Universidade Federal de Minas Gerais
Instituto de Ciências Biológicas
Departamento de Botânica
Programa de Pós-Graduação em Biologia Vegetal

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Ecofisiologia evolutiva de sementes de Melastomataceae de campos rupestres

Orientador: Prof. Dr. José Pires Lemos Filho
Co-orientador: Dr. Geraldo Wilson Afonso Fernandes

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Belo Horizonte – Minas Gerais

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Tese apresentada ao Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais como requisito parcial para a obtenção do título de Doutor em Biologia Vegetal.

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DEDICO ESTA TESE INTEGRALMENTE AOS MEUS PAIS

“What you do for yourself dies with you. What you do for others lives on forever”
Albert Pike

AGRADECIMENTOS (Oh, I get by with a little help from my friends!!!)

Jamais poderia imaginar que depois de tanta leitura, tanto delineamento experimental, trabalho de campo e laboratório, tanta tabulação de dados e análises estatísticas, a parte mais difícil da tese seria esta. Tenho razoável noção de delineamento experimental e estatística, conheço relativamente bem a biologia das plantas que estudei e a literatura acerca do tema investigado, mas tenho muito pouca idéia de como agradecer a cada um dos elementos que me ajudaram a construir esta tese. Não sei a quem devo agradecer primeiro, e vagueio ao tentar descobrir o porquê de toda minha gratidão. É altamente provável ($p< 0,001$) que tenha "me esquecido de lembrar" de alguém, e adianto minhas sinceras desculpas. Provavelmente meu esquecimento se deve ao "excesso de falta" de tempo para adiantar a defesa a tempo. Tentei ser breve e direto em meus agradecimentos, já que explicitar todas as razões duplicaria o tamanho deste documento.

À minha querida e amada família de natureza. Pai, Mãe, Juninho e Nanda! Vocês não acharam suficiente me amar e me apoiar em todos os momentos, como também acreditaram que era preciso por a mão na massa: foram ao campo, digitaram dados, discutiram idéias! Em especial à minha querida mãe que durante este percurso, venceu uma de suas maiores batalhas. A sua vitória me serve de inspiração!

Ao Prof. Pires, não apenas por sua orientação leve, sensata e provocativa, mas também pela sua serena forma de conduzir o trabalho. Pela sua agradável convivência e amizade, agradeço à sua atenção, dedicação e confiança a mim dispensadas. Você foi muito além do que o orientador desta tese, e se tornou um orientador e modelo para toda vida. Ao Prof. Geraldinho, agradeço pelos mais de 10 anos de convivência e por sempre me surpreender com um novo ponto de vista. Muito aprendi neste tempo em que estamos juntos.

A todo o corpo docente do Departamento de Botânica da UFMG. Vocês foram mais que professores e mestres! À Profa. Denise, coordenadora do curso, que me conquistou de imediato por todas suas qualidades e por ter me atendido com a maior presteza em tudo que me foi necessário. Ao Prof. Eduardo Borba, pelas suas sugestões iniciais no projeto de tese e pela seriedade e profissionalismo nas quais tento me espelhar. Em especial, à Profa. Rosy que apostou em mim há mais de 10 anos atrás. Um agradecimento especial ao Prof.

Evandro Gama (Centro Universitário UNA) por todos os ensinamentos em ocasiões formais e informais.

A todos que me ajudaram em trabalhos de campo e experimentos no laboratório. Destaco aqui a Débora Amarante, Mariana Ubaldino, Rosa Oliveira, Lívia Gilberti, Pedro Mafia e Luiza Fonseca. Em especial, agradeço aos meus braços direito e esquerdo, Natália Sales e Natália Robles. À Rafaella Ribeiro por me ancorar no solo quando os passos eram maiores que as pernas.

A todos os colegas da PG em Biologia Vegetal, do LEEB e do LFV. Sinto-me honrado de ter partilhado alguns anos da minha vida com vocês! A vocês, expresso minha admiração, carinho e agradecimentos por tantos momentos de convivência, alegria e aprendizado. Fica a saudade e nostalgia de deixar um ambiente tão amistoso. Obrigado Nara e Mari Augsten pelas sementes coletadas!

Aos meus amigos de toda vida. Por motivos de espaço, seria impossível mencionar a razão da minha gratidão a cada um de vocês. Cada nome, cada gesto e palavra estão marcados indelevelmente em mim.

À Camila, pelos anos de convivência ao meu lado e por ajudar a contar “estas sementinhas chatas”! Lesinha continua sendo uma pessoa especial e muito querida. À sua família, agradeço a acolhida.

À Luiza e sua família, por muita coisa. Lu, obrigado por ser minha força quando ela se esvaiu! Obrigado por me ouvir, me apoiar e me apresentar ao novo mundo da poesia. Sem seu companheirismo, parceria, lucidez e senso estético esta tese não seria o que foi.

Mientras estuve de estáncia en Madrid, me tocó aprender a comer, hablar y caminar otra vez, o sea volví a ser niño. Solo pudo convertirme en adulto porque había muy buena gente acercándome. Muchas gracias al Prof. Fernando Valladares por aceptarme en su grupo de investigación y dirigir parte de esta tesis doctoral. Sobre todo, te agradezco por tu solicitud y por invertir tiempo y atención a tu tocayo. Ha sido un honor y un gusto que tengas compartido conmigo ideas y experiencias. A todos mis compis del Laboratorio de Ecología Mediterránea (Consejo Superior de Investigación Científica): Prof. Mario Diaz, Teresa, Alicia, Anita, Elena G., Elena C., Elena B., Raquel, Jesús, David, Sonia, Dulce y por supesto, a Pillar, la profa de Taichi. Habéis sido más que compañeros, sino que amigos y maestros de vuestro eterno giri hippie. A Oscar Godoy e a Ruben Milla (Universidad Rey

Juan Carlos) por echarme una mano con los análisis filogenéticos y al Prof. Antonio Delgado (Estación Biológica de Zaidin) por recibirme y prestarme asistencia con lo de los isotopos. A mi familia madrileña: gracias a Davi Rossatto, Gui Fogaca, Maria de Andrés, Angelito, Felipe, Frida, João, Fábio y a mis tres hermanitas: Paula, Carol y Nati. Hoy me doy cuenta de cuánto he sido feliz y cuanto sois importantes para mí. Me lo he pasado genial no solo desde el punto de vista profesional sino que tambien personal. Tengo memorias maravillosas del tiempo en que viví en esta auténtica ciudad maravillosa. Gracias a todos los españoles que construyen diariamente una muy bonita y alentadora nación. “Nunca tan poco fue tanto”. Os echo mucho de menos.

Aos membros da Banca Examinadora da tese (Dr. Renato Goldenberg, Dra. Maíra Goulart, Dr. Fernando Vale e Dr. Marcel França) e da Qualificação (Dra. Cláudia Jacobi, Dr. Marco Pizo e Dr. Marcos Rodrigues) que se dispuseram a criticar o trabalho, complementando-o com importantes informações, correções, sugestões e *insights*. A todos meus co-autores e também aos colegas que se dispuseram a ler o primeiro artigo antes da submissão (Dra. Carol Baskin, Dr. Augusto Franco, Dr. Leandro Assis).

Aos professores Renato Goldenberg, Angela Martins e Rosana Romero pela identificação das espécies. Ao Daniel Vilela do CETAS/IBAMA por permitir a utilização das aves nos experimentos de frugivoria. Aos meus alunos e colegas professores, enquanto estive no Centro Universitário UNA e a equipe Biotrópicos (especialmente Quincas e Rafael) por compreenderem os momentos de sufoco e aperto.

Às agências de fomento CAPES e FAPEMIG pelas bolsas concedidas e ao CNPq pela bolsa sanduíche (201275/2010-4). Este projeto foi financiamento pelos processos CNPq (309633/2007-9, 304716/2008-1) e FAPEMIG (EDT465/07). A todos os brasileiros que pagaram com seu suor, os pesados impostos que foram revertidos no financiamento deste projeto e nas minhas bolsas. Espero retornar-lhes um pouco do que investiram em minha formação.

Às Melastomataceae de campos rupestres, cujas mais de 100.000 sementes possibilitaram a realização dos experimentos.

Embora esta tese seja uma construção coletiva, eu me responsabilizo integralmente por todos os erros. A todos vocês, declaro a minha dívida que não poderei pagar. Não seria exagero dizer que sem vocês esta tese não sairia do plano das idéias e tão pouco estarei sendo sincero ao dizer que esta é a minha tese. Por isto, alego: esta é a NOSSA tese de doutorado!

INTRODUÇÃO GERAL

Por que as espécies ocorrem no seu espaço atual? Por que e como algumas comunidades sustentam maior diversidade de espécies que outras? Qual o papel do clima, topografia e interações como fatores limitantes da distribuição de uma espécie? Estas ambiciosas e amplas perguntas determinam o foco de investigações científicas de ecólogos e biogeógrafos em todo o globo, em parte por que são altamente relevantes para a vida humana, em parte porque fascinam os cientistas há séculos. Há várias hipóteses propostas para explicar a distribuição dos organismos, mas até hoje nenhuma delas sozinha foi capaz de se sustentar de maneira satisfatória para se consolidar como uma teoria unificadora. Nesta tese, investigo os mecanismos fisiológicos que podem contribuir para explicar padrões de abundância, distribuição e coexistência de plantas em comunidades ricas em espécies. Aqui, utilizo a expressão "distribuição de plantas" para me referir à distribuição geográfica (escala global) - padrões que vão desde microendemismos até plantas amplamente distribuídas na América do Sul - e para referir-me à distribuição em microhabitats (escala local), que diz respeito à distribuição em sítios distintos em suas propriedades físicas e químicas em ambientes espacialmente heterogêneos.

A abordagem ecofisiológica (fusão entre a Ecologia e a Fisiologia) empregada aqui objetiva utilizar explicações fisiológicas para explicar os padrões ecológicos de organismos, espécies ou populações. Tal abordagem está longe de representar alguma novidade científica, uma vez que estudos em nível de comunidade foram realizados em diversos biomas do mundo a fim de correlacionar traços fisiológicos e a capacidade de colonização e sobrevivência a ambientes específicos. Entretanto, a maior parte dos estudos ecofisiológicos realizados ao nível de comunidade tem ignorado um importante aspecto que pode causar ruído nas análises: o efeito da filogenia (veja detalhes em Felsenstein 1985). Apenas recentemente no Brasil, o paradigma da integração da informação filogenética vem sendo considerado para a compreensão da evolução da forma e função de plantas (Hoffmann & Franco 2008). De fato, os estudos iniciais de C. Darwin em fisiologia de plantas foram conduzidos com o objetivo de testar a falsibilidade da descendência com modificação (Kutschera & Niklas 2009). Portanto, a abordagem evolutiva para o estudo da fisiologia vegetal deixou de ser uma novidade há mais de 150 anos. Dada a enorme lacuna (eu diria até negligência) na interpretação evolutiva de dados fisiológicos, Kutschera & Niklas (2009) chamam a fisiologia vegetal evolutiva da “síntese esquecida de Darwin”.

Obviamente, a ecofisiologia evolutiva moderna não é feita da mesma forma como nos tempos de C. Darwin. Atualmente, está disponível uma pletora de métodos estatísticos para analisr os resultados de estudos experimentais e as informações funcionais dos organismos são interpretadas sob a luz da perspectiva filogenética. Dentro desta agenda, as pesquisas têm como objetivo investigar seleção natural, restrições filogenéticas e evolução de traços ecofisiológicos (Ackerly et al. 2000). Atualmente, sabe-se há mais de 25 anos que utilizar testes estatísticos rotineiros como ANOVA não são suficientes para determinar como traços variam entre espécies, grupos funcionais ou mesmo comunidades vegetais. Métodos comparativos são, portanto, (algumas vezes obrigatoriamente) complementares às análises tradicionais. Somente através integração da informação histórica revelada pela filogenia, é possível inferir sobre evolução de traços ecofisiológicos.

Desta forma, utilizei uma abordagem baseada em métodos filogenéticos comparativos atuais para (1) corrigir o efeito da dependência filogenética, (2) mapear a evolução de caracteres ecofisiológicos ao longo da história evolutiva das plantas estudadas e (3) avaliar a evolução coordenada de caracteres ao longo da história evolutiva dos taxa. A incorporação da informação filogenética caracteriza um significativo avanço nas pesquisas ecofisiológicas realizadas no Brasil. A integração da informação filogenética possibilita encontrar padrões que muitas vezes estariam ocultos caso, tal informação não fosse incorporada (Hoffmann & Franco 2008) e podem revelar a inexistência de padrões funcionais que não poderiam ser detectados através de estatística convencional (Ackerly 2009a).

