactions

Figura 3

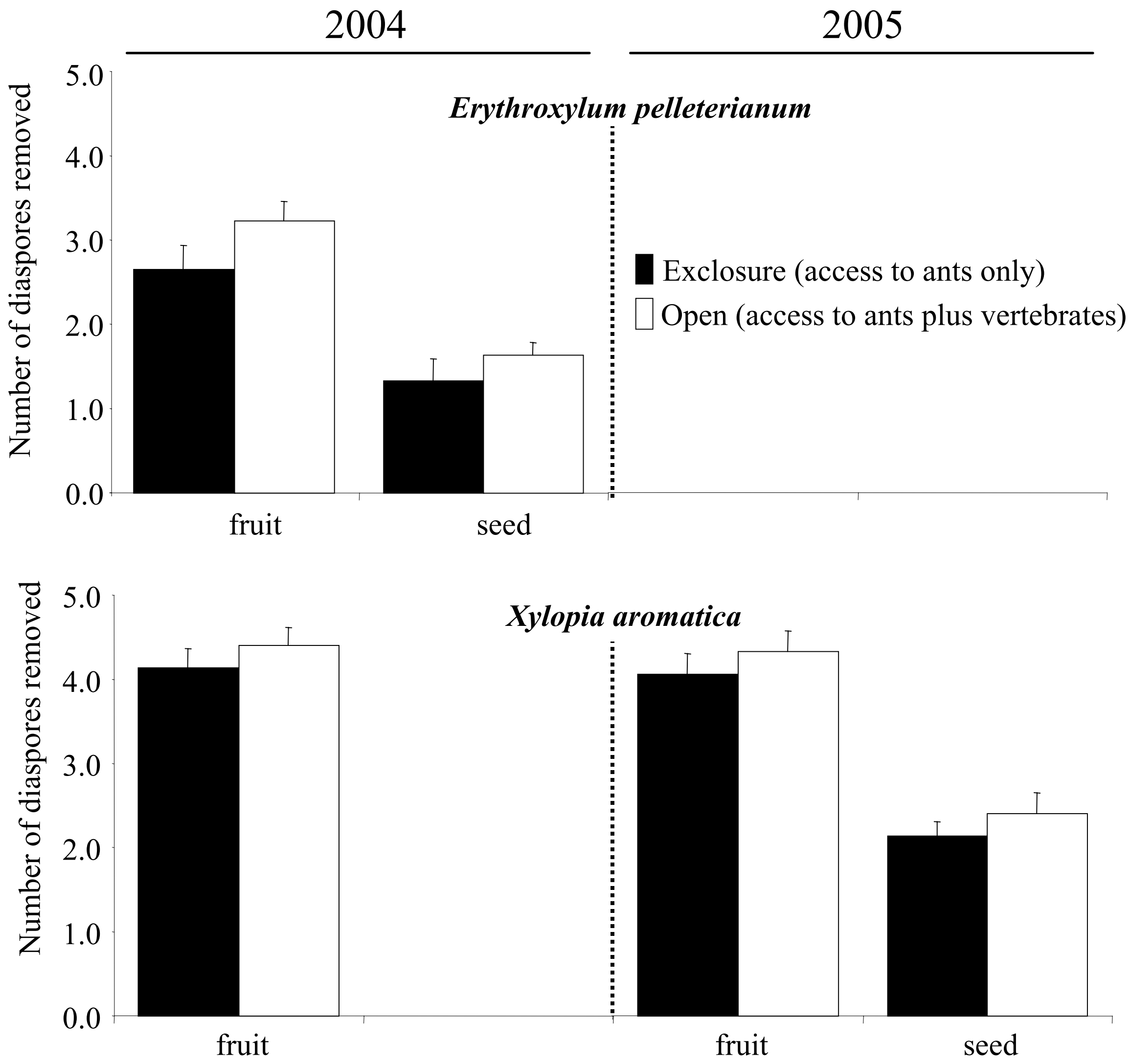

2004
2005
Erythroxylum pelleterianum
Number of diaspores removed
5.0
4.0
3.0
2.0
1.0
0.0
■ Exclosure (access to ants only)
□ Open (access to ants plus vertebrates)
fruit
seed
Xylopia aromatica
Number of diaspores removed
5.0
4.0
3.0
2.0
1.0
0.0
fruit
fruit
seed

Figure 4

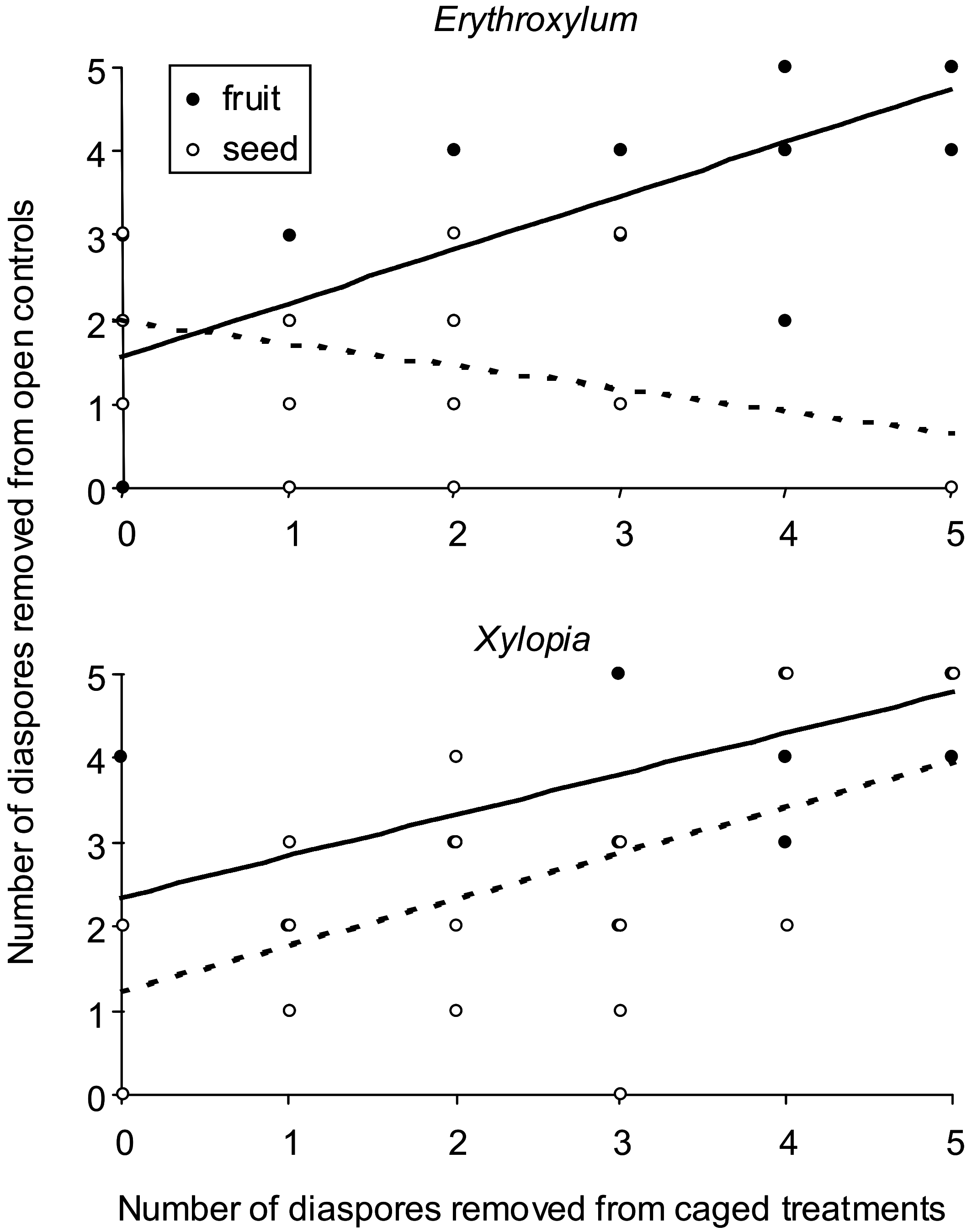
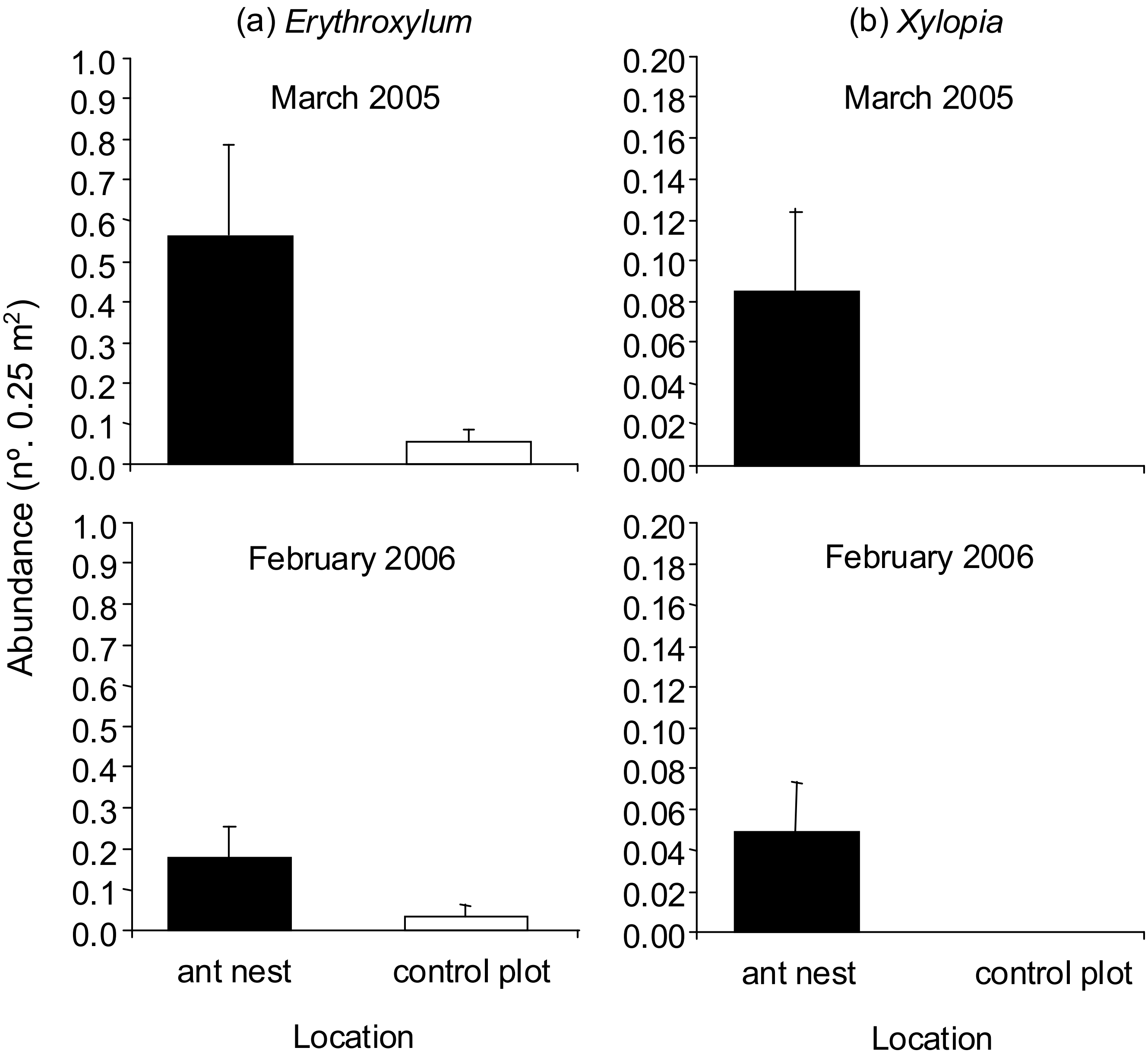

Erythroxylum
fruit
seed
Xylopia
0
1
2
3
4
5
Number of diaspores removed from open controls
Number of diaspores removed from caged treatments

Figure 5


(a) *Erythroxylum*
(b) *Xylopia*
March 2005
February 2006
Abundance (nº. 0.25 m²)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
ant nest
control plot
Location

Capítulo 4

Relative importance of primary and secondary seed dispersal to *Miconia rubiginosa*, a diplochorous melastome from a neotropical savanna

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Running-title: Primary and secondary seed dispersal in a savanna

Key-words: ant-fruit/seed interaction, Cerrado, diplochory, myrmecochory, seed germination

Abstract

We investigated the diplochorous seed dispersal system of the fleshy-fruited plant *Miconia rubiginosa* (Melastomataceae) in a neotropical savanna (cerrado) in south-east Brazil. We estimated the relative contribution of birds (phase one of dispersal) and ants (phase two) for the seed dispersal of this plant over two fruiting seasons. Birds (13 species) removed 20 to 25% of total diaspore production, but they dropped many diaspores beneath the mother plant while foraging. From 11 to 19% of the total fruit crop fall beneath the parent plant as ripe fruit. Ants (12 species) were responsible for most removal of fallen diaspores, but removal rates varied between fruiting seasons. Average distance of diaspore displacement by ants was shorter (6.5 m) than birds, which removed seeds at least 3-fold farther. Leaf-cutter ants *Atta* sp. were the most common ants recorded in interaction with fallen diaspores, and also removed bird droppings with seeds. Fruits of *Miconia* comprised up to 96% of the food items taken to the nests by *Atta sexdens* in the peak of fruit production. However, a mean of 46% of these fruits were rejected by the ant colony and discarded around nest entrance. There was no differences in seed germination among seeds taken at ants nests refuse piles, bird feces and controls. Birds are more likely to provide effective long-distance dispersal allowing plant colonization of new sites for recruitment, while ants reshape the seed shadow at a finer scale, delivering seeds to specific sites (i.e. close to the ant nest). Differences between bird- and ant-dispersal may translate in distinct patterns of recruitment and plant dispersal. We propose that the complex seed dispersal system of *Miconia* is likely due to an indirect effect of the heavy chemical defenses of plant leaves from this savanna, which constrain ants to rely on fruit harvesting for fungus cultivation.