Nesta tese foquei em traços relacionados à biologia de sementes de Melastomataceae de campo rupestre (Figura 1). Estou convencido que a grande diversidade de espécies e microhabitats do campo rupestre representa um modelo ideal para estudos que integralizam a ecologia, filogenia e fisiologia. A variação nos atributos ecológicos, morfológicos e fisiológicos de Melastomataceae complementa de forma fascinante a heterogeneidade ambiental dos campos rupestres. Em minha pequena sapiência, desconheço uma família que apresente tamanha variação em história de vida, habitat, biogeografia e, sobretudo, compreenda elevada diversidade filogenética. Todos estes são aspectos-chave para entender a evolução da germinação e dormência. No meu ponto de vista, o casamento da Ecologia, Fisiologia e Filogenia só pode ser rivalizado pela elegante sintonia entre Melastomataceae e campo rupestre. Estou certo que os cientistas brasileiros têm em mãos um modelo biológico extraordinário que pode gerar informações valiosas para o entendimento do funcionamento da vida na Terra, bem como dados que possam ser efetivamente empregados na conservação da biodiversidade e restauração ecológica.

Esta tese foi escrita na forma de capítulos. No primeiro capítulo, fizemos uma revisão teórica sobre a ecofisiologia de sementes e plântulas de Melastomataceae neotropicais. Espero que esta revisão possa fornecer a base e dar suporte a outros estudos relacionados à biologia de plantas Neotropicais. O segundo capítulo trata dos efeitos da passagem pelo tubo digestivo na germinação de sementes de espécies ornitocóricas. O terceiro capítulo investiga a relação entre o nicho de regeneração (respostas germinativas a fatores ambientais) e a distribuição de plantas em escala local e global. O quarto descreve a evolução de dormência fisiológica e as pressões seletivas que favoreceram a evolução desta estratégia. No quinto capítulo, exploro as relações evolutivas e ecológicas da germinação de sementes em grupos funcionais através dos métodos comparativos filogenéticos. Finalmente, informações sobre o banco de sementes são apresentadas no sexto capítulo. Infelizmente, os dados de fenologia reprodutiva quantitativa, tão importantes para os demais capítulos, não puderam ser incluídos na tese e espero trabalhar neles em breve. Ainda há um longo caminho a ser trilhado e entendo que esta tese toca apenas na superfície do problema.

Uma conseqüência da formatação em capítulos é que a descrição da área de estudo, das espécies estudadas e alguns métodos foi repetida em cada capítulo. O atual formato digital de publicações permitiu a criação de arquivos suplementares que contém informações importantes, mas não essenciais para o entendimento do artigo. Para os capítulos que contém arquivos suplementares, estes documentos são apresentados individualmente, embora contenham dados repetidos em grande parte. Por motivos de economias de folhas (leia-se recursos naturais), todas as referências bibliográficas foram agrupadas em uma lista apresentada no final da tese. O primeiro capítulo foi submetido para o *Annals of the Missouri Botanical Garden*, o segundo está submetido ao *Plant Ecology and Evolution*, o terceiro foi submetido para o *Journal of Ecology* e o quarto está publicado no *Seed Science Research*. Para os dois trabalhos que ainda não foram enviados, temos em mente algumas idéias de revistas, mas neste documento todos os capítulos estão formatados de forma padronizada.

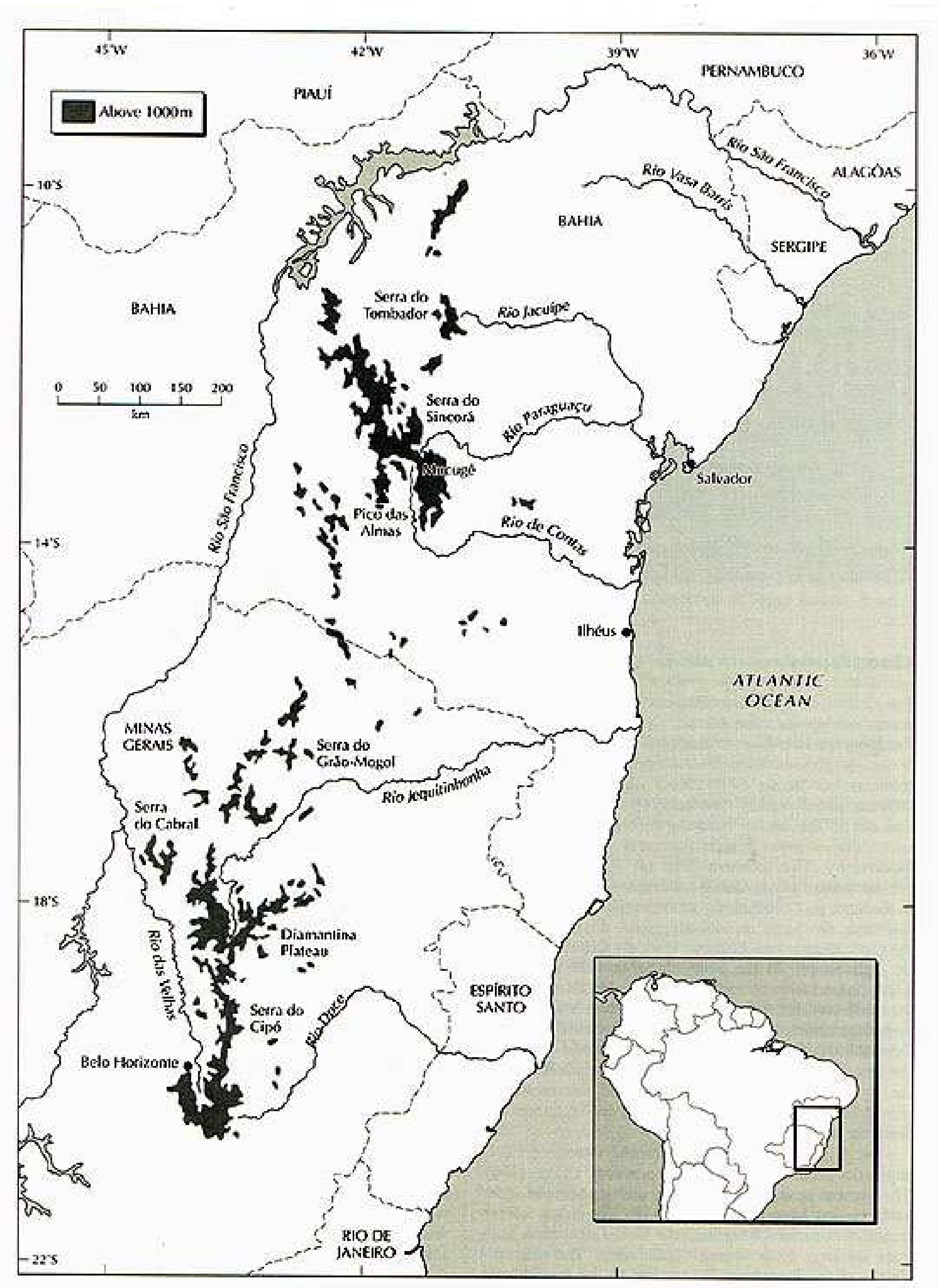

Figura 1. Distribuição dos campos rupestres ao longo da Cadeia do Espinhaço (http://botany.si.edu/projects/cpd/sa/map56.htm).

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RESUMO

Sementes apresentam um importante papel na ecologia e evolução de plantas. Os benefícios conferidos pelas sementes foram cruciais para o surgimento e dominância das espermatófitas no globo. Sementes são de grande importância para o sucesso reprodutivo e seus traços estão sob forte pressão seletiva, porque sem o estabelecimento de plântulas após a germinação, nenhum fenótipo pós-germinação pode ser expresso. Além disto, traços de sementes estão associados com a fecundidade, amplitude geográfica, segregação de habitat, abundância de adultos, persistência no solo, capacidade de dispersão e sobrevivência das plântulas. Neste estudo, nós empregamos métodos comparativos filogenéticos para investigar a evolução do tamanho da semente e traços germinativos nos campos rupestres. A heterogeneidade micro-espacial dos campos rupestres associada a uma diversidade de padrões biogeográficos de suas espécies permite explorar a associação entre traços de sementes e distribuição geográfica de plantas em escala global e local. Nosso foco foi em Melastomataceae porque esta família de plantas pioneiras compreende um dos clados mais diversos em termos funcionais e ecológicos de Angiospermas. No primeiro capítulo, nos revisamos a ecologia funcional das transições de ciclos de vida de Melastomataceae em dois hotspots, o Cerrado e a Mata Atlântica. Existe uma grande variação na história de vida de Melastomataceae no que diz respeito à fenologia e estabelecimento, mas não em termos de dispersão e germinação. Todas as espécies estudadas produzem sementes pequenas, fotoblásticas que se incorporam em bancos de sementes. Em relação à frugivoria, os frutos ricos em carboidratos e água são consumidos por uma ampla gama de frugívoros, principalmente aves. Embora seja frequentemente argumentado que as Melastomataceae vão se beneficiar com mudanças antrópicas, reduções na germinação e estabelecimento causadas por aumento de temperatura e freqüência e intensidade de fogo, provavelmente superarão os aumentos de densidade em bordas de florestas. Finalmente, as implicações para a ecologia da restauração são discutidas. No segundo capítulo, examinamos se a passagem pelo tubo digestivo de aves afeta a germinação de sementes de 8 espécies de Melastomataceae. A limpeza das sementes é um pré-requisito para a germinação, uma vez que a germinação foi nula ou menor que 4% dentro de frutos intactos. Efeitos mecânicos/químicos do tubo digestivo não foram significativos em sua maioria, e poucas respostas foram espécie-específicas. Nossos resultados sugerem que os variáveis efeitos

resultantes das complexas interações fruto-frugívoro potencialmente afetam o recrutamento de Melastomataceae no Cerrado. No terceiro capítulo, avaliamos o papel das sementes na distribuição geográfica e amplitude ecológica. Utilizamos diversas abordagens estatísticas para encontrar possíveis relações entre traços de sementes e a distribuição global (endêmicas vs. amplamente distribuídas) e local (microhabitats xéricos vs. mésicos). A germinabilidade e amplitude térmica variaram entre as espécies, mas não foram relacionadas ao endemismo. Desta forma, propomos que fatores relacionados ao estabelecimento de plântulas controlam a distribuição geográfica das espécies endêmicas. A amplitude térmica de plantas de ambientes mésicos foi maior que aquelas em ambientes xéricos, embora as plantas higrófilas estejam sujeitas a menor flutuação diária de temperatura. Diminuições na germinabilidade sob altas temperaturas sugerem fortes impactos negativos na germinação com aumento das temperaturas globais causadas por ações antrópicas. O quarto capítulo registra a evolução da dormência em Melastomataceae tropical. Estudos anatômicos e experimentos de germinação com sementes coletadas em diferentes locais e anos indicam a ocorrência de dormência fisiológica em quatro das 50 espécies estudadas. A posição filogenética das espécies dormentes sugere que a dormência evoluiu múltiplas vezes em espécies e populações dispersando sementes em ambientes xéricos durante períodos desfavoráveis para o estabelecimento. Assim, propomos que a dormência evoluiu como uma resposta à alta mortalidade induzida pela seca. No capítulo 5, investigamos a evolução do tamanho da semente e traços germinativos em 50 espécies dos campos rupestres. Correlações entre traços foram significativos para a germinabilidade e a sincronia de germinação, mas não para o tamanho da semente e requerimento de luz que foram considerados filogeneticamente conservados. Encontramos um padrão consistente de divergências entre Merianieae e Miconieae e dentro de Miconieae e um padrão consistente de convergências entre Melastomeae e Microlicieae e dentro de Microlicieae. Estes padrões indicam diferentes pressões seletivas governando a evolução das estratégias germinativas destes grupos e a evolução coordenada da germinação com o modo de dispersão e forma de vida. No último capítulo, avaliamos o comportamento de sementes de 18 espécies artificialmente enterradas em solos associados aos afloramentos rochosos e campos graminosos em intervalos regulares de seis meses por um período de até 18 meses. A dormência secundária e ciclos anuais de dormência são registrados pela primeira vez na

família. Sementes enterradas apresentaram alta longevidade, mas a emergência não dependeu do tipo de substrato ou condições desfavoráveis para o estabelecimento. Estes resultados sugerem que filtros ecológicos e o conservatismo de nicho controlam a manutenção da distribuição em manchas em habitats complexos. Os resultados desta tese contribuem para o entendimento da evolução da dormência e germinação de sementes nos neotrópicos e também ajudam a compreender o papel das sementes na determinação da distribuição de plantas em habitats complexos.

Palavras-chave: bancos de sementes, correlação de caracteres, dispersão de sementes, dormência fisiológica, ecofisiologia vegetal comparada, ecologia de sementes, frugivoria, germinação de sementes, métodos comparativos filogenéticos, nicho de regeneração.

ABSTRACT

Seed biology plays a central role in plant ecology and evolution. The multiple benefits provided by seeds to seed plants were crucial in their rise and dominance throughout the globe. Seeds are of primary significance for reproductive success and seed traits are under strong selective pressure because without successful establishment following germination, no postgermination phenotype can ever be expressed. Seed traits are associated with fecundity, range size, habitat segregation, adult plant abundance, seed persistence in the soil, dispersal ability and seedling survival. In this study, we implemented phylogenetic comparative methods to investigate the evolution of seed size and germination traits in Neotropical montane savannas (rupestrian fields). We take advantage of micro-environmental spatial heterogeneity and contrasting geographic distribution patterns in plant communities of rupestrian fields to explore the association of seed traits and plant distribution in both global and local scales. We focused on Melastomataceae because this pioneer-dominated family comprises one of the most functionally distinctive, ecologically diverse and species-rich clade of Angiosperms. In the first chapter, we reviewed the functional ecology of life cycle transitions in Melastomataceae in two Neotropical hotspots, namely the Brazilian Cerrado and the Atlantic Rain Forest. The life-history traits of Melastomataceae are very variable in terms of fruiting phenology and seedling establishment, but not in terms of dispersal ecology and seed germination. All examined

species produce small-sized, photoblastic seeds, dominant across Neotropical seed banks. With regards to frugivory, a taxonomic wide range of frugivores (mainly birds) consume the water- and sugar-rich berries. Although it is frequently argued that melastomes are expected to benefit from human-induced changes, decreases in germination and establishment caused by high temperatures and enhanced fire frequency are likely to override possible increases in plant density in forest edges. We discuss how seed- and seedling-based restoration techniques can help assist ecosystem recovery in restoration ecology. In the second chapter, we examined whether gut passage affects seed germination in ten melastome species. We compared the dispersal quality of seven bird species by contrasting seed germination of hand-extracted, gut-passed seeds and seeds within intact fruits. Seed cleaning is required prior to germination for all species, because germination within intact fruits was < 4% across species. Most mechanical/chemical action of guts on the seeds was non-significant and a few responses were species-specific. Our results suggest that the variable outcomes resulting from complex fruit-frugivore interactions potentially affect the recruitment of Cerrado melastomes. In the third chapter, we addressed the role of seeds in determining the geographic range and ecological breadth. We used a multitude of statistical approached in order to find whether seed germination requirements are associated with plant distribution in global (endemics vs. widespreads) and local scales (xeric vs. mesic sites). Germinability and temperature range greatly varied among species, but variation was not related to plant endemism. Therefore, we argued that factors controlling seedling establishment, rather than seed germination, constrain the geographic expansion of endemic species. Temperature ranges were higher for plants in mesic microhabitats compared to plants associated with xeric microhabitats, though hygrophilous experience lower daily soil temperature fluctuations. Decreased germination under high temperatures suggests major negative impacts during regeneration from seed as global air and soil temperature increases. In the fourth chapter, we provide the framework for the evolution of seed dormancy in tropical Melastomataceae. Anatomical analysis and germination experiments with seeds collected in different years and sites indicated the occurrence of physiological dormancy in four out of 50 species. Phylogenetic reconstruction methods allowed us to detect multiple evolution of physiological dormancy in species and populations dispersing seeds in xeric microhabitats during unfavourable

times for establishment. Therefore, drought-induced mortality may have been a strong selective pressure favouring the evolution of dormancy in Melastomataceae. In chapter 5, we have investigated the evolution of seed size and germination traits in 50 species in Neotropical montane savannas. Trait-trait correlations along the phylogenetic tree of Melastomataceae were significant for germinability and germination synchrony, but not for seed size or light requirement, since these two traits were phylogenetically conserved. We found a consistent pattern of divergences in germination traits between Merianieae and Miconieae and within the Miconieae whereas coordinated patterns of convergences in germination traits were found between Melastomeae and Microlicieae and within Microlicieae. These contrasting patterns indicate different selective pressures driving the evolution of seed strategies in different groups and coordinated evolution of dispersal mode and growth-form. In the last chapter, we have evaluated seed behaviour of 18 melastome species artificially buried in soils associated with rocky outcrops and grassy fields for regular intervals of six months up to 18 months. Secondary dormancy and annual emergence cycles are documented for the first time for the family. Buried seeds are long-lived but emergence was independent of microhabitats or favourable conditions for establishment, suggesting that ecological filtering and niche conservatism both operate in maintaining the patchy distribution in complex habitats. Overall, our data contribute to the understanding of the evolution of seed dormancy and germination in the Neotropical area and also shed light into the roles of seeds in determining plant distribution in complex habitats.

Key-words: comparative plant ecophysiology, frugivory, phylogenetic comparative methods, physiological dormancy, regeneration niche, seed banks, seed dispersal, seed germination, seed ecology, trait-trait correlation.

CAPÍTULO I

A REVIEW ON SEED AND SEEDLING ECOPHYSIOLOGY OF NEOTROPICAL MELASTOMATACEAE: IMPLICATIONS FOR CONSERVATION AND RESTORATION OF SAVANNAS AND RAIN FORESTS

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ARTIGO SUBMETIDO AO ANNALS OF THE MISSOURI BOTANICAL GARDEN

ABSTRACT

We review the ecophysiology of life cycle transitions (patterns of fruit maturation, dispersal, germination, seed bank formation and seedling establishment) of Melastomataceae in two Neotropical hotspots, the Brazilian Cerrado and the Atlantic Rain Forest. Studies on seed biology of Melastomataceae are relevant because this family is ubiquitous, species-rich and dominant in these two biodiversity hotspots and its increased relative importance is anticipated under the current scenario of habitat loss and forest fragmentation. The life-history traits of this pioneer-dominated family are very variable in terms of fruiting phenology and seedling establishment, but not for dispersal ecology and seed germination. The seed biology is used to infer on the predicted impacts of global change and forest fragmentation for forest and Cerrado melastomes. Species in Melastomataceae are key in restoration ecology and we discuss how seed- and seedling-based restoration techniques can assist ecosystem recovery.

RESUMEN

Revisamos la ecofisiología de transiciones de ciclos vitales (maturación de frutos, dispersión, germinación, banco de semillas y establecimiento de plantas) de Melastomataceae en dos biomas brasileños, el Cerrado y la Mata Atlántica. Estudios de semillas de Melastomataceae son importantes porque la familia presenta alta diversidad y es dominante en un gran rango de hábitats. Además, resulta que la familia tiene prevista su importancia aumentada en escenarios de fragmentación de bosques. Las características de historia de vida de esta familia de plantas pioneras es muy variable con respecto a la fenología reproductiva y establecimiento de plántulas, pero no con respecto a la ecología de dispersión de semillas y germinación. La biología de semillas embasa la discusión sobre los impactos de los cambios globales y fragmentación de bosques en Melastomataceae de sabanas y bosques tropicales. Especies de Melastomataceae son claves para la ecología de la restauración y discutimos como las técnicas de restauración basadas en semillas y plántulas pueden ayudar en la restauración. Los impactos de los cambios globales y fragmentación de hábitats en la biología de semillas de Melastomataceae son discutidos. Finalmente, son discutidas como los rasgos funcionales de las semillas de Melastomataceae pueden ayudar en la restauración de ecosistemas naturales.

Key words: forest fragmentation; global change; seed germination; seed bank; seed dispersal; seedling establishment.

Introduction

The patterns of seed germination have important ecological and evolutionary implications. At the population level patterns of seed germination have been shown to be determinant of regeneration strategies (Tomback et al., 2001), and to affect geographic distribution (Donohue et al., 2010), abundance (Howard & Goldber, 2001), and population dynamics (Garcia & Oliveira, 2007), ultimately leading to changes at the community level. Differences in germination timing have been shown to affect plant performance and fitness, modifying the phenotypic expression of important life-history traits (Verdú & Traveset, 2005; Donohue et al., 2010). The patterns and processes related to seed germination have also been used to explain intra-specific competition (Verdú & Traveset, 2005), coexistence of sympatric species (Daws et al., 2002), ecological breadth and geographic range (Donohue et al., 2010), hence highlighting the role of germination in structuring plant communities. Moreover, the knowledge of seed biology is amongst the key elements to understand processes such as plant establishment and natural succession (Vásquez-Yanes & Orozco-Segovia, 1993), providing a theoretical framework for restoration ecology.

In the face of increasing pressure on natural environments, seed biology has been recently attracting the interest of the scientific community because of its implications in restoration ecology. Here, we review the available data on seed development, dispersal, germination and seedling ecophysiology of Cerrado and Atlantic Rain Forest Melastomataceae. We also interpret the available data to build a comprehensive understanding on the selective pressures that drove the evolution of melastome seeds. Finally, we discuss how knowledge on seed and seedling ecology of melastomes can be applied to conservation and restoration practices.

THE CERRADO AND THE ATLANTIC FOREST

The Brazilian Cerrado and the Atlantic Forest have both experienced a considerable area loss due to human activities (Myers et al., 2000). The Cerrado biome covered nearly 2 million km^2, representing 22% of the land surface of Brazil, occupying an altitudinal range from near sea-level to 1,800 m (Oliveira-Filho & Ratter, 2002). The Cerrado developed on old, Al-rich, highly weathered, deep or shallow soils, mostly dystrophic, with low pH and Ca and Mg availability (Ratter et al., 1997). The climate is seasonal with dry winters from April-September and rainy summers (Oliveira-Filho & Ratter, 2002). The typical vegetation landscape of the Cerrado consists of a complex mosaic of savanna physiognomies on the well-drained interfluves with riparian forests following the watercourses. The physiognomies range from dense grassland, usually with a sparse covering of shrubs and small trees, to almost closed woodlands (Ratter et al., 1997). The woody layer is composed of small, gnarled trees coexisting with scattered shrubs whereas the herbaceous layer is made up of grasses, sedges and subshrubs with variations in continuity (Furley, 1999). The flora of both woody and ground layers evolved typical features of pyrophytic savanna vegetation. The trees are of a low contorted form with thick, corky, fire-resistant bark and sclerophyllous leaves and underground organs (Ratter et al., 1997; Oliveira-Filho & Ratter, 2002).

Soil erosion, land degradation, uncontrolled fires, the spread of exotic species, increasing cleared area for agriculture and pasture and charcoal production pose major threats to Cerrado biodiversity. Deforestation rates have been higher in the Cerrado than in the Amazon rainforest, whereas conservation efforts have been modest: only 2.2% of the Cerrado area is under legal protection (Klink & Machado, 2005).

The Atlantic Forest originally covered ca. 1 million km^2 extending throughout Brazilian coast. It is composed of two major vegetation types, the Atlantic Rain Forest which covers mostly the low to medium elevations along South America coastline and the Atlantic Semi-Deciduous Forest that extends across the plateau in the center and southeastern interior of the continent (Oliveira-Filho & Fontes, 2000; Morellato & Haddad, 2000). The orientation of the coastline and mountain ranges gives rise to orographic rains and a considerable winter rainfall. The result is an ever-wet rain forest climate with annual precipitation > 2000 mm. Relative humidity can be > 85% year-round and mean annual temperature is nearly 22ºC (Morellato et al., 2000).

The Atlantic Forest is constantly subjected to intense human pressures. Only a small portion of the original area (aprox. 11.4-16%) still remains in the form of small and isolated fragments (Ribeiro et al., 2009). Almost 60% of the Brazilian population lives within deforested areas of Atlantic Forest (Morellato & Haddad, 2000), which contributes to increasing levels of habitat loss and forest fragmentation.

THE CERRADO AND ATLANTIC FOREST MELASTOMES

Melastomataceae comprise shrubs, climbers, herbs, epiphytes or trees that occur from montane to lowland forests, savannas and disturbed vegetation (Clausing & Renner, 2001). This pantropical family comprises 4570 species in 150--166 genera arranged into nine tribes (Renner, 2004) and attains highest diversity in the Neotropics, with nearly 3000 species (Renner et al., 2001).

With nearly 521 taxa (Mendonça et al., 2008), the Melastomataceae is the fifhh most speciose family in the Cerrado. The diversity of melastomes in the Cerrado is high in both herbaceous (Filgueiras, 2002) and woody communities (Ratter et al., 1997). The family is one of the most important in terms of species number, occurrence, aerial cover (Filgueiras, 2002; Jacobi et al., 2007) and endemism (Romero & Martins, 2002). Representative taxa in the Cerrado include Miconieae, Microlicieae and Melastomeae. Miconieae is a monophyletic group characterized by small flowers with inferior or partly inferior ovaries that develop into baccate fruits (Michelangeli et al., 2008), and it is the most species-rich tribe. Nearly 90% of the 275--300 species of Microlicieae are endemic to the Cerrado, where it originated (Fritsch et al., 2004). Most threatened species belong to endemic Microlicieae (Mendonça & Lins, 2000).