Introduction

Recent studies have emphasized the often multi-step process of plant regeneration through subsequent conected stages of seed dispersal, a process known as diplochory (Vander Wall & Longland 2004). In such systems primary seed dispersers move diaspores (i.e. the unit of dispersal such as a fruit or seed) away from the parent plant (i. e. phase one of dispersal), whereas secondary seed dispersers take part after the diaspores reach the ground naturally, or embedded in faeces (i. e. phase two of dispersal), moving seeds to specific sites, such as below litter (e.g. Vander Wall *et al.* 2005). For instance rodents, beetles, or ants may cache seeds from mammal and bird feces across the forest, reshaping the seed shadow provided by primary dispersers, what may influence the spatial distribution and performance of seeds and seedlings (Forget & Milleron 1991, Vander Wall *et al.* 2005, Passos & Oliveira 2002, Andresen 2002). Recognition of diplochory chalenges the role played by primary seed dispersal, which cannot account solely for the ecology and evolution of plant traits linked with seed dispersal and plant regeneration (Kaufmann *et al.*1991, Aronne & Wilcock 1994, Wang & Smith 2002, Vander Wall & Longland 2004). Diplochory may have evolved because combining two ways of dispersal can increase benefits of seed dispersal over a single means of dispersal. However, there is a lack of knowledge about the prevalence and geographical occurrence of diplochory relative to other modes of seed dispersal, and the ecological setting that promoted the evolution of such complex seed dispersal systems (Vander Wall & Longland 2004). For instance, studies that partition seed mortality and plant fitness between phase one and two of dispersal are rare, and present conflicting results of the importance of each phase for plant recruitment (Böhning-Gaese *et al.* 1999, Passos & Oliveira 2002).

Leaf-cutter ants (Attini) are ubiquitous elements in neotropical forests and savannas where they collect a great amount of vegetative plant material daily for fungus culturing inside ant nests (Hölldobler & Wilson 1990). Leaf-cutters also harvest fruits and seeds, and are likely to influence the fate of large amounts of diaspores (Roberts & Heithaus 1986, Dalling & Wirth 1998, Leal & Oliveira 1998), most of them with no adaptation for ant dispersal. Until recently leaf-cutters were recognized only as seed predators, but field and laboratory studies indicated that attine ants can positivelly affect the recruitment of some plant species trough an increase in seed germination and seedling establishment (Oliveira *et al.* 1995, Farji Brener & Silva 1996, Leal & Oliveira 1998, Farji-Brener & Ghermandi 2004). Thus, leaf-cutters are better described as having a dual role as seed predators and dispersers (Levey & Byrne 1993, Retana *et al.* 2004).

In the cerrado savanna of central Brazil, ants are primarily responsible for the removal of small diaspores that fall to the ground, what may influence the regeneration of non-myrmechocorous plants (Leal & Oliveira 1998, Christianini *et al.* 2007). In this study we compared the potential contribution of phase one and two of seed dispersal for the regeneration of the fleshy-fruited melastome *Miconia rubiginosa* in the cerrado. Fruits of *M. rubiginosa* are consumed by birds in plant crown, and are frequently harvested by leaf-cutters once fallen to the ground. We specifically addressed the relative roles of phases one and two of dispersal in the quantity of seeds dispersed, and searched for patterns and consequences of each phase of dispersal. We show that phase two of dispersal plays a key role in the fate of diaspores of *M. rubiginosa* in the cerrado, and suggest a hypothesis for the widespread use of fruit by leaf-cutter ants in this savanna.

Study site

Field observations and experiments were carried out from December 2003 to August 2005 in the reserve of the Estação Experimental de Itirapina (22^{o}12'S, 47^{o}51'W), a 200 ha fragment of cerrado in Southeast Brazil. Average annual rainfall is 1,190mm, concentrated in the warm and wet season (December to March). A dry and cold season occurs from April to November. Mean annual temperature is 19.7ºC. The vegetation at the study site is the cerrado *sensu stricto*, the typical fire-prone savanna that grows on sandy, nutrient poor soils of the cerrado domain, characterized by 50% to 80% of ground cover by small palms, shrubs and trees (Oliveira-Filho & Ratter 2002). A fair amount of leaf litter and herbaceous vegetation still covers the soil. Trees can reach 4 to 6 m, with emergent *Dalbergia miscolobium* Benth. (Fabaceae) reaching up to 8 m. Details about the flora of the study site can be found in Giannotti (1988).

The plant

Miconia rubiginosa (Bonpl.) DC. (Melastomataceae) (hereafter referred only by its genus) was chosen for this study based on its great abundance at the study site (401 ind./ha, Giannotti 1988), and on its widespread occurrence in the cerrado. *Miconia* is a fleshy-fruited tree (crown radius 2.1 ± 0.9 m (mean ± SD)) that grows in patches exposed to full sun. Large fruit crops are produced anually from February-June. Diaspores are purple berries with a mean fresh mass of 0.12 g, each bearing a mean of 10 tiny, 1.2 mg seeds. *Miconia* have permanent seed banks in the study site. Fruit pulp of *Miconia* is rich in carbohidrates (87.3% dry mass), followed by a small amount of protein (8.3%), lipids (2.8%) and ash (1.6%) (A. V. Christianini & P. S. Oliveira unpubl. data). Like other melastomes (Loiselle & Blake 1999), *Miconia* is primarily dispersed by birds and present no morphological trait that may suggests secondary dispersal by ants or other animals.

Methods

Diaspore production and fate

In order to examine diaspore production by representative plant individuals, we arbitrarily selected ten trees of *Miconia* (5 in 2004, and other 5 in 2005) dispersed within a 4 ha plot in the savanna reserve. Each tree was isolated from the nearest reproductive conspecific by 5 to 30 m. Direct counts of fruits on tree crown were unreliable because of the huge fruit crop of trees. To obtain an estimate of fruit production we multiplied the mean number of fruits obtained by direct counts of 3 to 4 bunches of fruits collected randomly at each focal *Miconia* by the number of bunches visually estimated at the same tree at the beginning of the fruiting season (Blake *et al.* 1990).

Fruit traps were used to evaluate diaspore dispersal rates by primary dispersers, and diaspore fall to the ground. Traps consisted of 0.14 m^2 plastic trays lined with 0.2-mm nylon mesh placed at random below the crown of focal trees. Traps were kept 20 cm above ground by four stakes, each coated by a sticky resin (Tanglefoot®) to prevent ants from reaching diaspores. We placed 2-10 traps under each tree, covering up to 30% of the area beneath the tree crowns to catch fallen diaspores, as well as seeds embedded in bird feces. We removed fruit debris in the traps every 2-4 weeks throughout the entire fruiting season. Diaspores were then counted and classified as: (1) ripe (mature fruit with viable seed), (2) unripe (mostly aborted undeveloped fruits), (3) preyed on (open fruit with seeds showing signs of damage by predators), or (4) manipulated by primary dispersers (seeds embedded in feces or regurgitated/dropped by birds with fleshy matter still attached). Fruit traps allowed us to estimate how much of the crop was removed by birds, as well as the number of diaspores that reached the cerrado floor. Calculations were made as follows. We determined the number of non-dispersed seeds for each tree by dividing the sum of the sampled material in traps (within the four categories specified above) by the fraction of canopy area sampled (see Passos & Oliveira 2002). To estimate the number of seeds dispersed by birds, we subtracted the total material in traps from the total crop size estimated by the visual counts. For the purpose of this paper, seed dispersal efficiency will estimate the percentage of seeds that is dispersed away from the parent plant (see Jordano & Schupp 2000).

Observation of frugivorous visitors

To obtain information about frugivorous visitors we monitored fruiting trees of the focal species in the study plot. Observations were conducted along the day, between 05:50 h and 18:30 h, for 64.7 h in 2004 and 21.7 h in 2005 fruiting seasons, respectivelly (total of 86.4 h of focal tree observations). For each visitor we recorded the duration of the visit, seed handling behaviour, and counted the number of diaspores either dropped under the plant, or dispersed away from the tree crown. Seeds swallowed by birds that left the tree afterwards were considered dispersed away. We also recorded postfeeding flight distances of birds departuring from the focal tree until the first landing perch as an estimate of dispersal distance (Jordano & Schupp 2000). We used the following distance intervals: 0-1.9 m, 2-4.9 m, 5-9.9 m, 10-19.9 m, 20-39.9 m, and > 40 m. *Ad libitum* observations of birds interacting with fruits of *Miconia* in plant crown were also recorded to increase sample sizes.

Ant attendance to fallen diaspores, and secondary seed dispersal

To determine which ants interact with fallen diaspores of the focal plant species we recorded all ant-diaspore interactions observed throughout the entire fruiting seasons of 2004 and 2005. Systematic sampling was also carried out by placing marked diaspores at ground stations 10 m apart, 1-2 m off transects that crossed the study site. Two diaspores per sampling station were placed on white filter paper (4 x 4 cm) to facilitate visualization on the leaf litter. The filter paper had no detectable effect on ant behaviour (see Passos & Oliveira 2003). Diaspores were set at 08:00 and 18:00 h and checked through regular intervals through two hours during the fruiting season of each species. During observations we recorded the ant species attracted, and their behaviour toward diaspores. Ant behaviour was classified as follows: (1) remove whole fruit to nest (> 5 cm), (2) clean fruit pulp at the spot, no removal, (3) inspect or manipulate fruit, but without removal (< 5cm). We followed ants carrying diaspores until they entered their nests or disappeared in the leaf litter. The distance of fruit displacement was then measured. Voucher specimens of the ants are deposited in the collection of the Universidade Federal Rural do Rio de Janeiro (CECL).

Since leaf-cutter ants (*Atta* sp.) were commonly found in interaction with fallen fruits of *Miconia* (see below) we recorded the foraging activity of three colonies of *Atta sexdens* throughout a 24 hour cycle in the peak *Miconia* fruiting period in 2004. Activity measurements were made at 2-h intervals beggining surveys at 04h00 and ending at 02h00 the following day (11-12 April). At each sampling period we counted for 15 min the number of ants laden with *Miconia* fruits or other plant material returning to the nest entrance hole of an active foraging trail. During previous observations we noted that some harvested fruits were rejected by the ant colony, and were taken out of nest and deposited in a refuse pile around the entrance hole. To obtain an estimate of the proportion of fruit harvested that was discarded in the refuse pile we also counted the number of ants ladden with *Miconia* fruits leaving the nest and depositing the fruit in the refuse. We estimated the total daily input of harvested items through extrapolation.

To examine how patterns of seed shadow are altered by secondary dispersal and predation on the soil surface, we measured fruit removal rates below the crown of focal trees over two fruiting seasons (2004 and 2005). The relative contribution of ants and vertebrates was assessed by performing an exclosure experiment during the fruiting period of the focal species. Vertebrates were excluded from diaspores with the aid of a wire cage (17 x 17 x 8 cm), fenced on top and sides with mesh (1.5 cm) and staked to the ground (see Roberts & Heithaus 1986). Ten diaspores of *Miconia* were set out at about 08:00 h at each paired-treatment placed at random beneath fruiting trees (n = 30 in each fruiting season). Diaspores were marked with a small dot of enamel paint (Testors, Rockford, USA) to distinghish them from naturally fallen ones. Each paired-treatment consisted of ten diaspores placed directly on the floor under a wire cage, and ten exposed diaspores. After 24 h, we recorded the ant species interacting with diaspores, and the number of diaspores missing in each group. A diaspore was considered removed if not found within a 30 cm radius from its original location. We kept a minimum distance of 20 m between replicates to increase the probability of independent discoveries by different ant colonies (see Levey & Byrne 1993). The experiments were restricted to fruit removal only, because of the tiny size of seeds (ca. 1 mm). Moreover, because seeds could be carried by wind once on the floor, removal rates of seeds obtained from experiments would be unreliable for this plant. Data on diaspore removal were analysed using factorial analysis of variance. The dependent variable was the number of diaspores removed per tree, while the independent variables were year and exclusion type (caged versus uncaged). Fruiting season (2004 or 2005) was considered as a fixed factor to investigate temporal variation in the removal of fallen fruits and interactions with exclusion type. Data was square root (x + 1) transformed prior to the analyses to improve normality and homocedasticity.

Seed germination

Previous greenhouse germination experiments indicated that removal of pulp material (simulating seed cleaning by ants) increased germination of *Miconia* up to 77% compared to intact fruits (Christianini *et al.* 2007). To examine the effect of seed handling by ants on germination of *Miconia*, we obtained seed samples from five nest refuse piles from each of two ant species (*Odontomachus chelifer* and *A. sexdens*) that frequently interact with these fruits in the field (Christianini *et al.* 2007). Other samples were obtained from seeds embeded in fresh bird faeces (n = 15), as well as from control seeds removed from mature fruits from 10 plants. Seeds were rinsed using a 0.5% sodium hypochlorite solution to surface-sterilize seeds. Seed samples were placed in plastic trays, above regularly moistened filter paper kept in a germination chamber at 20ºC and constant light, and checked weekly for germination. Comparisons between groups were done by G-tests.

Results

Diaspore production and fate

Estimates obtained for 2004 and 2005 indicated great variation in seed output within the species (Table 1), but diaspore production did not vary greatly between years. In both years, most diaspores reached the ground beneath rather than away from the tree crown. Fruits fell beneath the tree spontaneously, or dropped by birds (Table 1). On average, 11%

to 19% of the diaspores that fall under crown were intact ripe fruits. Abortion and pre-dispersal seed predation accounted for the single largest category of fate, with 60% to 64% of the diaspores produced. These figures indicated that many diaspores are available to secondary dispersers under trees at each fruiting season.

Frugivorous visitors: phase one of dispersal

We observed 13 species of birds feeding on fruits of the focal plant species (both fruiting seasons pooled, Figure 1). Several birds acted as legitimate dispersers by ingesting the whole diaspore, and afterwards defecating or regurgitating intact seeds (e.g. *Cyanocorax cristatellus*, *Elaenia flavogaster*). Many diaspores, however, were also dropped beneath the parent plant by birds that act as pulp consumers and provide no dispersal away from the parent plant (e.g. *Tangara cayana*). Some of the latter species, however, may disperse the seeds when they remove the fruit and fly to feed on it in another tree, acting as pulp consumers and seed dispersers (Figure 1). Four among five of the most common bird visitors dropped most fruit they interacted with, and as consequence, half of the total number of fruits removed by birds were dropped under the mother tree (Figure 1). Distances of seed dispersal provided by birds based on mean flight distances from fruiting trees were 19.8 ± 8.7 m (mean ± SD) (Figure 2).

Ant attendance to fallen diaspores: phase two of dispersal

Twelve ant species (7 genera) were attracted to fallen diaspores, but four species that always transported the diaspores to ant nests concentrated 83% of interactions (Figure 1). Results from surveys indicated that Attini ants (mainly *Atta laevigatta* and *A. sexdens*) were responsible for 66% of the interactions recorded (n = 55), including many records of removal of seeds embedded in bird feces. *Atta* workers were occasionally observed climbing *Miconia* trees to remove diaspores, however the bulk of fruits harvested were collected on the floor. Ants displaced fallen diaspores to 6.54 ± 4.08 m (Figure 2).

An *Atta sexdens* colony was likely to collect fruits of one to five trees across the fruiting season (A.V. Christianini pers. obs.). Fruits of *Miconia* comprised a relevant proportion of the items taken by *A. sexdens* to their nests, although there was some variation among ant colonies (Table 2). Estimated daily inputs of *Miconia* reached more than a thousand fruits per ant colony. This is probably a conservative estimate since an ant colony could have several active foraging trails at a time (see Wirth *et al.* 2002). Fruits represented an average of more than two-thirds of all plant material harvested, and unripe fruits comprised a minor proportion of it (Table 2). An average of 47% of the ripe fruits harvested were rejected and deposited intact in the refuse pile around the nest entrance hole (Figure 3), but there was considerable variation among ant colonies (18% of the ripe fruits harvested were discarded in refuse pile by *Atta* colony 2, 32% by colony 1, and 96% by colony 3).

Removal of fallen diaspores did not differ between caged and open treatments, but removal decreased two-fold from 2004- to 2005-fruiting seasons of *Miconia* (from ca. 32% to 11% of fallen fruits removed within 24 h) (Table 3, Figure 4). The effect of fruiting season was consistent for cage and open treatments (Table 3, Figure 4).

Seed germination

The germination experiments indicated that seeds taken from ant nest refuse piles or bird feces had similar germination performances compared to those taken directly from plants (Table 4). However, we found no seedlings of *Miconia* growing in ant nests in the field.

Discussion

The view that fruits falling under tree crown would be a waste of plant resources is not true for *Miconia* in the cerrado. Ripe fruits dropped under the mother tree are promptly harvested by ants, especially *Atta* sp., which also removes bird feces with seeds embeded. Our results demonstrate that birds remove up to a quarter of total seed production from the plant crown, while ants reshape the seed shadow by depositing fallen seeds in disproportionate numbers in particular locations on the cerrado floor (i.e., close to the nest). Bird- and/or ant-dispersal of *Miconia* may lead to differences in the spatial patterns of plant recruitment and dispersal (Horvitz & Le Corff 1993), and in the genetic structure of plant populations (Kaliz *et al.* 1999, Jordano *et al.* 2007).

Although birds are not responsible for the removal of the majority of diaspores produced they provide plants with longer distances of dispersal. Differences among bird species in relation to habitat preferences and the distance of dispersal achieved are likelly to be important. For instance, Jordano *et al.* (2007) found that small birds disperse seeds of *Prunus mahaleb* to short distances (e.g. < 51 m) and into covered microhabitats, while medium-sized birds and carnivorous mammals are responsible for most seeds dispersed from neighbour populations (> 1500 m) into open microhabitats in southern Spain. A similar mechanism may operates for *Miconia* in the cerrado, since small birds (e.g. *Thraupis sayaca* and *Zonotrichia capensis*) prefer more open cerrado (A. V. Christianini pers. obs.) and are likely to provide shorter distances of dispersal (e.g. < 40 m) while medium-sized birds (such as *Turdus leucomelas and Cyanocorax cristatellus*) should carry diaspores farther away and may be responsible for most seeds dispersed among plant populations (Ortiz-Pullido *et al.* 2000).

The role ants are playing in the complex dispersal system of *Miconia* can be sumarized in two mais effects: (1) extend the tail of the seed shadow further than that provided by phase one of dispersal; (2) reshape the seed shadow locally to specific microhabitats. Extending the tail of the seed shadow may be important for plant diaspores that need to land in particular microsites for regeneration, increasing the probability of a seed hitting a safe site (Green 1983, Murray 1988). This may be particularly important for species like *Miconia* in the cerrado, which takes advantage of uncovered microhabitats for germination and establishment (Hoffmann 1996). Although seed dispersal distances provided by ants worldwide are usually short, around 1 m (reviewed in Gómez & Espadaler 1998), the mean distance of dispersal provided by ants (manly *Atta*) in the cerrado is much larger and reach a third of the estimated mean dispersal distance provided by birds (see Figure 2). Longer distances of seed transport by *Atta* (up to a 100 m) are often reported in the literature (Dalling & Wirth 1998, Leal & Oliveira 1998, Christianini *et al.* 2007). Moreover, ants can behave as true dispersers of *Miconia* even in the absence of bird fruit removal, since diaspores may potentially be transported beyond the parent tree's crown area where density-dependent seed and seedling mortality is increased (Augspurger & Kelly 1984, Retana *et al.* 2004). On the other hand, *Atta* workers should produce a more highly aggregated seed distribution pattern than those provided by birds (Dalling & Wirth 1998, Dalling *et al.* 1998), what may negativelly affect the fitness of this tree as it increases the possibilities of density-dependent mortality and sibling competition among seedlings (Augspurger & Kelly 1984). However, several diaspores are often dropped and not recovered during ant transport to the nest (Dalling & Wirth 1998, Leal & Oliveira 1998), what may decrease the pattern of seed clumping and spread out seeds at variable distances from the mother tree. Despite seedlings have not been found on active *Atta* nests, abandoned nest sites may be excellent sites for the regeneration of this tree, as leaf-cutter ants often prune the vegetation above the mound and around the nest entrances, creating "bottom-up gaps" in the vegetation (Farji-Brener & Illes 2000).

Birds and ants are legitimate dispersers of seeds, since they do not decrease levels of seed germination. Ants (mostly *Atta*) possibly play a greater role in seed bank dynamics than in germination of *Miconia* in the cerrado. Indeed, with the small-seeded pioneer *Miconia argentea*, ants create a spatial mosaic of variable seed densities at surface and deep levels into the forest soil (Dalling *et al.* 1998). Since *Miconia* species have permanent seed banks, the uneven spatial distribution of seeds provided by ants may be a mechanism of dispersal in time and space for this species (Farji-Brener & Medina 2000). Many seeds of *Miconia*, however, probably receive no benefit from the interaction with ants because they rest too deep into the nest soil to germinate (Dalling & Wirth 1998), or are killed during fungus grow. This reinforces the dual role of *Atta* as a seed predator and disperser of seeds (Retana *et al.* 2004).

Most removal of diaspores of *Miconia* in the floor of cerrado may be assigned to ants, since there was no difference in removal rates between caged and open treatments. Because ants quickly remove seeds from beneath the plant crown, they may reduce seed losses to vertebrate predators (Beattie 1985). However, it is known that some of the tiny seeds of *Miconia* are likely to pass the digestive system of small rodents undamaged (Magnusson & Sanaiotti 1987), and thus some secondary dispersal of seeds by rodents may also occur. Removal rates were subjected to temporal variation leading to significant differences between fruiting seasons. Ant activity can be greatly influenced by the abiotic setting (Hughes & Westoby 1990). The 2005 fruiting season experienced conspicuous lower temperatures than the 2004 fruiting season, what may have lead to a decrease in ant activity in the floor of cerrado, with a subsequent decrease in removal rates of diaspores.

The role of fruits such as *Miconia* as substrate for fungus culturing by *Atta* in the cerrado seems to be more pronounced than at rainforest sites. For instance, Dalling & Wirth (1998) reported that fruits of *Miconia argentea* comprise just 2% of the total foraging effort of *Atta colombica* throughout the fruiting season at Barro Colorado Island in Panamá. On the other hand, *A. sexdens* harvest diaspores of at least 19 plant species in the study site of cerrado on a year basis (A. V. Christianini & P. S. Oliveira unpubl. data). Why leaf-cutter ants harvest so many fruits in the cerrado? It is well know that leaf-cutters choose among the available plant material for fungus-culturing, and that the chemical composition of plant matter may play a role in this choice (Howard *et al.* 1988, Farji-Brener 2001, Wirth *et al.* 2002). Many plants in the cerrado produce leaves heavily protected by toxic compounds, high toughness, and with poor nutrient content (e.g. Marquis *et al.* 2002). For instance, terpenoids, which are toxic to leaf-cutters and their fungus (Howard *et al.* 1988) are common among certain plant clades in the cerrado (e.g. Gottlieb *et al.* 1975). Carbohydrate-rich and watery fruits probably provide a better substrate to cultivate the fungus inside ant nests than the cerrado foliage. The fleshy part (aril or pulp) of fruits can be highly susceptible to fungus growth (Oliveira *et al.* 1995, Leal & Oliveira 1998, Ohkawara & Akino 2005, Christianini *et al.* 2007). Indeed, non-leaf plant material, such as flower parts, fruits and seeds, as well as insect and vertebrate feces, comprised most material collected by attine ants in a year-round basis in another cerrado locality (Leal & Oliveira 2000). The participation of leaf-cutter ants in the phase two of dispersal of carbohydrate-rich fruits like *Miconia* may be driven by preference patterns constrained by the ecological setting, which imposes limits to the harvesting of leaf material for fungus grow as it occur in other neotropical sites (Farji-Brener 2001). Thus, the complex seed dispersal system of *Miconia* including bird- followed by attine ant-dispersal may have evolved by an indirect influence of plant defenses against herbivores in the cerrado.

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Table 1: Diaspore production and fate for *Miconia rubiginosa* across 2004-2005 fruiting seasons in a cerrado savanna in Southeastern Brazil. Values express the relative importance of each fate category in relation to total fruit production of 5 different plants in each year.

Fate category		2004	2005
Dropped under crown	Mature	10.7%	19.2%
	Immature, preyed on, or rotten	64.4%	60.0%
Dispersed away from crown		24.9%	20.8%
Total diaspore production		192,879 ± 118,470	96,347 ± 130,741
Range		32,462-318,368	4,186-325,864

Table 2: Daily pattern of fruit harvesting by three *Atta sexdens* colonies in the peak of *Miconia* fruiting season in the cerrado vegetation. Values indicated the number of fruits brought to the ant nest during a 24-h cycle, the number of all forage items collected by ants and the percentage of total forage composed by *Miconia* fruits. Daily inputs were obtained through extrapolations of 15 min observation sessions at two-hour intervals across 24 h.

Ant colony	Daily input *Miconia* fruit (% unripe)	Daily input all forage	*Miconia* (%)
1	1136 (1.4)	1176	96.6
2	1360 (12.4)	1424	95.5
3	1040 (0.8)	3264	31.9
Average ± SD	1179 ± 164 (4.8 ± 6.5)		74.7 ± 37.1

Table 3: Two-way ANOVA results from diaspore removal experiments in a cerrado savanna in Southeast Brazil. Factors included in the analyses were fruiting season (2004 or 2005), and exclusion (access to ants only, or open to all animals). Significant differences are in bold ($p < 0.05$).

Efect	df	MS	F	P
Fruiting season	1	7.456	7.354	**0.008**
Exclusion	1	1.649	1.626	0.21
Fruiting season x Exclusion	1	0.008	0.008	0.93
Error	122	1.014		

Table 4: Results from seed germination experiments performed with *Miconia* under constant light and temperature (20º C). Seeds used in the experiments were obtained from ant nest refuse piles, fresh bird feces, or taken directly from plants (control). Comparisons of germination frequencies were performed with G-tests (all non-significant).

	Nests or feces		Controls		
Source of seeds	Sowed	Germinated (%)	Sowed	Germinated (%)	G-value
Atta refuse piles	207	39 (18.8)	113	20 (17.7)	0.064
Odontomachus refuse piles	220	81 (36.8)	112	38 (33.9)	0.271
Bird feces	197	40 (20.3)	225	58 (25.8)	1.774

Legend to figures

Figure 1: The contribution of the frugivorous bird and ant assemblages to the interactions with fruits of *Miconia rubiginosa* in the cerrado savanna across phase one and two of seed dispersal. (A) relative importance of bird-diaspore interactions in plant canopy (phase one of dispersal). Birds may take fruits and drop seeds under crown, or swallow the fruit and defecate seeds away; (B) relative importance of ant species interacting with fallen fruits (phase two of dispersal). Ants may remove fruit pulp at the spot or carry the whole diaspore to the ant nest. See Figure 2 for distances of seed displacement achieved in each phase of dispersal.

Figure 2: Comparative distances of seed displacement during phase one and two of seed dispersal of *Miconia rubiginosa* in a cerrado savanna, southeast Brazil. Distribution of distances of diaspores carried away by birds in first flight from feeding trees (phase one dispersal), and carried away by ants on the floor (phase two of dispersal). n = number of independent records of seed displacement by ants, or number of flights observed for birds. The figure does not include data of seeds that were not dispersed (i.e. seeds cleaned on spot by ants with no displacement, or dropped under the plant crown by birds). See text and Figure 1 for further details.

Figure 3: Harvesting of ripe fruits of *Miconia rubiginosa* vegetation (mean ± SE) by three *Atta sexdens* colonies throughout a 24-h cyle during the peak of fruit production in cerrado (continuous line, filled circles). Harvested fruit was sometimes rejected, and deposited in the refuse pile around nest entrance (dashed line, empty circles).

Figure 4: Removal rates of fallen diaspores of in a cerrado savanna in Southeast Brazil, in caged treatments (accessed by ants only) and paired open controls (accessed by ants and vertebrates). Mean (± SE) removal rates of fruits of *Miconia rubiginosa* in 2004-2005 fruiting seasons.