Melastomataceae is the second most important family in southeastern Atlantic Rain Forest and the fifth one in the Atlantic Semi-Deciduous Forest. *Miconia* stands out as the second most speciose genus in both physiognomies. *Tibouchina* is also important, with 28 species in Atlantic rain forest and 14 in semi-deciduous forests (Oliveira-Filho & Fontes, 2000). The family is diverse in both lowland and hinterland montane forest enclaves, i.e. the “brejos de altitude” in northeastern Atlantic Forest (Cavalcanti & Tabarelli, 2004). The Microlicieae is species-poor in the Atlantic Forest. Understorey gap-specialists shrubs and shade-tolerant trees account for the highest diversity.

FROM FLOWER TO FRUIT

The pollination biology and breeding system of melastomes are very diverse and have been thoughtfully reviewed by Renner (1989). Miconieae shows a disproportional occurrence of apomictic species (Goldenberg & Shepherd, 1998; Goldenberg & Varassin, 2001), and seeds obtained from apomictic species are able to germinate normally. Polyembryony is reported for *Miconia rubiginosa* (Bonpl.) DC. (Goldenberg & Shepherd, 1998).

Fruit and seed maturation studies provided the initial basis for understanding germination ecology. The heterogeneity of melastome capsules and berries suggests that both structures may have evolved independently several times within the family. Fruit character plasticity in Melastomataceae must relate to shifts in seed dispersal mechanisms, which in turn relate to the type of habitat and of dispersal agent. Thus, changes from fleshy berries to dry capsules may correlate with colonization of more open habitats where wind dispersal may be less costly than animal dispersal or where frugivorous understory dispersers may be less abundant. Alternatively, capsular species may undergo selection for increased exocarp and placenta fleshiness upon entering more closed forest habitats where vertebrate dispersal predominates (Clausing et al., 2000). In general, animal-dispersed melastomes are most diverse in lowlands and montane forests, whereas capsular-fruited tribes are richer in savannas (Renner, 1989; Stiles & Rosseli, 1993).

The extent of berry maturation greatly varies among melastomes, with most species showing irregular fruit maturation within the canopy and inflorescence (Pereira & Mantovani, 2001). The period of fruit and seed maturation is 100-220 days after anthesis (DAA) for fleshy-fruited species (Carreira, 2004). Germination inhibitors occur in unripe and ripe fruits of *Miconia cinnamomifolia* (DC.) Naudin (Amaral & Paulilo, 1992), *Miconia albicans* (Sw.) Steud. and *M. rubiginosa* (Carreira, 2004), thus requiring seed cleaning by frugivores prior to germination (see Dispersal Ecology section). Capsule maturation period is more variable compared to berries. In *Tibouchina mutabilis* (Vell.) Cogn., 10% of dry seeds are viable 14 DAA, and a high percentage of dehydrated and viable seeds is observed 21 DAA (Simão et al., 2007). However, for *Tibouchina granulosa* (Desr.) Cogn., mature seeds can be harvested only between 84-105 DAA (Lopes et al., 2005).

In fleshy-fruited species fruit growth precedes color change (Pereira & Mantovani, 2001; Carreira, 2004; Lopes & Soares, 2006; Cortez & Carmello-Guerreiro, 2008). As fruits mature, fruit tissues remain hydrated (from 70 to 80% of water content) and accumulate sugars (Maruyama et al., 2007). At this stage, germination is prevented due to the high-water content of immature seeds (Pereira & Mantovani, 2001; Lopes & Soares, 2006). Seed water content at maturity is 27% for *M. albicans* (Carreira, 2004), and 23% for *M. cinnamomifolia* (Lopes & Soares, 2006) and *T. granulosa* (Lopes et al., 2005). Recalcitrance has been reported for *Melastoma malabathrichm* L. from tropical swamp forests (Farnsworth, 2000). The desiccation-tolerant post-harvest behavior of melastome seeds contributes to the formation of persistent seed banks (see below).

Melastome seeds are small to minute in size and lack endosperm (Baumgratz, 1985). Most seeds are typically between 0.4--2mm, but in some genera seeds are often smaller and few larger. Seed mass is generally < 0.01 g. There are six morphologically different seed types: cochleate, obpyramidal, alate, ovate-oblong-obovate, orbicular plano-convex, and clavate. Fully developed embryos are differentiated in cotyledons and hypocotyl (Baumgratz, 1985) and are often bent, folded, investing, linear and spatulate (Baskin & Baskin, 2007). Melastome seeds have full embryos that completely fill the seed with a high embryo:seed ratio (Forbis et al., 2002).

Malformed and well-developed but embryoless seeds (WELS) are a commonplace in the family (Baumgratz, 1985). Quantitative data are lacking perhaps due to the difficulty in evaluating viability in small-seeded species. High levels of WELS are recorded across lineages of melastomes including *T. mutabilis* (60 to 80% of WELS; Simão & Takaki, 2008), *Tibouchina pulchra* Cogn. and *T. granulosa* (70 to 80%; Zaia & Takaki, 1998), *Trembleya laniflora* (D. Don) Cogn. (86.5%; Rodrigues & Silveira, in press.), *Miconia ferruginata* DC. (79.7%; Mendes-Rodrigues et al., 2010), and *Marcetia* (ranging from 2.4% in *M. candolleana* to 54.4% in *M. harleyi* Wurdack; Vasconcelos et al., 2007). High levels of WELS can potentially lead to erroneous conclusions on the occurrence of seed dormancy (Lopes et al., 2005, Mendes-Rodrigues et al., 2010).

FROM THE MOTHERPLANT TO SOIL

Seed dispersal is strongly influenced by life-history traits and fruit morphology. Melastome fruits are structurally diverse and dispersal mode varies as a function of fruit morphology (Clausing et al., 2000). Species in Miconieae are animal-dispersed, whereas Melastomeae and Microlicieae are abiotically dispersed. Nearly 40% of Neotropical melastomes have capsular fruits while 60% have endozoochorous berries (Renner, 1989). Apart from differences in fruit morphology, melastomes are also diverse in reproductive phenology.

FRUITING PHENOLOGY

Fruiting phenology of Atlantic Forest trees is aseasonal (Morellato et al., 2000). In the lowland Atlantic Forest, *Leandra* aff. *sublanata* (Gridi-Papp et al., 2004), *Miconia dodecandra* Cogn. and *Tibouchina pulchra* Cogn. fruit year-round, whereas *Miconia prasina* (Sw.) DC., *Miconia rigidiuscula* Cogn. (Talora & Morellato, 2000), *M. cinnamomifolia* (Mantovani et al., 2003), *Miconia cinerascens* Miq. (Gridi-Papp et al., 2004) and *Miconia hypoleuca* (Benth.) Triana (Galetti & Stotz, 1996) fruit during the dry season. *Miconia cabucu* (Mantovani et al., 2003) and *M. urophylla* DC. (Manhães et al., 2003) in turn, fruit during the rainy season.

The aseasonal fruiting phenology of melastomes in the Atlantic Rain Forest suggests that climatic factors do not limit fruit production in forest tress. Rather, competition for frugivores seems to be a stronger selective pressure and in both seasonal and aseasonal forests and fleshy fruits are available year-round for frugivores (Morellato et al., 2000). Snow (1965) proposed that displaced fruiting seasons in species of *Miconia* that share the same dispersers have evolved as a response to competition for seed dispersers. Melastome berries are regarded as a key resource for Neotropical frugivorous birds for providing them with water and sugars-based resources when they are limited (Stiles & Rosselli, 1993; Galetti & Stotz, 1996). Fruiting phenologies of forest *Miconia* from other sites have also been reported to be segregated in time (Poulin et al., 1999), providing support for the hypothesis that segregated fruiting evolved through interspecific competition (Snow, 1965).

At the Cerrado, however, seedling establishment is assumed to be constrained during the dry winters and therefore, the time and duration of reproductive phenophases should have been selected to adjust for dispersal during the onset of the rainy season. Thus, the synchronization of seed dispersal near or during the onset of the rainy season (the period of high soil moisture) is assumed to increase seedling survival and avoid drought-induced mortality (Oliveira, 2008). As a consequence, dry-fruited species from Cerrado shed seeds during the dry-wet transition and are expected to produce non-dormant seeds that promptly germinate at the beginning of the rainy season (in October). In contrast, zoochorous species disperse fruits during the mid- or late rainy season and are expected to have evolved dormancy that would prevent germination during the dry season. Species with dormant seeds are expected to synchronize germination with the onset of the following rainy season (Oliveira, 2008).

The greater seasonality in the Cerrado may have selected for more adjusted fruiting phenologies when compared to the more aseasonal forest species. However, the few available data on melastome phenology from the Cerrado prevents testing the Oliveira’s (2008) predictions. Data from scattered studies provide partial support to Oliveira’s hypothesis (Antunes & Ribeiro, 1999; Lopes et al., 2005; Montoro & Santos, 2007; Simão et al., 2007; Carreira, 2004; Garcia et al., 2009), but shedding light on how biotic and abiotic pressures drove melastome phenology can only be achieved by conducting comparative studies designed to include a larger array of sympatric species. The current data suggest that competition for frugivores rather than abiotic factors have shaped fruiting phenology of fleshy-fruited taxa. In contrast, the requirement of low air moisture to promote opening of dry dehiscent capsules coupled with improved seedling establishment during wet season may have been more important for capsular-fruited taxa.

DISPERSAL ECOLOGY

Data on abiotic dispersal of capsular fruits are scarce and focus will be given on biotic dispersal. Even though most capsular-fruited melastomes are thought to be wind-dispersed, rain is necessary to release the seeds from the capsules of *Bertolonia mosenii* Cogn. and probably other Bertolonieae (Renner, 1989; Pizo & Morellato, 2002). The distance of the anemochoric dispersal for melastomes is currently unclear. The striking disjunct distributions of populations of wind-dispersed species (e.g. *Marcetia taxifolia* (A. St.-Hil.) DC.)) could attest to good dispersability (Renner, 1989). Mature berries from riparian *Tococa* species that fall to the water and float into the forest could represent another example of abiotic dispersal. These fruits are water-dispersed and pubescent seeds could be an adaptation to water and/or fish dispersal (Michelangeli, 2005).

Fleshy berries of Miconieae (hereafter berries) usually have fleshy placentas and fused tissues or carpels and other accessory structures (Cortez & Carmello-Guerreiro, 2008) with high water- and sugar-content in which numerous and small seeds are embedded (Maruyama et al., 2007). Small berries are of outstanding importance for small frugivorous and omnivorous birds (Renner, 1989; Michelangeli, 2005). Berry size ranges from 5--35 mm in diameter (Renner, 1989). Melastome berries fall into the category of generalist fruits, which is typically characterized by high fecundity, small-sized seeds and low nutritional value (Mckey, 1975). The community of frugivores exploiting melastome berries encompasses birds in several families which provide them with low dispersal quantity (Mckey, 1975; Schupp et al., 2010).

In Costa Rica and Amazonian forest, Tyrannidae (fly-catchers), Pipridae (manakins), Turdidae (thrushes) and Thraupidae (tanagers) represent the major frugivores (Loiselle & Blake, 1999). The spectrum of bird families consuming fruits in the Atlantic Forest and Cerrado is wide; these four families comprise more than 84% of the frugivores (Silveira et al., in prep.). However, Pipridae is species-poor in the Cerrado (Macedo, 2002). Large birds such as Cracidae, Columbidae and Ramphastidae (Ragusa-Netto, 2008) play a minor role in fruit removal. Interestingly, fruits of *Mouriri elliptica* Mart. (formerly Memecylaceae) are thought to be anachronic (unfit fruits with apparent exadaptations to the extinct South American megafaunal community of frugivores; Guimarães et al., 2008).

Compared to pollination ecology, bird-fruit interactions are less specific and their coevolution is nearly always diffuse (Stiles & Rosseli, 1993). If competition for frugivores has significantly shaped Miconieae phenology determining niche differentiation in time, we should expect some degree in diet overlap among frugivores. Studies addressing the basis of fruit choice among birds suggest that both fruit and bird traits affect bird decision (Griddi-Papp et al., 2004).

In Costa Rica rainforests, understory melastome shrubs have a relatively small set of seed dispersers in contrast to melastome canopy trees. The set of effective dispersers is essentially composed of two or three species and plant-bird interactions may be much more specific in forest understory than in species-rich canopies (Loiselle & Blake, 1999). At these sites, there is a significantly greater niche overlap in the diet of two manakins (Loiselle et al., 2007). Manakins and tanagers are common dispersers in the Neotropics, but Brazilian melastome shrubs are visited by a larger assemblage of frugivores and plants are not particularly associated with a particular set of frugivores (Galleti & Stotz, 1996; Pereira & Mantovani, 2001; Marcondes-Machado, 2002; Manhães et al., 2003; Michelangeli 2005).