Figure 1

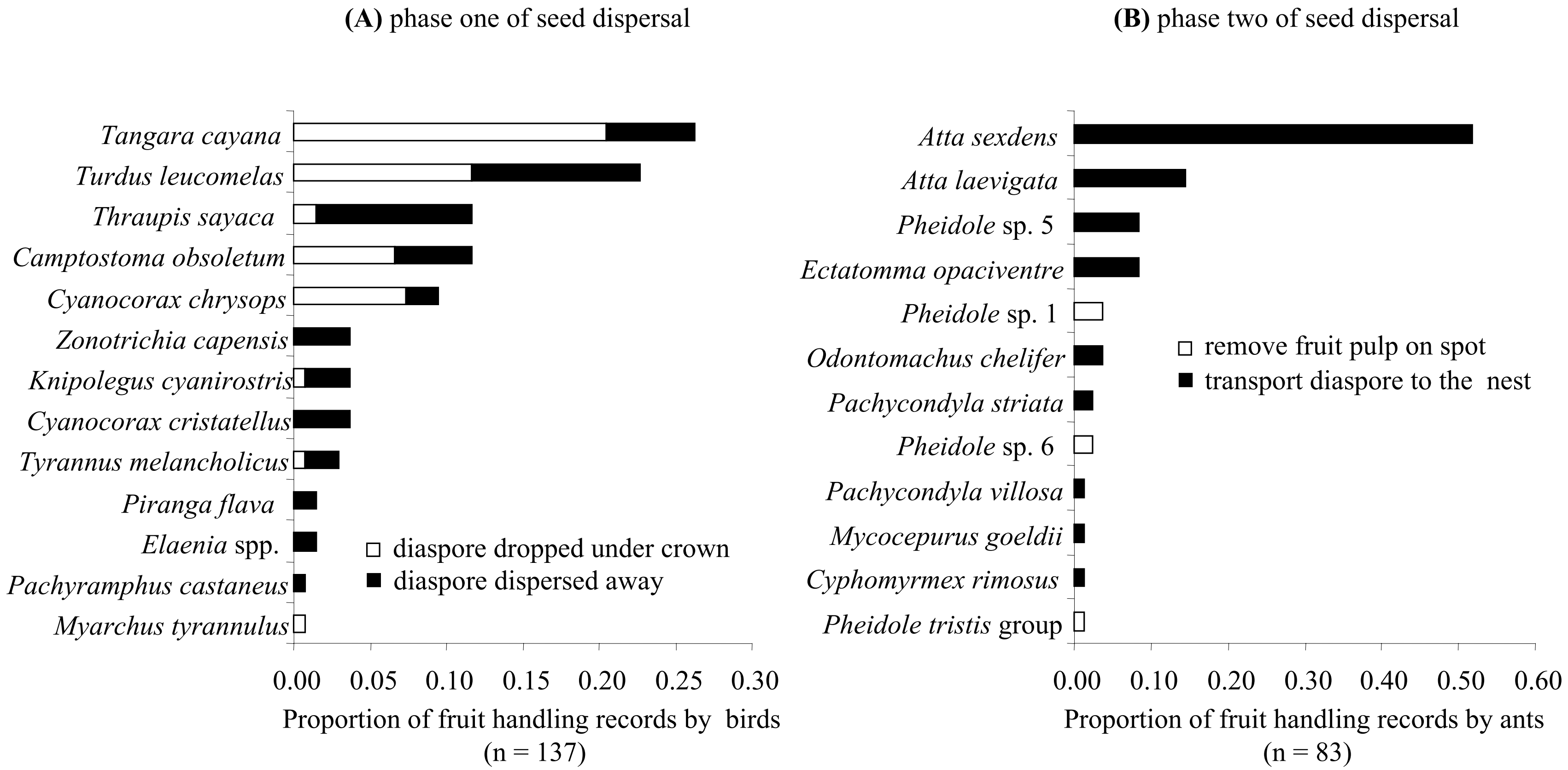

(A) phase one of seed dispersal
Tangara cayana
Turdus leucomelas
Thraupis sayaca
Camptostoma obsoletum
Cyanocorax chrysops
Zonotrichia capensis
Knipolegus cyanirostris
Cyanocorax cristatellus
Tyrannus melancholicus
Piranga flava
Elaenia spp.
Pachyramphus castaneus
Myarchus tyrannulus
□ diaspore dropped under crown
■ diaspore dispersed away
0.00 0.05 0.10 0.15 0.20 0.25 0.30
Proportion of fruit handling records by birds (n = 137)
(B) phase two of seed dispersal
Atta sexdens
Atta laevigata
Pheidole sp. 5
Ectatomma opaciventre
Pheidole sp. 1
Odontomachus chelifer
Pachycondyla striata
Pheidole sp. 6
Pachycondyla villosa
Mycocepurus goeldii
Cyphomyrmex rimosus
Pheidole tristis group
□ remove fruit pulp on spot
■ transport diaspore to the nest
0.00 0.10 0.20 0.30 0.40 0.50 0.60
Proportion of fruit handling records by ants (n = 83)

Figure 2

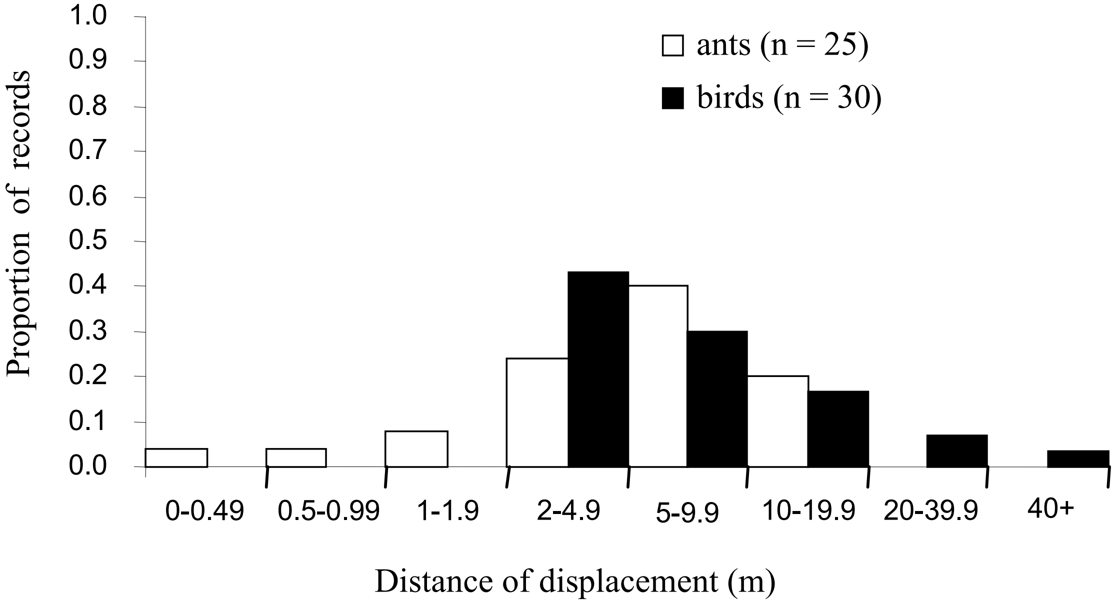

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Proportion of records
□ ants (n = 25)
■ birds (n = 30)
0-0.49
0.5-0.99
1-1.9
2-4.9
5-9.9
10-19.9
20-39.9
40+
Distance of displacement (m)

Figura 3

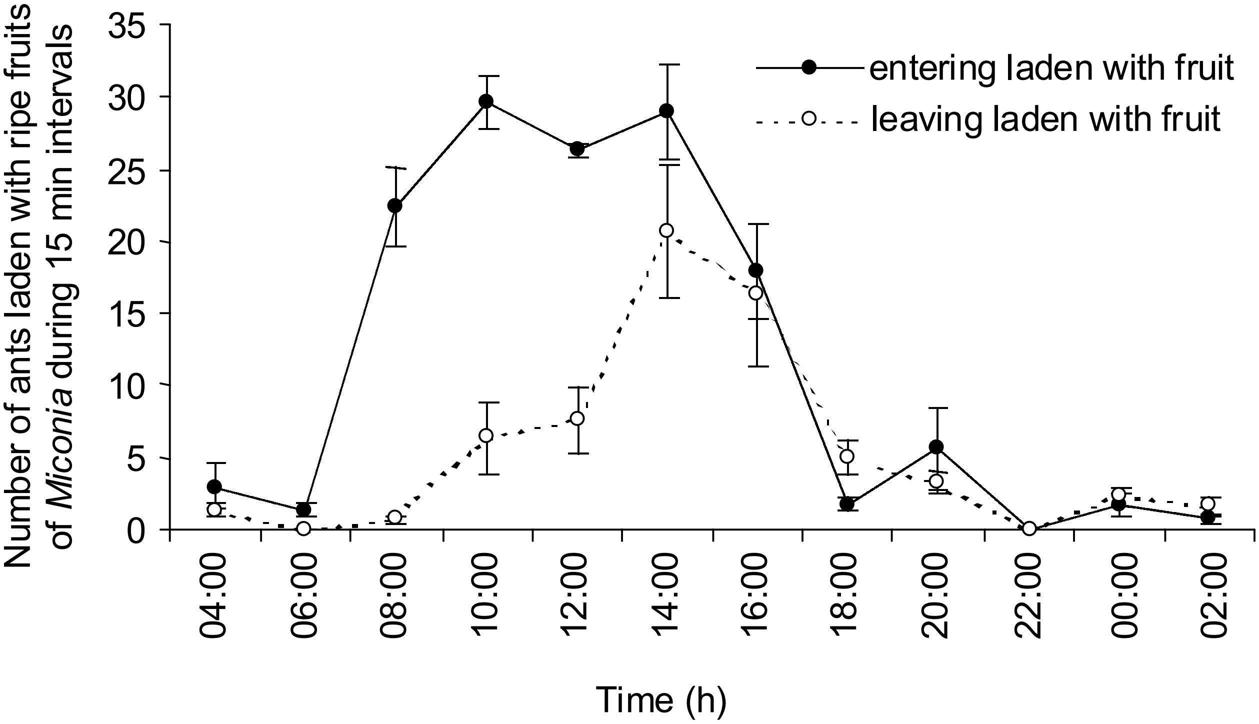
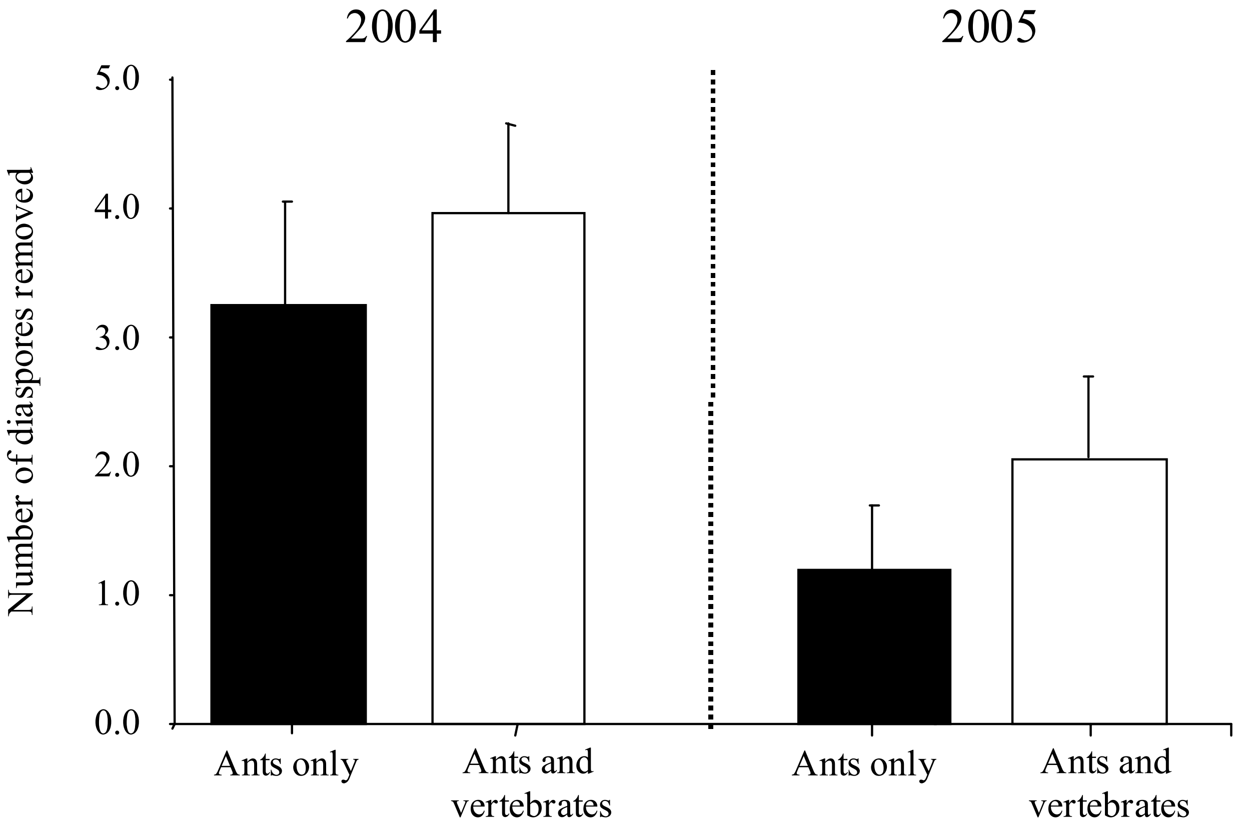

Number of ants laden with ripe fruits of *Miconia* during 15 min intervals
35
30
25
20
15
10
5
0
entering laden with fruit
leaving laden with fruit
04:00
06:00
08:00
10:00
12:00
14:00
16:00
18:00
20:00
22:00
00:00
02:00
Time (h)

Figura 4


2004
2005
Number of diaspores removed
5.0
4.0
3.0
2.0
1.0
0.0
Ants only
Ants and vertebrates
Ants only
Ants and vertebrates

Capítulo 5

Edge effects decrease benefits obtained by plants in interaction with their ant mutualists in a neotropical savanna *

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Running title: *Edge effects in ant-plant mutualism*

Key words: Cerrado, *Erythroxylum*, diplochory, habitat fragmentation, plant reproduction, plant recruitment, seed germination, seed dispersal, secondary seed dispersal, seedling survival

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Abstract

Edge effects may lead to changes in abiotic conditions and in mutualistic plant-animal interactions, such as seed dispersal, in the borders of habitat remnants. We evaluated the occurrence of edge effects and its consequences for the regeneration of *Erytroxylum pelleterianum* (Erythroxylaceae), a shrub that benefits from the interaction with seed dispersing ants in the cerrado. We compared air temperature, relative humidity and vapour pressure deficit, as well as the outcome of ant-diaspore interactions between edge and interior of a fragment of cerrado in southeast Brazil. Despite great within habitat variation, the inner portion of cerrado was likely to be moister than its borders. Seed production and germination did not differ between edge and interior of cerrado. Fallen diaspores of *E. pelleterianum* were removed by ants at greater rates near fragment edges. However, ant-diaspore interactions at edges were dominated by small Myrmicinae, which are likely to provide few benefits to seeds. Seedlings of *E. pelleterianum* growing close to ant nests showed higher survival than seedlings growing away from nests in the interior of cerrado, but such effect disappeared near edges. Widespread seedling mortality due to a higher evaporative demand at edges may partially account for this effect. Furthermore, ant nests also showed a lower residence time near edges, decreasing possible benefits derived from ant colony activity such as nutrient enrichment and protection against insect herbivores. Edge effects could potentially change the structure and dynamics of vegetation in cerrado fragments, due in part to the collapse of the mutualistic interactions demonstrated here.