The low values of bird-melastome dependence (Stiles & Roselli, 1993) for tropical forests reflect either the relative rarity of birds or a low preference for the typically low energy and nutrient content melastome fruits. Few species are highly dependent upon melastomes. In a lowland montane forest, one *Miconia* and one *Leandra* share only two frugivores (Griddi-Papp et al., 2004). In the Amazon Forest, tanagers and manakins show low fruit removal (Blendinger et al., 2008), suggesting they are not food-limited and are not likely to engage interespecific competition. A broad spectrum of bird species consuming melastome berries would intuitively suggest an overall high overlap in fruit consumption. However, the low dispersal quality (low removal rates) may offset diet overlap and decrease competition probabilities. If competition is or has been a significant force in determining the phenology of melastomes remains uncertain.

Effects of frugivores on seeds and seedlings

Fruit consumption by frugivores does not necessarily mean that seeds are dispersed because ingested seeds can be negatively impacted by gut passage. Therefore, experimental evidence is needed to determine the effects of gut passage on seed germination and seedling establishment.

The effect of dispersal can be measured in quantitative and qualitative components. Dispersal quantity refers to the number of visits and number of seeds removed/visit whereas dispersal quality refers to the condition of seeds following seed handling and probability of seeds surviving to later stages (Schupp et al., 2010). The outcome of fruit-frugivore interaction depends on intrinsic bird and berry traits. Birds are not equivalent in their ecological roles as seed dispersers for Melastome shrubs because they differ in both dispersal quantity and quality (Loiselle & Blake 1999). Removal rates are typically low by manakins and tanagers (Blendinger et al., 2008), but they differ in feeding ecology, as the formers are gulpers, eating the whole fruit (high dispersal quantity) and the latter act as mashers and drop seeds near the parent plant (low dispersal quantity; Fig. 1A, B) where survival is reduced due to density-dependent mortality (Stiles & Roselli, 1993).

Seeds can benefit from frugivores through numerous mechanisms. Firstly, seed cleaning by frugivores improve dispersal quality through the de-inhibition effect. The inhibition effect arises from high osmotic pressure caused by the high sugar content of ripe fruits, by light-blocking pigmentation that prevents enough light reaching the seeds and from secondary metabolites that directly inhibit seed germination (Samuels & Levey, 2005). Germination inhibitors in fruits of several *Miconia* species (Amaral & Paulillo, 1992; Carreira, 2004) suggest the widespread occurrence of these compounds in the family. The pulp removal and consequent release of the seeds from this kind of inhibition are provided by gulpers and to a less extent by mashers. Seeds concealed in intact fruits only rarely germinate (Silveira et al., in prep.; Fig. 1F), making seed cleaning a vital step for recruitment. Finally, the removal of the fruit pulp also decreases the probability of pathogen-induced seed death.

Secondly, seed passage through bird guts differently affects germinability (final germination) and germination speed according to the structure of seed coat. Also, seed retention time inside the disperser, the morphology and physiology of the digestive tract all affect the survival probability of the ingested seeds in different ways (Traveset el al., 2007). There are reports of intact seeds of *Tococa* following gut passage of fishes (Michelangeli, 2005) and coatis (*Nasua nasua*) (Alves-Costa & Eterovick, 2007), but effects on recruitment have not been properly evaluated because no germination experiments have not been conducted. The studies of gut passage effects of mammal dispersers on melastome seeds were done with marsupials and rodents. Germinability of seeds from scats was not affected by rodents (Lessa & Costa, 2009), but it was significantly improved after marsupial gut passage (Lessa & Costa, 2010).

In contrast to mammals, more information is available for the effects of avian gut passage on melastome seed germination. For Costa Rican understory melastomes Elisson et al., (1993) have found divergent results for the effects of fruit ingestion by the red-capped manakin, *Pipra mentalis*. The effects of gut passage ranged from positive to negative for germination of *Clidemia*, *Miconia* and *Conostegia* species. For the Cerrado, seed passage through the guts of seven bird species had minor effects on germinability of *Clidemia* and *Miconia* species (Silveira et al., in prep.).

The seeds of *Clidemia hirta* (L.) D. Don are argued to be dormant and require bird ingestion for dormancy break (Pereira-Diniz, 2003). For this species, there is a positive effect of gut passage on germination (Linnebjerg et al., 2009), but data do not support the hypothesis that its seeds are dormant. Our survey indicates that to date no Brazilian melastome is dormant (see Seed Dormancy section), but we still need data concerning the effects of frugivores on seed coat scarification. Overall for forest species, seed passage through bird gut has minor effects on germination (Manhães et al., 2003; Alves et al., 2007; Gomes et al., 2008). Even though birds do not increase germinability or germination speed of Neotropical species, fleshy-fruited melastomes clearly benefit from bird dispersal through seed cleaning and are likely to escape of density-dependent mortality and mortality .

Other primary and secondary dispersers

Although birds are the main dispersers of melastomes berries, mammals also play an important role in primary dispersal. Whilst most Miconieae produce small red or blue berries, a few have much larger yellow ones adapted for dispersal by bats or frugivorous monkeys (Renner, 1989; Galleti & Stotz, 1996; De Figueiredo & Longatii, 1997; Garcia et al., 2000; Lapenta & Procópio-de-Oliveira, 2008), rodents, marsupials (Magnusson & Sanaiotti, 1987; Lessa & Costa, 2009, 2010) and coatis (Alves-Costa & Eterovick, 2007). Bats and monkeys are not amongst the more important consumers perhaps due to the predominant understory habitat of Melastomataceae and preference of monkeys for acidic fruits (Renner, 1989).

A large part of the fruit produced by Miconieae is not removed by primary dispersers. Fallen fruits to the ground are eaten by turtles, lizards, tapirs (Renner, 1989) and armadillos (Baumgratz et al., 2006). Nevertheless, ants are the most important seed consumers of fallen fruits (Fig. 1D, 1E). Ants in several subfamilies actively forage on soil in search for small-sized diaspores (Rico-Gray & Oliveira, 2007) or even climb in the plants cutting fruit stalks (Fig. 1C). Attini ants directly disperse *Miconia* fruits in the

Cerrado (Christianini et al., 2007; Christianini & Oliveira 2009; Lima et al., in prep.) and also remove seeds from bird feces (Leal & Oliveira, 1998; Christianini & Oliveira, 2009). Interactions between ants and fallen melastome fruits may be especially common in lowland rain forests in which the abundance of ants is coupled with the year-round availability of fleshy fruits (Galetti & Stotz, 1996; Dalling & Wirth, 1998; Pizo & Oliveira, 2000; Pereira & Mantovani, 2001).

Potential benefits to nonmyrmecochorus melastomes include seed cleaning, increased germination success, reduced damaged to seeds and seedlings and reduced sibling competition (Rico-Gray & Oliveira, 2007). As seeds within fallen fruits are unlikely to germinate (Fig. 1F), pulp removal and subsequent reduction in fungal infestation (Samuels & Levey, 2005; Rico-Gray & Oliveira, 2007) maybe an important benefit emerging from ant-melastome interaction. Fruit displacement may also increase the chances of seeds reaching safe sites and seed deposition in the refuse piles may provide seedlings with a suitable soil for establishment (Christianini et al., 2007). Since seed delivery patterns by ants may differ from that of birds, ants may have a strong potential in the local population dynamics and spatial distribution of nonmyrmecochorus melastomes (Christianini & Oliveira, 2009).

FROM SEED TO SEEDLING

EFFECTS OF LIGHT AND TEMPERATURE

Light and temperature are among the most important abiotic factors controlling germination (Heschel et al., 2007). The physiological mechanisms employed by seeds to detect changes in light and temperature and how these factors control germination have been comprehensively discussed elsewhere. In this review, we focus on the light and temperature requirements of melastome seeds and the implications of seed responses to these abiotic stimuli to understand the ecology of those species.

Despite the low number of species reviewed (29 species in nine genera), general pattern for the Melastomataceae germination arises. Under controlled conditions, seeds of all studied melastomes are positively photoblastic. Germination under dark conditions was null or insignificant irrespective of phylogenetic position, geographic distribution, or temperature (Table 1).

Table 1. The effects of light and temperature on germination of Melastomataceae seeds from Brazilian Cerrado and Atlantic Forest.

Biome	Tribe	Species	Maximum G(%)[a]	Temperature range	Optimum temperature[b]	Light response[c]	Dormancy type[d]	Reference
CE	Melastomeae	*Marcetia taxifolia*	51	15-30	15-25	L > D	ND	Silveira et al. 2004
CE	Microlicieae	*Lavoisiera cordata*	~85	15-30	20-30	L > D	ND	Ranieri et al. 2003
CE	Microlicieae	*Lavoisiera francavillana*	~35	15-30	25	L > D	ND	Ranieri et al. 2003
CE	Microlicieae	*Trembleya laniflora*	16,5	15-30	20	L > D	ND	Rodrigues & Silveira, in press
CE	Melastomeae	*Tibouchina multiflora*	73	15-30	25-30	L > D	ND	Garcia et al. 2006
CE	Melastomeae	*Tibouchina papyrus*	70*	NA	NA	NA	ND	Montoro & Santos 2007
CE	Miconieae	*Miconia albicans*	~80	15-35	20-30	L > D	ND	Carreira & Zaidan 2007
CE	Miconieae	*Miconia stenostachya*	~65	15-35	20-30	L > D	ND	Carreira & Zaidan 2007
CE	Miconieae	*Miconia langsdorfii*	~65	15-30	25	L > D	ND	Carreira & Zaidan 2007
CE	Miconieae	*Miconia rubiginosa*	~30	20-35	25	L > D	D*	Carreira & Zaidan 2007
CE	Melastomeae	*Heterocentron elegans*	~85	20-30	30	L > D	ND	Carreira & Zaidan 2007
CE	Melastomeae	*Tibouchina gracilis*	~75	15-35	25-30	L > D	ND	Carreira & Zaidan 2007
CE	Miconieae	*Miconia theazans*	98	15-32.5	15-32.5	L > D	ND	Godoi & Takaki 2007
CE	Melastomeae	*Marcetia macrophylla*	1	NA	NA	NC	D*	Vasconcelos et al. 2007
CE	Melastomeae	*Marcetia shepherdii*	2	NA	NA	NC	D*	Vasconcelos et al. 2007
CE	Melastomeae	*Marcetia harley*	2	NA	NA	NC	D*	Vasconcelos et al. 2007
CE	Melastomeae	*Marcetia candolleana*	2	NA	NA	NC	D*	Vasconcelos et al. 2007

CE	Miconieae	*Clidemia hirta*	96	NA	NA	L > D	D*	Pereira-Diniz 2003
CE	Miconieae	*Miconia ferruginata*	59.5	NA	NA	L > D	D*	Mendes-Rodrigues et al. 2010
AF	Miconieae	*Miconia cinnamomifolia*	58.7	NA	25	L > D	ND	Pereira & Mantovani 2001
AF	Miconieae	*Miconia cinnamomifolia*	~60	20-30	25-30	L > D	ND	Amaral & Paulilo 1992
AF	Melastomeae	*Tibouchina mutabilis*	~80	15-35	25-30	L > D	ND	Simão & Takaki 2008
AF	Melastomeae	*Tibouchina pulchra*	30.9	NA	NA	L > D	ND	Zaia & Takaki 1998
AF	Melastomeae	*Tibouchina granulosa*	24.9	NA	NA	L > D	ND	Zaia & Takaki 1998
AF	Melastomeae	*Tibouchina granulosa*	17	NA	NA	L > D	ND	Lopes et al. 2005
AF	Melastomeae	*Tibouchina moricandiana*	~70	20-30	25	L > D	ND	Andrade 1995
AF	Melastomeae	*Tibouchina benthamiana*	~70	20-30	30	L > D	ND	Andrade 1995
AF	Melastomeae	*Tibouchina grandifolia*	~60	15-35	25-30	L > D	ND	Andrade 1995
AF	Miconieae	*Leandra brevifolia*	78	NA	NA	L > D	ND	Andrade 1995
AF	Melastomeae	*Tibouchina sellowiana*	NA	NA	30	L > D	ND	Barbosa et al. 1988
AF	Miconieae	*Miconia urophylla*	~60	NA	NA	L > D	ND	Manhães et al. 2003
AF	Miconieae	*Miconia chamissois*	90	NA	NA	L > D	ND	Valio & Scarpa 2001

a- percentages were not correct for viability; b- defined as the temperature that allowed maximum germinability in less time; c- L, light and D, dark; d- ND, nondormant; D, dormant. NC – non-conclusive, NA – data not available. * indicates that dormancy is plausible but data are insufficient to confirm and determine dormancy type.