Introduction

Habitat fragmentation may result in extensive disturbance of vegetation in the neotropics (Murcia 1995, Laurance & Bierregaard 1997, Laurance *et al.* 1998). Several studies suggest that reductions in the area of habitat remnants and the proximity to habitat edges may influence plant-animal interactions, particularly those related to plant recruitment, such as pollination, herbivory, seed predation and dispersal (Aizen & Feinsinger 1994, Santos & Telleria 1994, Jules & Ratchke 1999, Restrepo *et al.* 1999, Benitez-Malvido 2002). Some studies suggest that changes in key plant-animal mutualisms following habitat fragmentation, such as seed dispersal, may lead to recruitment declines and long-term impoverishment of plant species in fragments (Turner *et al.* 1996, Cardoso da Silva & Tabarelli 2000). As a result, it is frequently hypothesized that plant populations in habitat fragments will be reduced in size and show an increased probability of stochastic extinction (Jules 1998, Young *et al.* 1996, Honnay *et al.* 2005). Despite this widespread assumption, few studies have investigated the mechanisms under these plant population declines (but see Bruna 1999, Jules & Ratchke 1999, Benitez-Malvido 2002), and they are biased to forest formations. Plant physiological responses to edge formation may sometimes compensate a higher population mortality rate in fragments, through an increase in flower production and reproductive output (Aizen & Feinsinger 1994, Restrepo *et al.* 1999) in response to increased light availability near edges.

The highly diverse tropical savannas of central South America, the cerrado, have been extensively converted to cropland in the last forty years (Myers *et al.* 2000, Cavalcanti & Joly 2002). Nowadays, small and isolated habitat fragments, subjected to an increased susceptibility to edge effects, comprised most habitat remnants in large extensions of the original distribution of the cerrado (Cavalcanti & Joly 2002). However, the cerrado occurs in a naturally patchy, mosaic-like type of vegetation physiognomies from open grassland (*campo limpo*) to a short tall, dry forest (*cerradão*) (Oliveira-Filho & Ratter 2002). Thus, great natural variation in temperature and humidity should be observed inside patches of cerrado, independent of anthropogenic disturbance. Plants and animals in the cerrado evolve in a scenario naturally subjected to great spatial variation in environmental conditions (e.g. temperature and humidity), and this may be one of the reasons for the unusual investigation about edge effects in the cerrado. Although edge effects are currently unrecognized, changes in plant-animal interactions at edges are suggested by the spatial patterns of plant-hemiptera interactions (Del-Claro & Oliveira 1999) and by the influence of dirty roads on the selection of nest sites by alate queens of leaf-cutter ants in the cerrado (Vasconcelos *et al.* 2006).

Environmental conditions may influence the diversity and activity patterns of ants, because they are under greater risk of desiccation in dry or highly seasonal environments (Kaspari 1993), as is the case of the cerrado (Franco 2002). Edge effects may partially account for the negative effects of habitat fragmentation on ant communities (Carvalho & Vasconcelos 1999). Furthermore, edges could negatively influence seed dispersal by ants in forests (Guimarães & Cogni 2002, Ness 2004). In the cerrado, ants are important agents of secondary seed dispersal and may influence the regeneration of many plant species, through removal of fallen diaspores to ant nests were the survival of seedlings is increased (Leal & Oliveira 1998, Christianini *et al.* 2007). However, benefits achieved by plants interacting with ants are frequently contingent upon the ant species involved (Christian 2001, Ness 2004). Ant derived benefits to seedlings may also be linked with the time of residence of the ant colony, which is correlated with the level of soil nutrient enrichment achieved near the nest (Hughes 1990). If ant communities and the turnover rate of ant colonies change in the borders of cerrado in response to edge effects, we expected changes in the recruitment of plants that interact with seed-disperser ants at edges.

We investigated how edges may influence the benefits achieved by *Erythroxylum pelleterianum* (Erythroxylaceae) interacting with secondary seed-dispersing ants in the cerrado. First we compared the variation in abiotic variables (temperature, relative humidity and vapor pressure deficit) relevant for the performance of plants and ants between edges and the interior of cerrado. Using an observational/experimental approach we estimated seed production and germination, as well as the relative contribution of different ant species to the interactions with fallen diaspores, seedling survival, and the time of residence of the ant colonies in these two contrasting habitats in a fragment of cerrado. We showed that edge effects modulate the outcome of the mutualistic ant-plant interactions in our study system, what may influence the long-term vegetation dynamics of the cerrado fragment.

Methods

Study site and system

The study was conducted in the reserve of the Estação Experimental de Itirapina (22°12'S, 47°51'W), southeast Brazil. The study site is a ca. 200 ha fragment of cerrado isolated by dirty roads and fire breaks in a landscape dominated by *Pinus* sp. and *Eucalyptus* sp. timber plantations and pastures. The fragment is protected from fire since at least twenty years (D. Zanchetta pers. comm.). Mean annual rainfall is 1360 mm, concentrated in summer (December to March), and mean annual temperature is 21.8ºC (data from 1994-2004 from a local climatological station). Cerrado *sensu stricto* covers the study site, the typical fire-prone savanna that grows in sandy- and nutrient-poor soils of central Brazil (Oliveira-Filho & Ratter 2002). Plant cover varies from 50% to 80%, mainly by small palms (*Syagrus petraea* (Mart.) Becc., *Attalea geraensis* Barb. Rodr.), shrubs (*Miconia albicans* (Sw.) Triana, *Campomanesia pubescens* (DC.) O. Berg), and 4-6 m trees (*Miconia rubiginosa* (Bonpl.) DC., *Xylopia aromatica* (Lam.) Mart., *Pouteria torta* (Mart.) Radlk). Herbs are also abundant (e.g. *Echinolaena inflexa* (Poir.) Chase, *Paspalum* spp., *Bromelia balansae* Mez). Details about local vegetation can be found in Giannotti (1988).

We selected a locally abundant plant species for the study, the perennial shrub *Erythroxylum pelleterianum* A. St.-Hil (Erythroxylaceae) (hereafters referred by its genus). At the study site fruiting occurs from October to December. *Erythroxylum* produces single-seeded fruits (drupes) primarily dispersed by birds, and is dependent on seeds for regeneration (A.V. Christianini & P. S. Oliveira unpubl. data). Ants fastly remove plant diaspores fallen to the ground (Christianini *et al.* 2007) acting as secondary seed dispersers. Ants are key mutualists in the seed dispersal system of *Erythroxylum*, because they provide an escape from vertebrate seed predators and deliver seeds to nutrient enriched microsites where seedling survival is increased (Christianini *et al.* 2007, chapter 3).

Abiotic variables

To investigate the influence of edge effects on abiotic variables in the cerrado we measured temperature and relative humidity of the air and calculated vapor pressure deficits (VPD) in the edges and in the interior of the fragment. Paired sampled stations (blocks) were set across ten transects that started in the surrounding fire break, 5 m out of fragment, and run perpendicularly into the reserve's interior. Minimum distance between transects was 38 ± 15.2 m (mean ± SD). Sampling stations were positioned along the transects 5 m out of fragment, and at random in edges and in the interior of cerrado. We arbitrarily defined edge as the belt of vegetation growing up to 10 m into the fragment. Interior sampling sites were at least 30 m away from the nearest border of the fragment. Transects were sorted, and after the abiotic variables were measured once in each sampling station in a random order within a transect, between 1000 h and 1600 h of August 2006, in the peak of the dry season. Measures were taken in two days, once in five different transects per day (total of 10 measures for each variable and habitat combination). Temperature and relative humidity readings were done with a digital thermometer (precision of 0.1ºC for temperature and 1% for relative humidity), maintained in the shade at ground level for three minutes. VPD was obtained from temperature and relative humidity data following Campbell (1977). Since there is evidence of facilitative effects of tree cover on seedling establishment in the cerrado (Hoffmann 1996) we used the readings taken out of fragment (i.e. in the fire break) as controls for the effects of vegetation cover on abiotic variables. We subtracted the readings of temperature, relative humidity and VPD in the fire break from the readings obtained for the edge and interior sampling stations within transect blocks. Comparisons between air temperature, relative humidity and VPD between edge and interior of cerrado in relation to the fire break were done with ANOVA, using transect (block) as a random effect factor and habitat (edge or interior) as a fixed factor for each dependent variable (Zar 1999).

Removal of fallen diaspores and ant-diaspore interactions

To examine the contribution of ants to the removal of fallen diaspores we performed field experiments with ripe fruits collected from plants established at edges and interior of cerrado. During the fruiting period of 2004 (October-December), diaspore removal by ants only (exclosure treatment) was compared to diaspore removal by ants plus vertebrates (open control) through a paired experiment on the savanna floor. Vertebrates were excluded from treated diaspores with the aid of a wire cage (17 × 17 × 8 cm), fenced on top and sides with mesh (1.5 cm) and staked to the ground. Pairs of diaspores were set out at about 08:00 h at five stations placed radially beneath fruiting trees at the edge ($n = 15$ trees) and interior ($n = 15$) of cerrado. Each pair consisted of a diaspore placed directly on the floor under a wire cage, and an exposed diaspore (control) placed outside the cage, 15 cm away. After 24 h, we recorded the ant species interacting with diaspores, and the number of diaspores missing in each group. A diaspore was considered removed if not found within a 30 cm radius from its original location. Experimental diaspores were marked with a small dot of a permanent ink marker (Testors, USA) to distinguish them from naturally fallen ones. The ink mark has no detectable effect on ant behavior (Passos & Oliveira 2002). We kept a minimum distance of 20 m between replicates to provide independent discoveries by different ant colonies (Levey & Byrne 1993). We also performed seed removal trials a few weeks later following the same procedure for fruits, in the same individual trees. Data on diaspore removal were analyzed using factorial analysis of variance. The dependent variable was the number of diaspores removed per tree, while the independent variables were habitat (edge or interior), exclusion type (caged versus uncaged), and presence/absence of a reward (fruit pulp or aril). All factors were treated as fixed factors. Data was square root ($x + 1$) transformed prior to the analyses to improve normality and homocedasticity.

To record the ant species interacting with fallen diaspores and possible seed fate we conducted systematic sampling. We placed diaspores of *Erythroxylum* on the floor of the edge and interior of the fragment, and recorded the ants interacting with them in diurnal and nocturnal samplings. We established 20 sampling stations per habitat (edge or interior), 10 m apart, 1-2 m off a transect that cross the edge and interior of the study site. Diurnal samplings began at 08h00, and nocturnal ones at 19h00. Each sampling station received two diaspores of *Erythroxylum* (20 stations x 2 diaspores x 2 sampling periods x 2 habitats = 160 diaspores in total). Diaspores were placed on a small piece of white filter paper (4 × 4 cm) to facilitate visualization. The filter paper apparently did not influence ant behavior (Passos & Oliveira 2002). At each period we walked slowly along the transect over 2 h and recorded the ants removing diaspores. We followed ants carrying diaspores until they reached the nest or disappeared in the leaf litter. The distance of displacement was then measured. At any time during the study, opportunistic observations of ants removing diaspores were also recorded. Voucher specimens of ants and plants were deposited in the entomological collection of the Universidade Federal Rural do Rio de Janeiro (CECL), and at the herbarium of the Universidade Estadual de Campinas (UEC), respectively.

Seed production and germination

To investigate how edge effects may influence fruit production of *Erythroxylum* we sampled fruiting plants at random in the edge and interior of the cerrado at the beginning of the 2004 fruiting season. Fruits were directly counted on plant crown. To control for the effect of plant size on fruit production we related the number of fruits produced with plant diameter at soil level (mm), using habitat (plants found in the edge (n =11) or interior (n =7)) as a covariate in ANCOVA.

To evaluate the influence of edges on seed germination we compared seed germination success in experimental seed banks located at edges and in the interior of the cerrado fragment. We collected ripe fruits from ten plants of *Erythroxylum* found in edges and interior of the fragment. Filled seeds were joined to form a bulk sample. Seeds were stored in a cool, dry place until the end of the fruiting season (December 2004), when they were sowed in the field. Thus, seeds were subjected to the same abiotic conditions as most naturally dispersed seeds. Seed were sowed in open-topped seed baskets (12 x 12 x 14 cm, 1 mm mesh size) (Christian & Stanton 2004) made of plastic mesh. Prior to planting seeds in experimental seed banks, we sieved the soil in two 15 x 15 x 15 cm areas side by side to remove any *Erythroxylum* seed present prior to the sowing. An open-topped basket made of plastic mesh was placed into each pit and then filled with the sieved soil (see Christian & Stanton 2004). Each basket was buried at 12 cm deep leaving 2 cm of the rim above the surface to facilitate visualization. Tanglefoot© was applied to the exposed rim to prevent the access of ants to the seeds. In each pair of baskets we sowed 20 fruits or cleaned seeds (i.e. with fruit pulp removed), 1 cm deep and 2 cm apart. A wire cage (20 x 20 x 10 cm) fenced on top and sides with mesh (1.5 cm) was staked to the ground covering each seed basket to prevent vertebrate access. Paired germination tests were performed at three locations at the edge and interior of the cerrado (n = 120 seeds per habitat; 240 seeds in total) where *Erythroxylum* seedlings occurred naturally. Seedling emergence was followed monthly for one year. Since the presence of fruit pulp has no effect of seed germination in the field (A. V. Christianini & P. S. Oliveira, unpubl. data), we used each treatment in a pair as a replicate to increase sample size and power in statistical comparisons. We evaluated seed germination between edge and interior using Mann-Whitney U-tests. To determine the fate of seeds that did not emerge as seedlings we sieved the soil from experimental seed banks to recover any seed remaining after one year (January 2006), and inspected them visually for viability.

Ant colony residence time, seedling distribution and survival

We did not follow the residence time of ant colonies such as those of *Pachycondyla* sp. because they usually had several nest entrances dispersed in a patch, what made a proper discrimination between independent nests difficult. We choose to follow the fate of *Odontomachus chelifer* colonies because they occur as discrete units in the field, and also because seedlings of *Erythroxylum* are found in higher numbers in the nests of this species (A. V. Christianini & P. S. Oliveira, unpubl. data). Ant nests were located by following laden ant workers that had been attracted to fallen fruits of *Erythroxylum*, or attracted to tuna baits placed on the ground. Nests were tagged, and the residence time of ant colonies followed during a year. Ant colony residence time was determined by direct inspections of marked nests throughout intervals of 1-5 months. During inspections we recorded signs of nest activity up to 25 cm of the original location such as the presence of an active entrance of the colony and fresh dump material deposited in the refuse pile. If no obvious sign of ant activity was found, we set up a sardine bait close to the original mark and observed it for ant activity during one hour. If ants or fresh dump material was observed the nest was recorded as active, otherwise the nest was considered inactive. This protocol did not allow us to assign an exact fate of the colony, if dead or moved to another place, but give a relevant information for the plant point of view. The number of *O. chelifer* colonies active or inactive after one year between edge and interior of cerrado was compared with a G-test (Zar 1999).