Extreme temperatures seem to be deleterious for melastome germination. Only 33% of Cerrado and 40% of forest species germinate above 30ºC (Table 1). Optimum temperature is between 20 and 30ºC for most species. Widespread species (*M. albicans*, *Miconia stenostachya* DC. and *Miconia theazans* Cogn. in Cerrado and *T. mutabilis* and *Tibouchina grandifolia* Cogn. in Atlantic forest) are eurythermic (germinate over a wide temperature range). But contrary to predictions of the niche breadth hypothesis (Donohue et al., 2010), endemic species (*T. laniflora*, *Lavoisiera cordata* Cogn. ex Glaz. and *Lavoisiera francavillana* Cogn.) are not stenothermic (with a narrow temperature range; Table 1). Therefore, the available data do not support the hypothesis that predicts a relationship between germination and melastome geographical distribution.

The photoblastic response of melastome seeds is typical of small-seeded Neotropical pioneer species, which produce seeds capable of germinating in forest gaps (Pearson et al., 2002, 2003; Daws et al., 2008). The control of seed germination by red and far-red light is an important phytochrome-mediated process. Seeds of Melastomataceae have Phytochrome B (phyB) controlling germination through low-fluence responses. PhyB is involved in the perception of red/far-red ratios associated with the presence of canopy gaps (Casal & Sanchez, 1998) and controls germination across a broad range of temperatures (Heschel et al., 2007). In the Atlantic Forest, the red to far-red irradiance (R:FR) ratio significantly drops from 1.21 in open areas to 0.28 under closed canopy (Zaia & Takaki, 1999). The low R:FR under closed canopy prevents germination, and photoblastic seeds accumulate in the soil seed banks until canopy opening. The R:FR threshold for melastome seeds can be as low as 0.12 for *Miconia argentea* (Sw.) DC. (Pearson et al., 2003). Not only light quality is important for germination, but also light quantity is of major importance. Overall, melastome seeds required more than 60 daily minutes of irradiation to germinate (Carreira & Zaidan, 2007). The lack of response to lower irradiance time may have evolved to prevent germination induced by sunflecks and consequently establishment under unfavorable conditions for growth and survival.

An overlooked question regarding seed germination is “why some seeds do not germinate”. Although non-germinated seeds could provide interesting insights into seed biology, they are seldom studied. There are three reasons for lack of germination: 1) physical conditions are not suitable; 2) high frequency of WELS or nonviable seeds; and 3) seed dormancy. Lack of germination when seeds are dispersed to optimum environmental conditions for germination, leaves open the two last possibilities. Disentagling WELS and nonviable seeds can be confounding when data on embryo morphology are lacking. If we assume that seed viability of surveyed species (Table 1) equals maximum germinability, viability of Miconieae (72.1 ± 6.54; n= 10; mean (± SE) was twice that of Microlicieae (35.9 ± 17.9; n=4), while Melastomeae had intermediate levels (44.2 ± 7.78; n=18).

This simple analysis is biased due to small and unbalanced sample sizes, but it points out the phylogenetic-dependent effects on seed development and germination. Microlicieae species occur in the altitudinal rocky grasslands which develop on shallow, Al-rich and nutrient-poor soils (Benites et al., 2007), whereas Miconieae usually colonize mesic and soil-rich habitats. Therefore, maternal effects on seed development may be the underlying cause of differences. When only *Miconia* species are compared (and thus allowing for phylogenetic control), habitat-mediated effects appear not to be important. Maximum germinability of Atlantic Forest *Miconia* (67.2% ± 7.6) did not differ from Cerrado *Miconia* (67.6% ± 11.19). On the other hand, at intra-specific level, germinability of *M. albicans* increased with increasing soil fertility in a Cerrado gradient. Germinability increased from *campo sujo* (open vegetation) towards cerradão (cerrado woodlands), and seeds from cerrado sensu stricto presented had values. As the pattern holds for 3 years, this study strongly suggests that undocumented maternal effects help control recruitment (Sales et al., 2010). Maternal effects also are suggested to be important for germination of *M. ferruginata* (Mendes-Rodrigues et al., 2010). Since these two *Miconia* species are apomictic (Goldenberg & Shepherd 1998), differences among individuals occurring at different sites can be attributed to maternal effects.

EFFECTS OF ALTERNATING TEMPERATURES

Temperature fluctuations can change the responses of seeds to light, so that photoblastic seeds can germinate in darkness when submitted to alternating temperatures (Pearson et al., 2002). Increased germinability of small-seeded species as a response to fluctuating temperatures is expected since seeds buried at low soil depths experience high daily thermal fluctuations compared to deeply-buried seeds. Forest gaps also promote increased daily temperature fluctuation in the upper soil layers compared to covered soil, and seeds may cue for this variation to trigger germination (Pearson et al., 2002, 2003).

Overall, alternating temperatures do not promote germination of Neotropical melastomes nor can diel fluctuations in temperature surrogate light requirement (Godoi & Takaki 2005, Carreira & Zaidan, 2007; Simão & Takaki, 2008). Among the reviewed species, *Miconia chamissois* Naudin is the only one benefiting from alternating temperatures under light conditions (Valio & Scarpa, 2001). Indeed, when extreme temperatures such as 35ºC are included in the pair of daily alternating temperatures, germinability of melastomes is not observed (Godoi & Takaki, 2005).

The inability of melastome seeds to respond to fluctuating temperature highlights the critical role of seed size in determining patterns of germination. Daily variation in temperatures has been shown to favor germination of tropical large-seeded pioneers (Valio & Scarpa, 2001; Pearson et al., 2002), but for Neotropical small-seeded species, alternating temperatures have a minor role in seedling emergence.

SEED DORMANCY

A completely nondormant seed has the capacity to germinate over the widest range of normal physical environmental factors that include water, oxygen and an appropriate temperature (Finch-Savage & Leubner-Metzger, 2006). A dormant seed is one that will not germinate under any combination of normal physical environmental factors that otherwise is favorable for its germination (Baskin & Baskin, 2004). The adaptive significance of seed dormancy is to distribute germination in time and space, so that seedling establishment takes place in suitable periods that insure survival and growth (Fenner & Thompson, 2005; Finch-Savage & Leubner-Metzger, 2006; Heschel et al., 2007). Plant lineages have independently evolved a great variety of mechanisms related to seed dormancy in response to different selective pressures. Current data indicate that about 60% of the seeds of tropical rainforest and about 50% of those of tropical semi-evergreen forest are non-dormant at maturity. In contrast, more than 70% of savannas seeds are dormant (Baskin & Baskin, 2004).

To date, there is no evidence supporting the occurrence of seed dormancy in melastomes (Miyanishi & Kellman, 1986; Carreira & Zaidan, 2007; Silveira et al., 2010) from Cerrado and Atlantic Forest (Table 1). The seed structure of melastomes does not offer resistance to germination due to a weak connection between the testal and the raphal sclerotic layer; the raphal part easily breaks off during germination (Cortez & Carmello-Guerreiro, 2008). Although there is a layer of lignified cells in the seed coat (Baskin & Baskin, 1998), seeds of melastomes do not have physical dormancy (Baskin & Baskin, 2000). Also, melastome seeds have fully-developed embryos (Forbis et al., 2002) thus lacking morphological or morphophysiological dormancy. Nevertheless, Elisson et al. (1993) reported on nondormant and physiologically dormant seeds of melastome shrubs from Costa Rica, but level of physiological dormancy was not provided. The temperate herb *Rhexia mariana* L. have nondeep physiological dormancy (PD) (Baskin et al., 1999). Andrade (1995) regards light as the main dormancy-breaking factor in Melastomataceae. However, if darkness (photodormancy) is considered a factor causing seed dormancy, then all melastome species produce dormant seeds.

PD is argued to have evolved in *C. hirta* (Pereira-Diniz, 2003) and *M. ferruginata* (Mendes-Rodrigues et al., 2010). However, the occurrence of PD in both species is dubious because germinability is typically high and germination promoters do not increase germinability. For two other Cerrado species (Table 2), low germinability cannot entirely be attributed to seed dormancy since embryo development (i.e., seed filling) has not been investigated. Lopes et al. (2005) observed low germinability for *T. granulosa* seeds and described dormancy, although no determination of the dormancy level was provided. However, embryo analysis was not undertaken and a distinction between dormant seeds and WELS is not possible. The high frequency of WELS in four *Marcetia* species (Vasconcelos et al., 2007) is insufficient to explain almost null germinability (Table 1), suggesting moderate levels of seed dormancy. Currently, there is no evidence of evolution of seed dormancy in Atlantic Forest melastomes (Table 1).

Table 2. Germinability (%) of *Comolia sertularia* and *Microlicia tetrasticha* seeds under different light and temperature conditions. Four replicates of 25 seeds were set to germinate in a 12-hr photoperiod (light) and continuous dark (dark) in constant temperatures of 15, 20, 25 and 30ºC for 30 consecutive days. (–) indicates data not available. Original data.

Species	Year of collection	Temperature (ºC)							
		15		20		25		30	
		Light	Dark	Light	Dark	Light	Dark	Light	Dark
C. sertularia	2001	0	0	1	0	3	2	-	-
C. sertularia	2002	0	0	1	0	0	0	1	0
M. tetrasticha	2001	2	0	0	0	0	0	-	-

The lack of dormant melastomes in the Cerrado contrasts with existing models of seedling establishment (Oliveira, 2008). The confusion may lie on the assumption that dormancy is required to seed bank formation, but it is not (Thompson et al., 2003). Hence, the underlying mechanism accounting for melastome seed persistence in seed banks is the light requirement rather than dormancy (see Seed Bank Formation section). Nondormant melastome seeds dispersed during mid- or late-rainy season may be incorporated in the soil seed banks during the dry season and germinate in the following rainy season.

SEED BANK FORMATION

In both Cerrado and Atlantic Forest physiognomies, melastome seeds are species-rich and abundant in seed banks. Studies carried out in gallery forests (Pereira-Diniz & Ranal, 2006), rupestrian fields (Medina & Fernandes, 2007), “campo cerrado” (Carreira, 2004) and cerrado sensu stricto (Sassaki et al., 1999; Carreira 2004) documented melastome seeds as one of the most important families in the soil seed bank communities. The importance of melastomes in seed banks is not only due to their abundance and diversity but also because seeds can be found at several soil depths (Pereira-Diniz & Ranal, 2006), in both dry and wet seasons (Sassaki et al., 1999), significantly accounting for a large part of emerging seedlings (Medina & Fernandes, 2007). *Miconia*, *Clidemia* and *Tibouchina* species are the dominant ones (Baider et al., 1999, 2001; Neto et al., 2007), whereas endemic Microlicieae are particularly important for the rupestrian fields (Medina & Fernandes, 2007).

In degraded areas of Atlantic Forest, Melastomataceae comes in the second place in terms of seeds/spores in the soil, after Dennstaetiaceae, which includes the high invasive *Pteridium arachnoideum* (Kaulf.) Maxon (Silva & Silva-Matos, 2006). During gap-phase regeneration, the seed bank is likely responsible for the establishment of Melastomataceae. Melastome seeds are found in seed banks in second-growth stages and in mature forest stands, but seed density decreases with increasing forest age (Aidar et al., 2001; Baider et al., 2001). These species constitute the early or pioneer communities, which are essential for the establishment of shade-tolerant trees and consequently for forest regeneration (Baider et al., 2001). The high richness of melastomes in the soil seed bank highlights their potential role in restoration ecology.

The typical small-sized seeds of Melastomes may be regarded as the main factor accounting for high seed longevity. Easiness for seed burial prevents germination and decreases predation, leading to a predictable and nearly universal relationship between small seed size and persistence in soil (Milberg et al., 2000; Fenner & Thompson 2005). In laboratory studies, artificially stored melastome seeds also have high longevity (Zaia & Takaki, 1998; Pereira-Diniz, 2003; Carreira, 2004). The positive photoblastism of melastome seeds prevents germination of buried seeds, and the light requirement is the physical factor that triggers germination when soil moisture is suitable. Under darkness and/or low R:FR ratio conditions seeds remain quiescent until optimal conditions for seedling establishment occurs.

Melastomes produce a great number of seeds (Galetti & Stotz, 1996; Dalling et al., 1998) in a short period of time and enter tropical seed banks together with other woody pioneer and herbaceous species (Baskin & Baskin, 1998), hence playing an important role in regeneration following disturbance (Swaine & Whitmore, 1988). The high seed longevity leading to a high potential for seed bank formation helps to explain why invasive melastomes such as the weeds *M. calvescens* and *C. hirta* can be difficult to control.

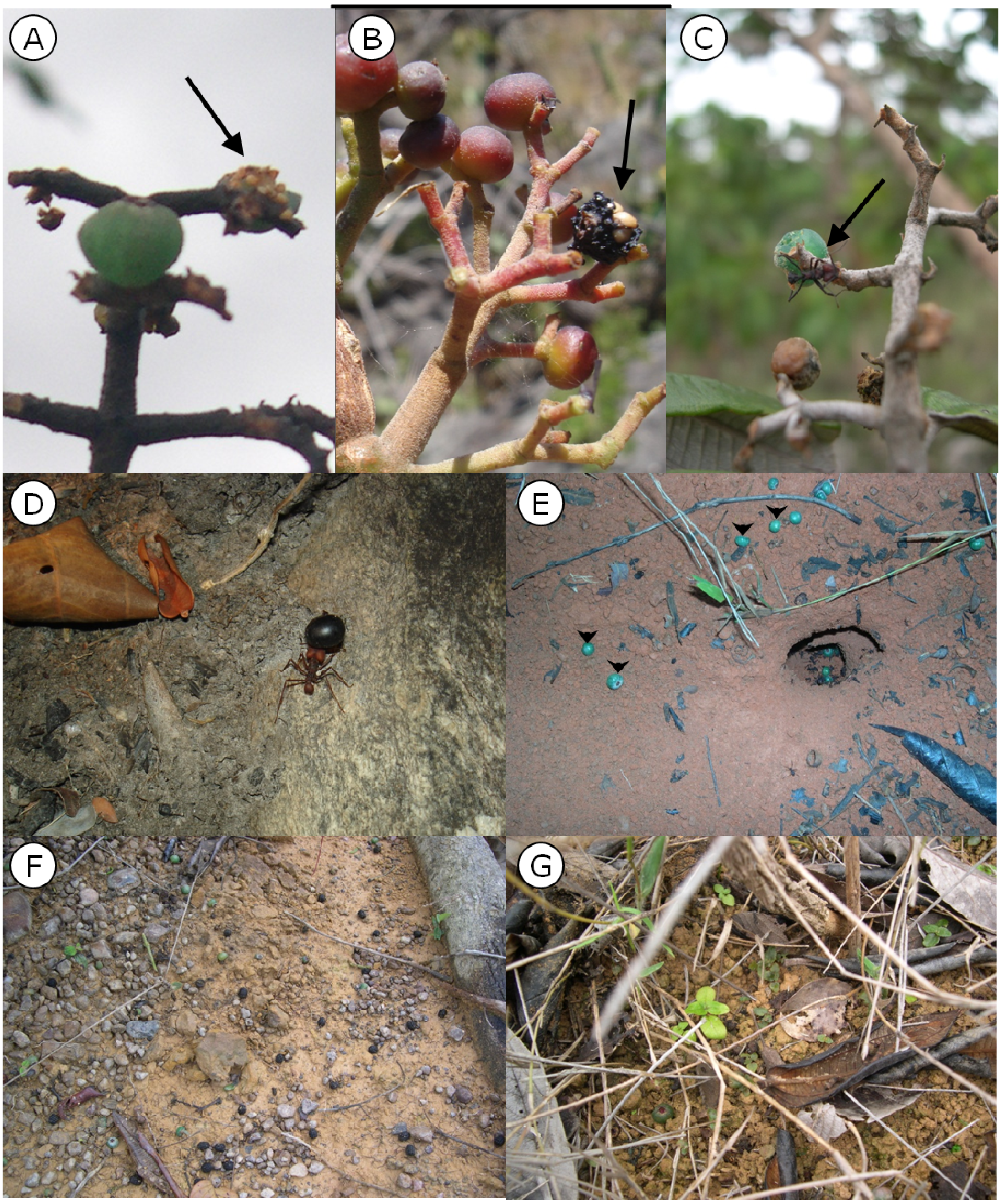

Figure 1. Bird and ant frugivory in *Miconia*. A- mashed fruit of *M. albicans*; B- mashed fruit of *M. irwinii*. C- Attini ant chewing on a fruit stalk; D- *Atta sexdens* soldier carrying *M. irwinii* berry; E- Fallen fruits of *M. albicans* near *Atta sexdens* nest; F- Failure in recruitment of seeds in fruits of *M. albicans*; G- Early seedling establishment in the campo sujo. Photo D by Mônica HC Lima.

SEEDLING ESTABLISHMENT

Seedling establishment is the most sensitive phase in the plant's life cycle. Due to high susceptibility to both biotic and abiotic stresses, understanding how selective pressures act on seedling response to multiple stresses is crucial in the conservation and management of plant populations and for restoration ecology. One would expect remarkable differences in recruitment between Cerrado and Atlantic forest species because these habitats provide seedlings with contrasting selective pressures during the establishment phase. Because studies on melastome seedling establishment are rare, the following discussion is based on studies conducted in the rainforests of Central America and the few studies conducted for Cerrado species.

Early accounts of germination and establishment of woody plants in the Cerrado indicated little success of sexual reproduction in spite of the high proportion of viable seeds and good germinability under laboratory conditions (Oliveira, 2008). These pioneer studies based on observations of a high proportion of species that resprout after fire suggested that fire-induced mortality would limit the role of regeneration from seeds (i.e., sexual reproduction).

The seasonal climate of the Cerrado prevents seedling establishment during the dry season (Fig. 1G). Drought, fire, cover effects and interespecific competition are important factors driving seedling establishment in the Cerrado. Water shortage during the dry season limits establishment to periods of high soil moisture that allow seed imbibition and seedling growth. Seedling roots tend to be restricted to the upper layers of soil during the first years of life, and therefore, they do not have access to the available water in deeper soil layers during the dry season (Goldstein et al., 2008). Pereira-Diniz & Ranal (2006) detected germinable seeds of melastomes in September, but this alone does not mean successful establishment as drought-induced mortality in early-wet season can directionally select for delayed germination until the arrival of perennial rains during mid-rainy season. Sporadic rains at the onset of the rainy season may not be enough to increase soil moisture above the threshold that ensures seedling establishment.

Soil evapotranspiration is high at the onset of the rainy season and water does not reach the lower soil layers to allow root growth. Irrigation positively increase seedling establishment of *M. albicans* (Hoffmann 1996). Recruitment begins in December, peaks in

February and decreases as the dry season proceeds (Carreira, 2004). Establishment during the mid-rainy season is thus an effective strategy to ensure seedling growth and root elongation that result in the typical low shoot:root ratio of Cerrado species (Hoffmann & Franco, 2003). Higher biomass allocation to roots allows for root growth at deeper soil layers where moisture remains high, even after several months without rains (Goldstein et al., 2008). High relative growth rates in melastomes may thus represent an effective strategy that increases net carbon gain during the early establishment phase.

Fire may also have selected for establishment during the rainy season. Fire negatively impacts sexual reproduction, destroys reproductive structures, decreases size-specific reproductive output and kills seedlings (Hoffmann, 1998). Forest species are fire-sensitive, whereas most Cerrado species are able to resprout following fire (Hoffmann, 2000). Fire-induced mortality is particularly costly for small-seeded species (Lahoreau et al., 2006). Fire-induced mortality is 100% for *M. albicans* seedlings, even for 2-year old seedlings (Hoffmann, 1998) and establishment of *M. albicans* and *Clidemia sericea* D. Don. peaked the second and third year after fire (Miyanishi & Kellman, 1986).

Cover effects on seedling establishment include canopy shading and litter cover. Canopy shading is usually neglected as a limiting factor in the Cerrado but it potentially restricts seedling growth during early establishment even in open grasslands and particularly in cerradão (cerrado woodlands; Franco 2002, Goldstein et al., 2008). The effects of canopy shading on germination are an overlooked component of regeneration dynamics. The grass layer reduces up to 70% of photosynthetic photon flux densities (Franco, 2002) and is very likely to alter R:FR ratio, hence hampering regeneration from seed.

Litter modifies conditions on the soil surface by intercepting light, reducing thermal amplitude, releasing nutrients and toxins and reducing evaporation (Fenner & Thompson, 2005). In woodlands, litter prevents the establishment of small-seeded species. In open grassland, however, scant litter positively affects establishment as it probably reduces soil temperature and desiccation (Hoffmann, 1996). Shallow-rooted herbaceous species die back during the 4- to 5-month-long dry season (Goldstein et al., 2008), but the effects of dry/death aerial biomass on seedling establishment remains unexplored.

Finally, grass root competition for water should be a critical factor during the wet season, even though the topsoil layers remain wet most of the time. Low shoot:root ratio and biomass allocation to roots (Goldstein et al., 2008) should allow roots to reach moist soil layers beyond the grass root zone. Also, with the buildup of underground energy reserves would allow regrowth of aerial biomass after fire or drought (Franco, 2002). It should be investigated, however, whether a low amount of food reserves in small seeds limits seedling root growth, which in turn, affects competition with grass roots.

Recruitment in forests drastically differs from that in savannas. The closed canopy of rain forests alters light quality and quantity (Zaia & Takaki, 1998; Pearson et al., 2003), thereby hindering regeneration from photoblastic seeds. The small light-demanding melastome seeds accumulate in the seed banks and germination is inhibited unless canopy gaps occur. However, canopy gaps not only changes light quantity/quality but also increase diel temperature fluctuations providing cues for seeds to detect a canopy opening. Small-seeded species (dry mass <1 mg) respond to an irradiance cue for germination, whilst only larger-seeded pioneers respond positively to an increasing magnitude of diel temperature fluctuation (Pearson *et al.*, 2002). Small-seeded forest pioneers show significantly greater germination in response to irradiance than in complete darkness (Pearson et al., 2002) and accordingly melastomes recruit in canopy gaps. In Costa Rica, *Miconia* seedlings were 20-fold more abundant in gaps than in understory, with emergence increasing with gap size, though survival success was low (<0.5%) irrespective of light environment (Elisson et al., 1993).

Because gaps severely reduce soil moisture, seedling mortality in gap-induced dry soils should select for germination in suitable microsites within the gap. These microsites must also be litter-free as a thick soil or litter covering prevents light penetration and reduces the R:FR to inhibitory levels for germination (Pearson et al., 2003). Melastome seedlings are unevenly distributed within gaps. They are frequent in root pit and mound (Elisson et al., 1993) and recruit preferentially in litter-free sites (Metcalfe et al., 1998; Dalling & Hubell, 2002). Small-seeded species germinate only in comparatively moist microsites, such as small canopy gaps, which may reduce the risk of drought-induced mortality (Daws et al., 2008).

Despite its importance, the role of biotic factors in recruitment has been neglected for melastomes. Fungal and oomyceteous pathogens are major cause of seed mortality in the soil for tropical pioneers including *Miconia* (Dalling et al., 1998) and may decrease recruitment from seed banks. On the other hand, the occurrence of mycorrizhae in Melastomataceae may be underestimated and their potential in increasing establishment has been overlooked (Elisson et al., 1993; Metcalfe et al., 1998; Urcelay et al., 2005; Matias et al., 2009), while nothing is known about the role of fungal endophytes.

SEED ECOLOGY OF MELASTOMES IN A CHANGING WORLD

EFFECTS OF GLOBAL CHANGE

There is certain agreement among scientists that increasing levels of carbon dioxide (CO_2) and temperature in the last decades result from human activities (IPCC, 2007). Potential effects of climate change on melastome seed and seedlings include changes in seed size and production, germinability, germination time, seed bank dynamics, seedling establishment and indirect effects on frugivores ecology. Global climate change effects on seed germination are unclear, and their effects are very speculative (Fenner & Thompson, 2005) since responses are species-specific. Most available data on the effects of climate change comes from studies conducted on temperate species (Fenner & Thompson, 2005). Thus, the following discussion is grounded on available evidence and should be viewed with caution, as no study to date has specifically focused on melastomes.

Current models predict an increase of ca. 0.2°C per decade for the next two decades for a range of emissions scenarios. In the best scenario, temperature increase at the end of 21 century is expected to be 1.8ºC above 2000 values. In the worst, a global increase of 4ºC is projected (IPCC, 2007). Regeneration from seed is generally expected to benefit from climate warming, but perhaps this is because attention has tended to focus on the colder parts of the globe (Fenner & Thompson, 2005). Because melastome germination is disfavored at high temperatures (Table 1), a negative impact of temperature raise is anticipated (Table 3).

With regards to CO_2, some studies have found little or no effect of elevated atmospheric global CO_2 on seed germination (Thurig et al., 2003; Stiling et al., 2004), while others have found a positive effect of elevated CO_2 in germination time, germinability and seedling emergence (Mohan et al., 2004; Zavaleta 2006). Studies investigating the combined effects of temperature and CO_2 on germination have provided evidence for significant interaction on reproductive allocation and germination parameters (Hovenden et al., 2008, Qaderi & Reid, 2008). Elevated CO_2 effects on melastome seeds are unpredictable but seedling growth may be enhanced as species evolved the C3 photosynthetic pathway (Table 3). There is some indication that climate change may have large indirect effects on seed banks (Fenner & Thompson, 2005). In advance, regeneration from seed banks of melastomes would also be compromised as high temperatures are inhibitory for seed germination and/or seedling survival.