To investigate how edge effects may influence seedling fate and the outcome of ant-diaspore interaction we marked seedlings found in sampling quadrats (50 x 50 cm) laid out in ant nests and in paired-control plots in the edge and interior of cerrado. Nests of all ant genera interacting with *Erythroxylum* were used. Sampling quadrats were centered on nest entrances or on the refuse piles if present (usually 10-15 cm from nest entrance). Control plots were established 2 m away, in a random direction (Passos & Oliveira 2002). Nest and control plots were surveyed in March 2005. Seedlings of *Erythroxylum* inside nest and control quadrats were individually marked with a numbered flag, and monitored for survival monthly until July 2005, and then every two months until February 2006. The 2005 seedling cohort of *Erythroxylum* was easily distinguished from juveniles (older than one year) because young seedlings had a single green stem while juveniles had a brown stem, frequently ramified. To increase the records of seedlings growing away from nests we also searched seedlings up to 1 m at both sides of a transect that crossed the edge and the interior of cerrado, and followed the same procedure as above. Seedling survivorship was compared with Peto & Peto's logrank tests (Pyke & Thompson 1986).

Results

Great within habitat variance in abiotic variables was observed. However, a gradient of decrease in temperature and increase in relative humidity of the air could be observed in the transition matrix → edge → interior of cerrado (Figure 1A). Using the readings in the fire-breaks as controls in the sampling blocks we observed differences in relative humidity (F = 9.0, P = 0.015) and VPD (F = 6.40, P = 0.032) between the edge and interior of cerrado, but there was no difference in air temperature (F = 6.27, P = 0.24) (Figura 1B). The interior of cerrado had 1.4ºC lower mean air temperature and 2% higher humidity than the cerrado edge (Figure 1A). This translated in a mean difference of almost 20% in the VPD (0.286 KPa) between the cerrado interior and its edge (Figure 1B), indicating that the cerrado interior was moister than the edge of the fragment.

Habitat type (edge or interior) had a significant effect on the removal of fallen diaspores of *Erythroxylum* (Table 1). Fruits and seeds were removed at greater rates at edges than at the interior of the fragment, but there was no interaction between habitat type and exclosure treatment or presence of reward (Figure 2) (the effect of other factors will be presented and discussed elsewhere). However, the ant community that interacts with fallen fruits of *Erythroxylum* at edges was different from that in the interior of cerrado (G = 12.94, df = 4, P = 0.012, Figure 3). Small Myrmicinae ants (*Pheidole* spp., *Solenopsis* spp. and *Wasmannia auropunctata)* dominated the interactions at edges, with 80% of the records. In contrast, ant-diaspore interactions in the interior of cerrado were more evenly distributed across several ant genera (Figure 3) (see Christianini *et al.* 2007 for a more detailed list of the ant fauna exploiting diaspores). Ponerinae ants (*Pachycondyla*, *Dinoponera* and *Odontomachus*) were responsible for 30% of the interactions with diaspores in the interior of cerrado, against 16% in the edge. Interactions of several ant genera increased six-fold in the interior of cerrado compared to the edge (other ants in Figure 3). Seeds were removed by ants to short distances at edges (mean 0.66 m, range 0.35 –1.1 m, N = 3). In the interior of cerrado ants removed diaspores to more variable distances (range 0.1-7.05 m, N = 19), and at a farther mean distance (1.45 m). However, this difference was not significant (Mann-Whitney test: U = 19.5, P = 0.41).

Fruit production of *Erythroxylum* did not differ between plants growing in the edges or in the interior of cerrado when controlling for plant size (ANCOVA: $F_{1,15} = 0.68$; df = 1; P = 0.42). The median number of emerging seedlings from experimental seed banks in the interior of the fragment was 5 (mean = 5.0 ± 2.3), which did not differ from the number of emerging seedlings at edges (median = 4.5, mean = 4.2 ± 1.5) (Mann-Whitney test: U = 15, N =12, P = 0.70). All seeds recovered from the germination experiments were damaged or rotten, and none was still viable. Thus, there is no persistent seed bank.

In the interior of cerrado, seedlings that grow associated with ant nests showed a 70% higher survival than seedlings growing away from nests (Peto & Peto log rank test: $\chi^2 = 5.2$; df = 1; $P < 0.05$) (Figure 4). However, this benefit provided by ants to seedling survival collapsed at edges, where there was no difference in the survival of seedlings growing close to or away from ant nests ($\chi^2 = 2.8$; df = 1; $0.05 < P < 0.10$), probably because of widespread seedling mortality during the dry season near edges (Figure 4). Seedlings growing away from nests showed higher survival in the interior of cerrado than near edges ($\chi^2 = 9.4$; df = 1; $P < 0.005$). Furthermore, we also observed a lower residence time of ant colonies near edges compared to the interior of cerrado. Of 9 colonies of *Odontomachus chelifer* found in the edge just 3 (33%) were still active after one year, against 11 (92%) active colonies in 12 originally marked in the cerrado interior (G-test: G = 8.4; P = 0.003).

Discussion

Results from this study with *Erythroxylum pelleterianum* indicated that some plants may not achieve benefits from secondary seed dispersal by ants at edges of cerrado as they do in normal conditions, i.e. in the interior of fragments. Seed dispersal, a key process for the maintenance of plant community diversity (Christian 2001), was especially affected by edge effects in the cerrado fragment. Changes in abiotic conditions near edges seem to have direct and indirect effects on seedling survival of *Erythroxylum*, indicating that edge effects can affect biotic interactions and plant regeneration in the cerrado in a similar way as they do in fragmented forests (Aizen & Feinsinger 1994, Jules & Rathcke 1999, Ness 2004).

The survey of abiotic variables indicated that plants growing more than 30 m away from the border of the fragment of cerrado are subjected to a lower water stress during the dry season than the plants growing up to 10 m from the border of the fragment. It is likely that water stress was the main source for widespread seedling mortality near the edge of the cerrado fragment, including seedlings growing in ant nests. Since adult plants from the cerrado have a deep root system, this effect should be more pronounced for seedlings and juveniles that have shallower roots (Franco 2002). Water stress is among the main mechanisms promoting seedling mortality worldwide (Moles & Westoby 2004).

Edges with dirty roads in the cerrado are known to increase the colonization rate by a key herbivore, the leaf-cutter ant *Atta sexdens*. Queens of *A. sexdens* find more suitable conditions for initial nest excavation in these edges due to a lower cover of soil by plant material (Vasconcelos *et al.* 2006). As a result, it is possible that herbivory by leaf-cutter ants will be enhanced in the borders of cerrado close to dirty roads, what may increase environmental changes induced by habitat fragmentation (Urbas *et al.* 2007). Edges also influence ant-diaspore interactions in forest formations close to the study site (Guimarães & Cogni 2002). Higher rates of diaspore removal by ants were observed at edges in the cerrado, and the ants that removed diaspores were quite different in relation to the interior of the fragment. Small Myrmicinae ants contributed disproportionately to ant-diaspore interactions near edges. Such small ants displace diaspores only to short distances (Ness *et al.*2004), and are recognized more as seed predators than dispersers (Hölldobler & Wilson 1990, but see Levey & Byrne 1993). Although there was no interaction between habitat and presence of fleshy portion on diaspore removal we suspect that most ant-diaspore interactions near edges lead to seed predation because of the ant species involved. Seedlings of *Erythroxylum* are not associated with nests of *Pheidole* sp. ants as they are with nests *of Odontomachus chelifer* (A. V. Christianini & P. S. Oliveira unpubl. data). On the other hand, Ponerinae ants, which had a greater contribution to the interactions recorded in the interior of cerrado are recognized as high quality seed dispersers. Seedlings growing in nests of Ponerinae ants such as *O. chelifer* and *Pachycondyla* sp. usually show better performance than seedlings growing away from ant nests (Passos & Oliveira 2002, chapter 3).

Edges did not decrease seed germination of *Erythroxylum* in the cerrado. This result contrasts with those obtained for plants inhabiting moist tropical forests (e.g. Bruna 1999, 2002). To our knowledge there are no other studies comparing seed germination in edges and interior of cerrado fragments, but we expect that seed germination of other plants will not differ as well in the germination at edges compared to the interior of cerrado. Plant-animal interactions would be more affected by edge effects than plant physiological responses, such as reproductive output and seed germination, because of the natural mosaic-like type of vegetation where the plants from the cerrado evolve (Oliveira-Filho & Ratter 2002). However, facilitative effects of vegetation cover on seed germination and seedling establishment of seven species of shrubs and trees were demonstrated for the cerrado (Hoffmann 1996). The amelioration of stressful factors, such as high temperatures and lower humidity may explain part of this effect (Hoffmann 1996). We suggest that edge effects may be important to constrain the regeneration of some plant species at the seedling stage, particularly of those plants with small seeds that are more prone to death due to a series of hazards, including desiccation (Moles & Westoby 2004). A lower *Erytrhoxylum* seedling recruitment indicated that edges may be habitats of poor quality for some plants from the cerrado, as they are for some shade-tolerant plants from forests (Jules 1998, Bruna & Kress 2002).

Differential diaspore production cannot compensate the higher seedling mortality in the border of the fragment, since plants of *Erythroxylum* produced an equivalent number of seeds in the edges and in the interior of cerrado. The absence of clonal propagation and a persistent seed bank may also decrease the chance of a rescue effect in the case of a failure in seedling recruitment at edges. Furthermore, there is no reason to suspect that directed seed dispersal by vertebrate frugivores drives a massive number of seeds to the edges (Restrepo *et al.* 1999). The collapse of mutualistic ant-plant interactions may partially explain the worst performance of seedlings near edges. After a year, seedlings associated with ant nests in the interior of the fragment achieved 70% higher survival than seedlings that grow away from ant nests, while in the edges seedling survival did not differ between ant nests and controls. At least part of this effect may be explained by the lower residence time of ant colonies near edges. Since levels of soil nutrient enrichment are linked with the time of residence of ant colonies (Hughes 1990) it is possible that refuse piles of ant nests at edges did not accumulated enough nutrients to increase water retention and soil fertility as in the interior of cerrado (A. V. Christianini & P. S. Oliveira, unpubl. data). Moreover, a lower residence time of ant colonies may also decrease benefits provided by ants through protection against insect herbivores. There is evidence that ants aggressively attack insect herbivores at seedlings close to the ant nests (Passos & Oliveira 2004).

Certain life-history traits may predispose some plants to suffer the negative effects of edge formation (Kolb & Diekmann 2005). For instance, plants that benefit from secondary seed dispersal by ants are candidates to be negatively affected by edge effects in the cerrado. In the long term we expect a lower recruitment of *E. pelleterianum* near the edges of fragments, and an increase in the recruitment of plants that are more tolerant to the conditions found there. Exotic African grasses such as *Brachyaria* spp. and *Melinis* *minutiflora* usually benefits from disturbances in the cerrado and respond positively to edge formation (Pivello *et al.* 1999). These grasses are fire resistant and outcompete native plants (D'Antonio & Vitousek 1992, Pivello *et al.* 1999). Thus, a synergistic effect of habitat fragmentation, collapse of important mutualistic interactions, and invasion by exotic plants may accelerate the erosion of biodiversity of this species rich savanna (D'Antonio & Vitousek 1992). This effects may be particular important for highly fragmented cerrado landscapes, such as the central plateau of São Paulo in southeast Brazil. The original area of cerrado in São Paulo was greatly reduced and are now dispersed over more than 8,353 fragments, over half of which are smaller than 10 ha (see Cavalcanti & Joly 2002), but that still support a floristically distinct type of cerrado (Ratter *et al.* 2003). Even moderate edge effects may be important in such small fragments. We recommend that practices to minimize edge effects in forests should also be employed in the cerrado, such as an active management of the matrix surrounding fragments and increased patch connectivity (Laurance & Yensen 1991, Honnay *et al.* 2005).

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Table 1: Results of the ANOVA testing for an effect of habitat type (edge or interior), experimental treatment (caged or uncaged) and presence of reward (pulp or seed) on the number of fallen diaspores removed in the cerrado of Itirapina, south-east Brazil.

Factor	df	MS	F	P
Habitat	1	0.830	4.21	**0.043**
Treatment	1	0.952	4.83	**0.030**
Reward	1	5.796	29.40	< **0.001**
Habitat type x Treatment	1	0.175	0.89	0.348
Habitat type x Reward	1	0.176	0.89	0.347
Treatment x Reward	1	0.033	0.17	0.685
Habitat x Treatment x Reward	1	0.021	0.11	0.744
Error	114	0.197		

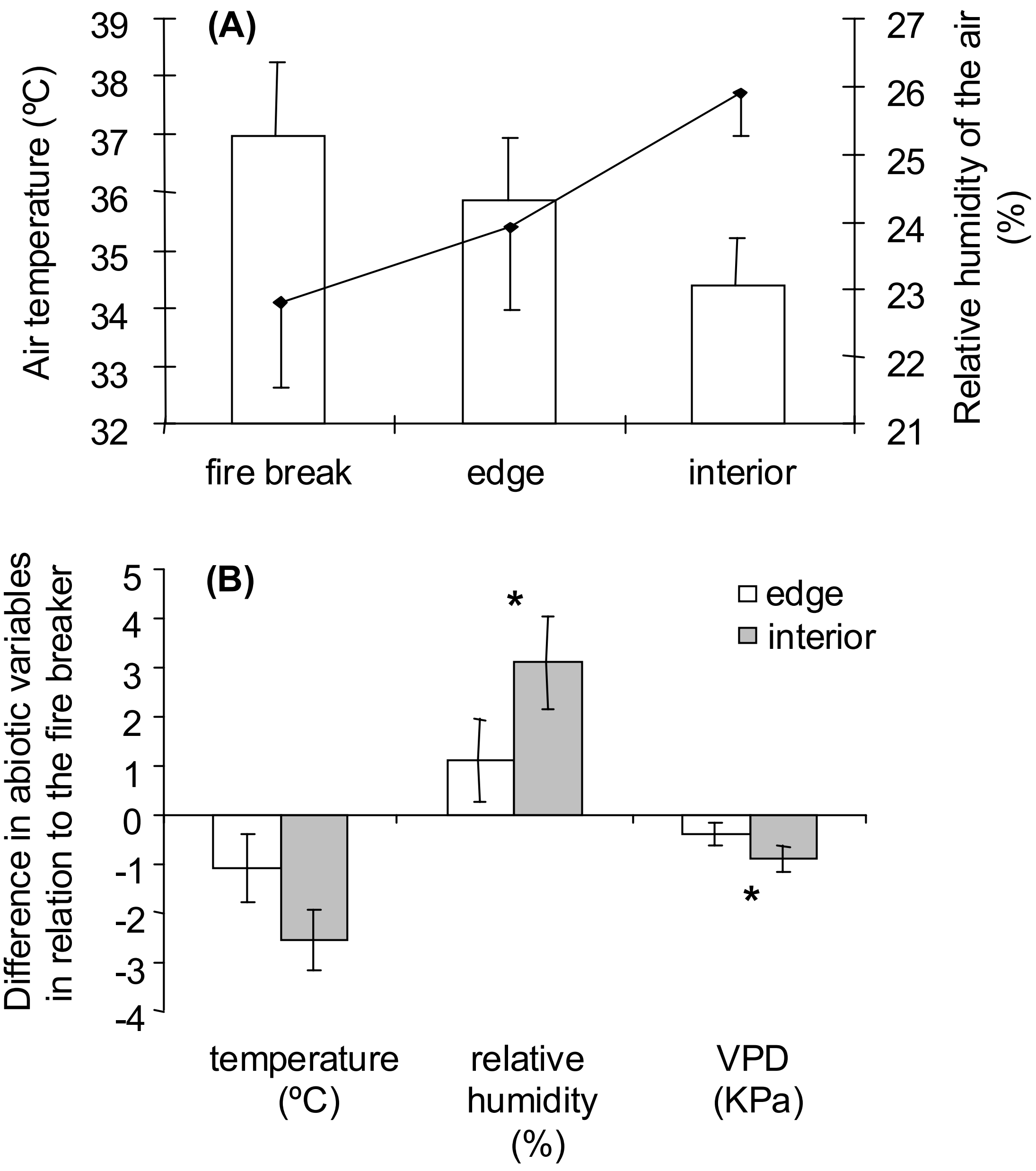

Figure 1: Abiotic variables at different habitat types in the cerrado (mean ± SE). Fire break = 5 m away from the fragment border; edge = 0-10 m into the fragment; interior = into the fragment > 30 m from the nearest edge. (A) air temperature (ºC) (white bars) and relative humidity (line) for each habitat type; (B) differences in the air temperature, relative humidity of the air and water vapor pressure deficit (VPD) between edge and interior of cerrado in relation to the fire break. *P < 0.05.

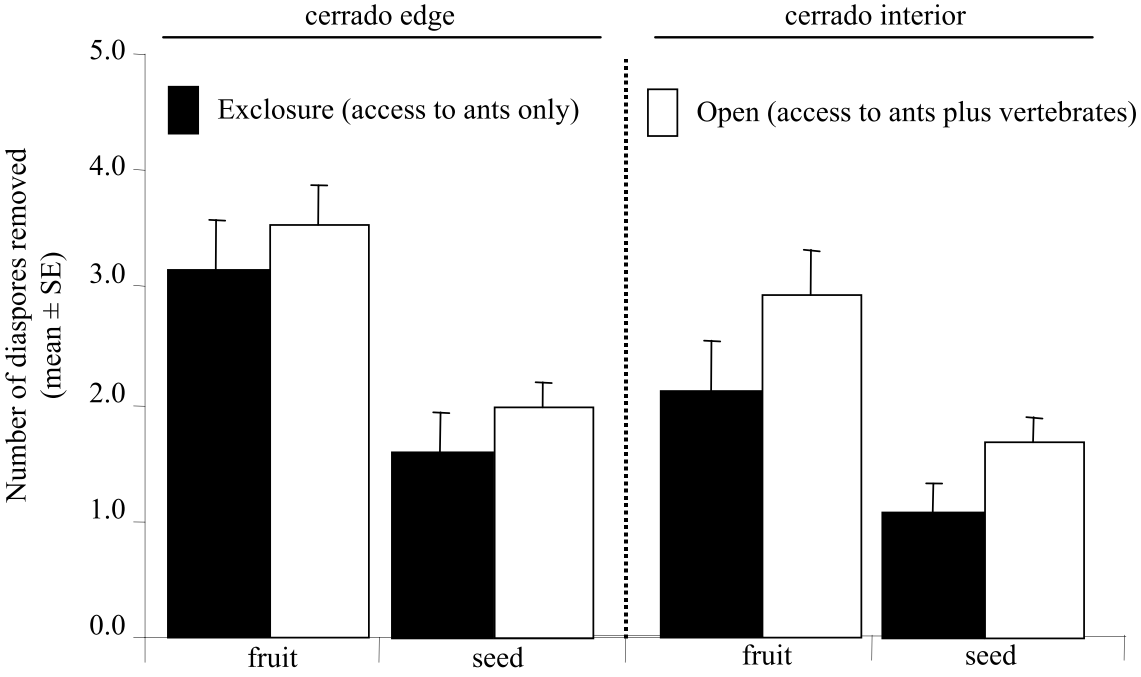

Figure 2: The mean number of diaspores (± 1 SE) of *Erythroxylum pelleterianum* removed from selective exclosure experiments performed in the cerrado edges and interior.

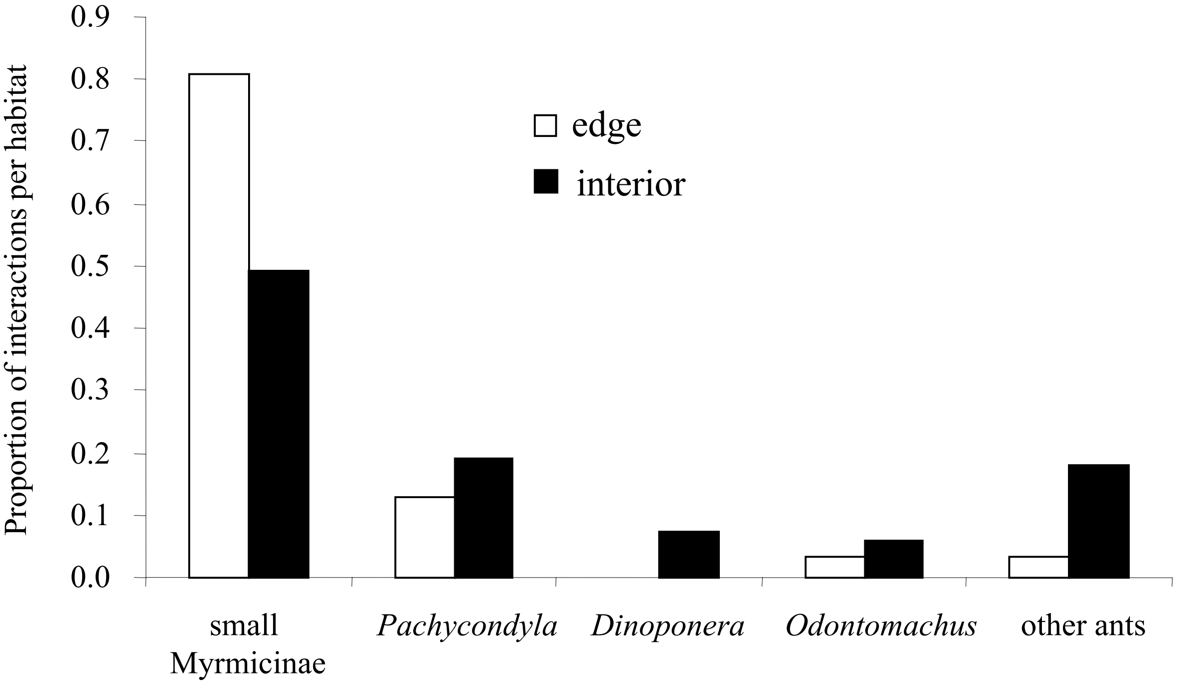

Figure 3: Relative importance of different ant groups to the interactions with diaspores of *Erythroxylum pelleterianum* on the floor of edges and interior of the cerrado savanna. The total number of interactions recorded was 114 (31 in the edges and 83 in the interior of cerrado). Small Myrmicinae includes the genera *Pheidole*, *Solenopsis* and *Wasmannia*, while other ants include manly Formicinae, Dolichoderinae and Attini ants. Ponerinae ants are indicated by their genera.

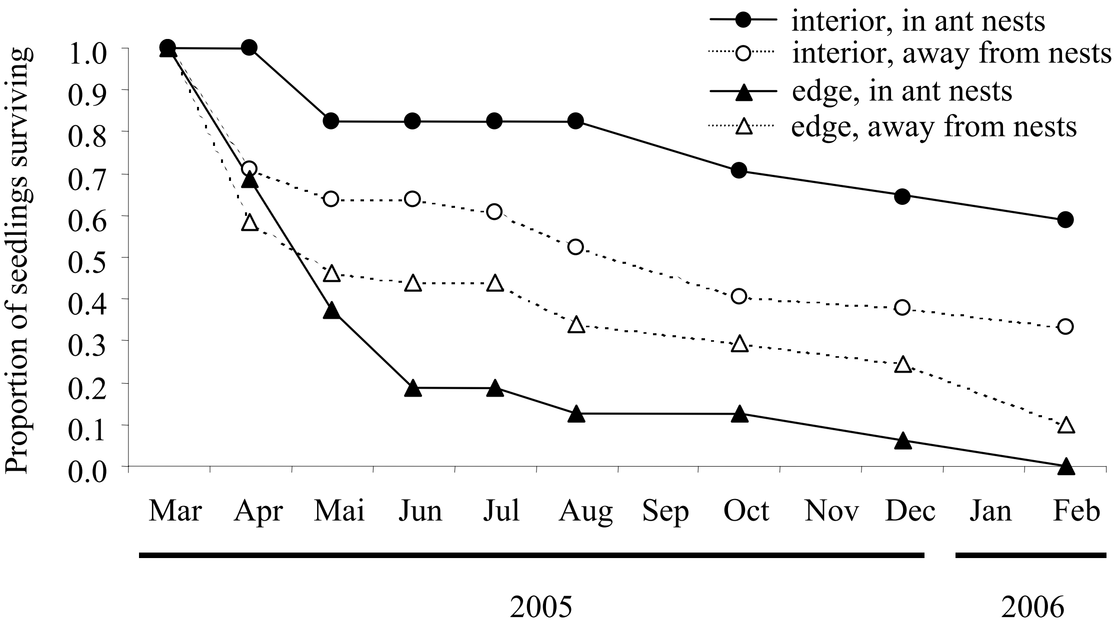

Figure 4: Survival of *Erythroxylum pelleterianum* seedlings (N = 143) in the cerrado across one year (March 2005 to February 2006). Survival was followed for seedlings growing in ant nests or control plots away from ant nests, in the edge or interior of cerrado.

Discussão Geral

As informações fornecidas neste trabalho indicam que o uso de frutos e sementes é bastante comum entre formigas no cerrado, envolvendo muitas espécies de diferentes subfamílias e com hábitos alimentares diversos. Entretanto, é possível reconhecer a existência de associações preferenciais entre determinados taxa de formigas e diásporos, provavelmente mediadas por características químicas dos frutos relacionadas com preferências alimentares e/ou necessidades nutricionais das formigas (Capítulo 1). Foi possível ainda comprovar a influência de características químicas e morfológicas dos diásporos no resultado das interações com formigas (Capítulo 2), demonstrar o papel das formigas na redistribuição espacial das sementes no solo (Capítulo 3-4) e sobrevivência de plântulas de espécies primariamente dispersas por aves (Capítulo 3), além de constatar a fragilidade destas interações frente à fragmentação do cerrado (Capítulo 5). Tomados em conjunto, os resultados deste estudo reforçam a importância da dispersão secundária de sementes ao longo do ciclo de regeneração de plantas no cerrado (Figura 1).

Como a maior parte das angiospermas se reproduz naturalmente apenas por meio de sementes (Howe & Smallwood 1982, Herrera 2002), a dispersão de sementes tem grande influência no valor adaptativo destas plantas, pois uma dispersão de boa qualidade (*sensu* Schupp 1993) aumenta muito as probabilidades de recrutamento de um novo indivíduo (veja Figura 1 da Introdução Geral) (Wang & Smith 2002, Nathan & Müller-Landau 2000, Christian 2001). Este estudo vem se somar a estudos prévios apontando que a dispersão de sementes de plantas tropicais é realizada por meio de um sistema complexo de interações subseqüentes, e que estas interações podem alterar as probabilidades de transição entre os diferentes estágios da regeneração de uma planta (Roberts & Heithaus 1986, Fragoso 2003, Andresen 1999, Böhning-Gaese *et al.* 1999, Passos & Oliveira 2002, 2003, 2004, Vander Wall & Longland 2004). Embora a importância das formigas para o recrutamento varie de acordo com a identidade das espécies em interação, ignorar a ação das formigas certamente significa perder uma visão mais abrangente do ciclo de regeneração de plantas no cerrado, impedindo o reconhecimento de alguns dos mecanismos responsáveis pelos padrões espaciais de recrutamento de plântulas e do banco de sementes no solo (Farji Brener & Silva 1996, Dalling *et al.* 1998, Böhning-Gaese *et al.* 1999, Passos & Oliveira 2002). O fato das plantas investigadas em detalhe não compartilharem um histórico evolutivo recente (até o nível de ordem), reforça a idéia de que a diplocoria possa ser um sistema de dispersão comum no cerrado.

Características que contribuíram para a seleção de sistemas complexos de dispersão de sementes provavelmente estão relacionadas à pobreza de alguns nutrientes no solo do cerrado em comparação ao solo associado aos ninhos de formigas. Condições semelhantes são apontadas para a prevalência de mirmecocoria em outras regiões do mundo (veja revisões em Giladi 2006, Rico-Gray & Oliveira 2007). A grande diferença do cerrado em relação às regiões do Velho Mundo, onde a dispersão de sementes por formigas é parte da dispersão primária, é a manutenção de uma fase de dispersão de sementes por vertebrados anterior à dispersão por formigas. Dois fatores potencialmente responsáveis por este padrão merecem destaque: (1) a necessidade da manutenção da dispersão de sementes a distâncias longas da planta-mãe para proporcionar o escape da predação e a colonização de novos hábitats (Howe & Smallwood 1982). Neste sentido, formigas seriam responsáveis por um ajuste fino da dispersão de longa distância, proporcionando uma dispersão direcionada aos ninhos aonde a performance de sementes e plântulas é melhor (Wenny 2001, Passos & Oliveira 2002, 2004, Giladi 2006) (Figura 2); (2) Boa parte da flora do cerrado é de origem relativamente recente. Por exemplo, altos níveis de endemismo são observados para espécies vegetais, mas não para categorias taxonômicas superiores (como gêneros e famílias) (Castro *et al.* 1999). Assim, mesmo que formigas tenham tido um papel importante na dispersão de sementes no cerrado, o tempo transcorrido a partir da inclusão destes agentes de dispersão pode não ter sido suficiente para moldar características dos diásporos que pudessem ser associadas a dispersão por formigas (como a presença de um elaiossomo), dado a grande inércia filogenética característica de atributos de frutos e sementes (Jordano 1995). Por outro lado, é possível que características químicas, ou particularidades morfológicas inconspícuas dos diásporos (veja Kaufmann *et al.* 1991, Aronne & Wilcock 1994) sejam suficientes para estimular o transporte de sementes pelas formigas e não sejam assinaladas por pesquisadores sem estudos mais detalhados.

Informações a respeito das interações entre formigas e diásporos não-mirmecocóricos em nível comunitário já estão disponíveis para quatro sítios de estudo, na floresta ombrófila densa (Pizo & Oliveira 2000), na mata de restinga (Passos & Oliveira 2003), na caatinga (Leal *et al.* 2007) e agora, para o cerrado (este estudo; veja também Leal & Oliveira 1998). Estudos comparando os padrões de utilização de diásporos não-mirmecocóricos por formigas e suas conseqüências em outras áreas seriam úteis a fim de compreender a generalização dos padrões encontrados até o momento, além de permitir uma maior compreensão dos fatores que mediam as interações entre formigas e diásporos, bem como as conseqüências para as partes envolvidas. Contudo, as informações acumuladas até o momento já permitem reconhecer uma série de padrões: (1) formigas dos mais diversos grupos taxonômicos utilizam-se de frutos e sementes caídos, possuindo repertórios comportamentais na interação com frutos consistentes entre as áreas investigadas (e.g. remoção *versus* limpeza *in situ* do diásporo); (2) o tamanho e a composição química dos diásporos permitem assinalar o grupo de formigas que majoritariamente interage com os diásporos caídos no solo, o que por sua vez irá influenciar no destino destas sementes (e.g. local de deposição das sementes) (Hughes & Westoby 1992b, Hughes *et al.* 1994, Pizo & Oliveira 2001); (3) há variações geográficas nos grupos de formigas responsáveis pela maior parte das interações com diásporos no solo (e.g. Ponerinae na Mata Atlântica *versus* Attini no Cerrado); (4) formigas podem influenciar a distribuição espacial e sobrevivência de plântulas de espécies primariamente dispersas por aves (Passos & Oliveira 2002, 2003, 2004, este estudo) (Figura 1), ou por agentes abióticos (Leal *et al.* 2007); (5) o resultado das interações entre formigas e diásporos não-mirmecocóricos é suscetível a efeitos de borda decorrentes da fragmentação de hábitats (Guimarães & Cogni 2002, este estudo).

Perspectivas

Várias perspectivas promissoras de investigação das interações entre formigas-diásporo são sugeridas a partir dos resultados deste estudo, juntamente com informações derivadas de estudos prévios. Dentre os possíveis caminhos de investigação merecem destaque as seguintes questões:

(1) Será que frutos e sementes cumprem um papel nutricional para as formigas que se alimentam no solo semelhante ao papel desempenhado por nectários extraflorais e hemípteros para as formigas que se alimentam sobre a folhagem no dossel de florestas tropicais? A análise da razão entre isótopos de nitrogênio ($^{15}N/^{14}N$) de formigas, insetos herbívoros e plantas poderia elucidar esta questão (Davidson *et al.* 2003, Capítulo 1).

(2) Quais compostos químicos do diásporo atuam na atração de grupos particulares de formigas, e como esta “escolha de parceiros” pode influenciar a evolução de características

de frutos/sementes dispersos por formigas (Hughes & Westoby 1994, Pizo & Oliveira 2001, Capítulos 1-4)?

(3) Quais são as conseqüências do alongamento das distâncias de dispersão de sementes proporcionada pelas formigas subseqüente a dispersão por vertebrados, independentemente do efeito proporcionado por uma eventual dispersão direcionada (Capítulos 3-4)?

(4) Como as características dos diásporos (além da abundância e distribuição espacial de recursos) influenciam a distância de dispersão das sementes pelas formigas (Capítulo 2)? Em especial para o cerrado, como a exploração de secreções açucaradas derivadas de plantas (via nectários extraflorais ou hemípteros) interfere nas interações entre formigas e diásporos caídos (Cuautle *et al.* 2005)?

(5) Como a dispersão direcionada de sementes e a dinâmica espaço-temporal dos ninhos de formigas influenciam a distribuição das plantas adultas (Hughes 1990, Capítulos 3-4)?

(6) A dispersão secundária de sementes por formigas é mais comum em clados filogeneticamente relacionados com a dispersão de sementes por aves (Capítulo 3)?

(7) As formigas podem amenizar o efeito da perda de vertebrados dispersores de sementes (devido à perda de hábitat e/ou caça) em áreas fragmentadas (Cordeiro & Howe 2003)?

(8) Qual a extensão de efeitos de borda no cerrado e qual o efeito de bordas nas interações formiga-planta em fragmentos de outros biomas (Guimarães & Cogni 2002, Capítulo 5)?

Estudos enfocando estes aspectos certamente irão proporcionar maiores subsídios aos padrões observados até o momento, ou permitirão elaborar hipóteses alternativas para melhor compreendermos a ecologia e evolução de sistemas complexos de dispersão de sementes nos trópicos. Além disso, o acúmulo de conhecimento derivado destes estudos poderá subsidiar melhores estratégias de conservação de espécies animais e vegetais, bem como das interações planta-animal importantes na manutenção da biodiversidade.

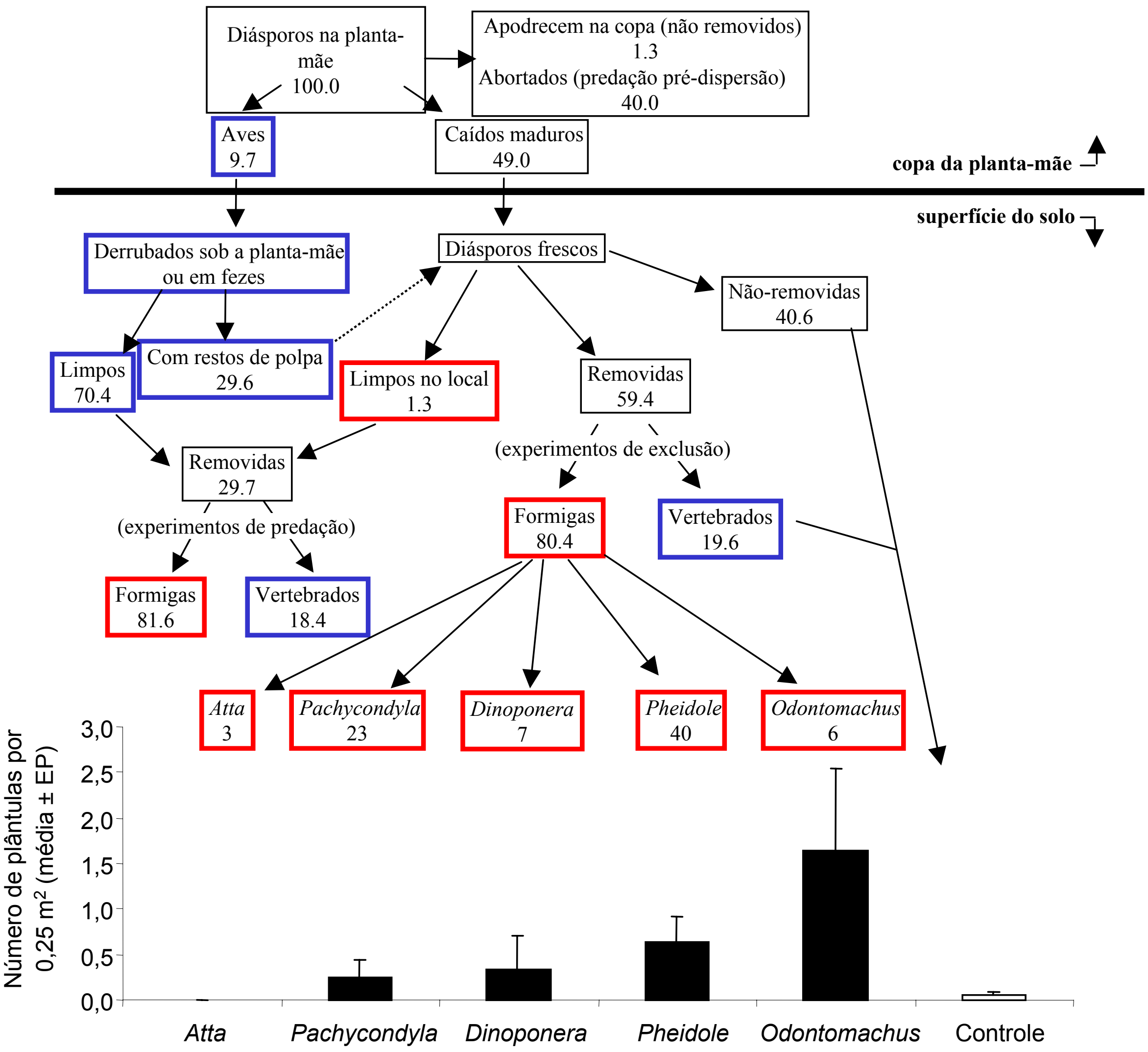

Figura 1: Diagrama esquemático da contribuição de vertebrados (caixas em azul) e formigas (caixas em vermelho) ao longo de diferentes etapas do ciclo de regeneração de *Erythroxylum pelleterianum* no cerrado até o estágio de plântula. Note que formigas interagem com diásporos apenas a partir do momento em que estes chegam ao solo. Apesar disso, plântulas estão mais associadas a ninhos de formigas do que áreas fora dos ninhos, embora a contribuição para este padrão varie de acordo com o gênero de formiga.

Figura 2

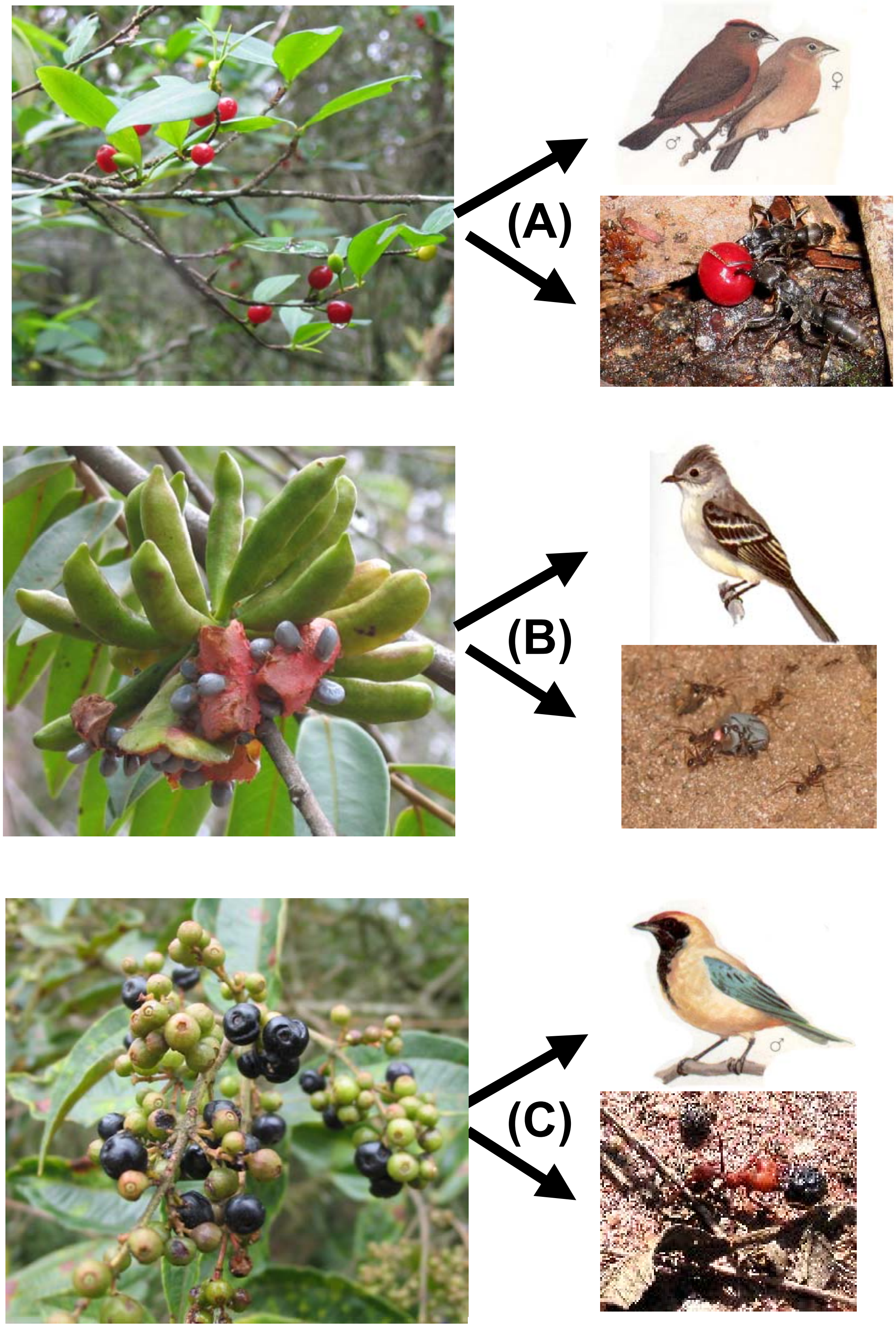

Figura 2: Exemplos de sistemas diplocóricos de dispersão de sementes no cerrado envolvendo aves e formigas com indicação das principais espécies envolvidas: (A) *Erythroxylum pelleterianum* (Erythroxylaceae) tem os diásporos removidos por 5 espécies de aves (em destaque *Coryphospingus cucullatus*) e 21 espécies de formigas (em destaque *Pachycondyla striata*); (B*) Xylopia aromatica* (Annonaceae) tem suas sementes removidas por 13 espécies de aves (em destaque *Elaenia flavogaster*) e 30 espécies de formigas (em destaque *Pheidole* sp.); (C) *Miconia rubiginosa* tem os diásporos removidos por 16 espécies de aves (em destaque *Tangara cayana*) e 13 espécies de formigas (em destaque *Atta sexdens*). Note a variação na morfologia e apresentação dos diásporos, bem como das aves e formigas que predominam nas interações (Capítulos 3 e 4).

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