Multi-models also project an altered pattern of precipitation worldwide due to global climate change. Data forecast reduced precipitation in both savannas and rainforests, with a 10-20% reduction in the Atlantic Forest and a reduction of more than 20% in precipitation for Cerrado during the dry season (IPCC, 2007). Conversion of Cerrado to grasslands increases mean surface air temperature and the frequency of dry periods within the wet season (Hoffmann & Jackson, 2000), a change that could be particularly damaging to the small-sized melastomes seedlings. Effects of changes in precipitation are hard to predict, partly because predicted patterns of future rainfall are less certain and partly because effects on plants may depend crucially on amount, timing and reliability of rainfall (Fenner & Thompson, 2005). However, there is little doubt that recruitment of melastomes may be constrained by a predicted shortened rainy season and decreased soil moisture (Table 3). Water shortage is critical for establishment in the seasonal Cerrado and the intensification of fire frequency may further impacts recruitment. At Cerrado/forest boundaries, enlarged fire seasons can be detrimental to ecotone-colonizing plants (Table 3).

Finally, climate change may indirectly disrupt fruit-frugivore interactions by simultaneously reducing bird distribution range and promoting frugivore extinction (Table 3). A conservative estimate addressing frugivores inhabiting flatland forests in the Amazon and Cerrado predicts that nearly 80% of manakins would lose their habitable area, and 20% of Cerrado manakin species would be potentially extinct from the biome under future climate changes (Anciães & Peterson, 2006). Following a loss of frugivores, a dramatic decrease in regeneration from seed is anticipated (Table 3).

EFFECTS OF HABITAT FRAGMENTATION

Habitat loss and fragmentation are the most pervasive form of human disturbance (Tabarelli et al., 2008). The increasing and unprecedented deforestation level is creating a landscape of edge-dominated forests where edges are more illuminated, desiccated and wind-exposed than forest interior. These microclimatic modifications strongly impact the structure of forest community and severely decrease functional diversity, driving the forest fragments towards early-successional conditions (Tabarelli et al., 2008). Throughout nearly 500 years, the Atlantic forest has been experiencing a massive slash-and-burn deforestation rate. Most Atlantic forest remnants are characterized by small (<50ha) and isolated patches corresponding to aprox. 11.4--16% of the original area (Ribeiro et al., 2009).

In forest edges, pioneer-dominated plant assemblages are markedly different in terms of tree species richness, proportion of pioneer, large-seeded and emergent species compared to the forest interior (Oliveira et al., 2004; Silva et al., 2007; Santos et al., 2008). Intense forest fragmentation, selective logging and hunting pressure will lead to regional extinction of large-fruited trees as a result of altered population dynamics and movement across the landscape of large fruit-eating vertebrates. Therefore, a dramatic shift in the plant community in the Atlantic Forest towards small-fruited and capsule-bearing species is anticipated (Silva & Tabarelli, 2000). This disturbance-driven plant community may favor small-seeded Melastomataceae, Rubiaceae, among other families (Silva & Tabarelli, 2001). In spite of apparent success of melastomes in disturbed habitats, mechanisms accounting for recruitment must be considered to accurately determine long-term changes.

The effects of fragmentation on bird abundance, richness and behavior have been investigated, but implications for seed dispersal remain unexplored. The following discussion explores whether forest fragmentation collapses fruit-frugivore interactions.

Bird response to fragmentation and consequences for melastome recruitment

Mechanisms accounting for differential bird distribution in edges vs forest interior (Restrepo & Goméz, 1998) include changes in microclimate and distribution of suitable habitats, distribution of parasites and predators and resource-based-driven mechanisms. Fragmentation can reduce bird abundance, richness and behavior (e.g. movement patterns, migration, and fruit consumption). Available data on fragmentation of Neotropical rainforests suggest two key findings: 1) responses are species-specific; and 2) landscape configuration is highly relevant. There are many reports on altered bird community structure and composition (Restrepo & Goméz, 1998; Ribon et al., 2003; Laurance 2004; Uezu et al., 2005; Ruiz-Gutiérrez et al., 2008) in fragments compared to pristine areas. Moreover, landscape metrics (such as connectivity, matrix type, fragment area, edge age) are also determinant of bird abundance and richness (Restrepo et al., 1999; Galetti et al., 2003; Martensen et al., 2008).

Studies report on higher frugivorous bird abundance in forest interior (Restrepo & Goméz 1998; Hasui et al., 2007), but evidence pointing to lack of variation is plenty as well (Laurance 2004; Uezu et al., 2005; Hasui et al., 2007). No general and clear pattern of bird responses to fragmentation emerges because edges resulting from anthropogenic disturbances influence the distribution of understory birds in complex ways (Restrepo & Goméz, 1998). Because numerous factors control bird distribution, they may be unresponsive to changes in fruit abundance in edges vs forest interior (Restrepo et al., 1999).

Despite the results of effects of fragmentation on frugivorous birds, few studies have looked at the effects of fragmentation on seed rain. Changes in the distribution and survival of frugivores suggest that seed dispersal may be influenced by fragmentation, leading to changes in the structure and location of edges through time and fruit removal rates (Restrepo & Goméz 1998; Galetti et al., 2003). Theoretically, the demography of generalist bird-dispersed small-seeded species, such as Melastomataceae and Rubiaceae, can be affected by fragmentation (Galetti et al., 2003). Since melastome species account for most records of fruit consumption (20% of fruit-frugivore interactions) in the forest edge, secondary-growth and also in the canopy and understory of mature forest Atlantic Forest (Silva et al., 2002), the impact should be noteworthy.

Negative effects of forest edge on fruit removal are not unequivocal (Restrepo et al., 1999). The probability of fruit consumption is lower in the interior than at the edge and less in small than in large fragments (Galetti et al., 2003). Conversely, higher seed rain of small-sized seeds at the Northeastern Atlantic forest edge explains continuous recruitment (Melo et al. (2006). However, large seeds are underrepresented at the forest edge vs. forest interior, as a consequence of reduced seed delivery services by vertebrates. In Central

America melastomes, removal rates were generally higher in young-secondary forest than in old-growth forest regardless of where plants grew. This was associated with higher sugar concentration in berries from young-secondary forest compared to those in old-growth forest species which could mean higher competition for frugivores (Lumpkin & Boyle, 2009).

The effects of fragmentation on seed rain are complex perhaps due to diversity of plant functional types in biodiversity-rich tropical forests, but it should not be assumed that decreased dispersal would necessarily result in lower recruitment. Despite negative effects of fragmentation on frugivory, plant fitness may remain unaffected (Valdivia & Simonetti, 2007).

Costa Rica melastomes have a relatively small set of seed dispersers and they maybe more vulnerable following the extinction of a seed disperser (Loiselle & Blake 1999). For the Atlantic Forest, large networks of seed dispersers (with higher ecological redundancy) may be robust to disturbance as a result of the large number of species with few interactions between the few super-generalists (Silva et al., 2007). Forest fragmentation is thought to favor small-seeded species (Silva & Tabarelli, 2001), and thus melastomes may not be negatively affected in the short-term. Small-seeded plant species are favored due to the overall dominance of small, habitat-generalist birds. Most of the melastome seed dispersers do well in degraded areas (Pizo, 2007) and disruption of fruit-frugivore mutualism may have only minor effects. However, this apparent resilience may not overcompensate for increasing habitat loss. Bird distribution is edge age-dependent, so it can be reasoned that time-lag responses may have gone undetected. Moreover, edge-mediate increased fire frequency will prevent seedling establishment in forest edges.

Ant response to fragmentation and consequences for melastome recruitment

Ants are the dominant organisms foraging on the forest floor and exploiting melastome berries. Ants can be of relevance for the rescue of fruits that cannot achieve high removal rates due to an impoverishment of vertebrate dispersal assemblages in fragmented or heavily hunted habitats (Christianini & Oliveira, 2009). Therefore, as habitat loss and fragmentation reduce abundance and richness of frugivorous birds, ant dispersal may become relatively more important for seedling establishment. Ant response to fragmentation thus, becomes critical to understand the impacts of fragmentation on melastome recruitment.

Table 3. Predicted effects of ongoing human impact in the life cycle transitions of Neotropical melastomes. + indicates a beneficial, - a deleterious, 0 a neutral and ? indicates an unpredictable response.

Life-history stage	**CO_2 increase**	**Temperature raise**	**Fire**	**Decreased precipitation**	**Fragmentation**
Seed production	?	?	-	-	?
Bird dispersal distance	0	-	0	-	-
Ant dispersal distance	0	?	0	0	-
Seed rain	0	-	0	-	-
Wind dispersal	0	0	0	0	?
Ant-fruit interactions	0	0	-	0	-
Germinability	?	-	-	-	+
Germination time	?	-	-	-	0
Seed bank dynamics	?	-	-	-	+
Seedling establishment	+	-	-	-	-
Seedling herbivory	-	-	0	0	-

Among seed-harvesting ants, leaf cutting ants (*Atta* and *Acromyrmex*) are of major importance (Dalling & Wirth, 1998; Leal & Oliveira, 1998; Christianini & Oliveira, 2009; Lima et al., in prep.), and they appear to benefit from fragmentation. Colonies of *Atta sexdens* and *Atta cephalotes* are more frequently found in forest edges and second-growth forest regeneration patches than in the forest interior. Shifts in both bottom-up and top-down controls have been invoked as the mechanism responsible for the higher ant abundance in disturbed habitats (Wirth et al., 2007; Silva et al., 2009).

As a result of ecological release – relaxation of top-down control – and increased abundance of less effectively defended and more palatable pioneer plants (Silva et al., 2009), increased herbivory in forest edge has also been documented (Urbas et al., 2007). In highly-disturbed sites, not only do colony density and herbivory increase, but foraging area of colonies dramatic reduces compared to forest interior (Urbas et al., 2007), suggesting that fragmentation indirectly reduces ant dispersal distance (Table 3). In disturbed areas, seed harvesting by ants would restrict seed dispersal to short-distances, promote seed clumping and reduce seedling survival beneath parents, overcoming any positive benefit plants would have from seed manipulation by ants (Guimarães & Cogni, 2002; Silva et al., 2007; Zelikova & Breed, 2008).

IMPLICATIONS FOR RESTORATION

As typical pioneer species, melastomes play a key role in restoration ecology (Swaine & Whitmore, 1988). Pioneers are able to colonize degraded areas and create suitable conditions for the establishment of secondary and late-successional species, improving natural regeneration. Moreover, some species are heavy metal accumulators and are potential candidates for restoration of mined areas (Jacobi et al., 2007; Rodrigues & Silveira, in prep). They also have been regarded as important sources of bioactive compounds (Cota et al., 2002) and in ornamentation (Ranieri el al., 2004), increasing income of landowners.

Their copious year-round seed production makes seed sowing a direct and practical alternative for ecological restoration. However, seedling mortality can be high and although cost-effective, seed sowing should not be employed as a single method (Table 4). Because melastome seeds and seedlings are fire-sensitive fire protection mechanisms are encouraged.

Although many new techniques have been developed, native tree planting from different functional groups is the most used in the Atlantic Forest (Rodrigues et al., 2009). In order to overcome early seedling mortality, sapling production following soil fertilization can be recommended, though it may be cost-prohibitive. At Cerrado, soil fertilization is not recommended as it increases the chances of invasion by exotic species (Barbosa et al., 2010).

Table 4. The advantages and disadvantages of employing Melastomataceae in the most recommended restoration techniques.

Restoration technique	**Advantages**	**Disadvantages**
Fire protection	A fire-free environment is likely to increase recruitment because of seed and seedling fire-sensitivity	May implicate in problems with farmers, requiring pre-action social mobilization
Perching	Profuse berry production is attractive for a taxonomic wide range of vertebrates, nucleation enhancement and low cost for implementation	None
Seed rain management	Large production of long-lived seeds, low cost for seed rain collection	No control of final seedling number
Seed sowing	Copious seed production and low cost for seed collection, handling and sowing	Low seedling recruitment No control of final seedling number
Litter and soil seed bank management	Ubiquity and abundance of melastome seeds in tropical seed banks and long-lived light demanding seeds spreads recruitment in time	May decrease regeneration in nearby fragments
Seedling resprout	Very limited technique	Melastome studied to date are obligate seeders following disturbance
Seedling transplanting	High seed production and rapid early seedling growth. Insures control of final seedling number	Relatively elevated costs for seedling production

Melastome seedlings have high growth rates (Carreira & Zaidan 2003; Ranieri et al., 2004), a desirable attribute for cover and soil conservation. Unfortunately, information on root biology is still needed. Lignotuber have been reported in *Marcetia taxifolia* (Melastomeae) populations growing in rupestrian fields but not in *restingas* (shrubby vegetation on coastal sandy plains) (Vale, 1999). The lack of well developed underground systems of melastomes places resprouting as an ineffective restoration technique (Table 4).

An alternative, less costly method to planting forest species is litter and soil seed bank management. By moderately increasing cover, seedling survival and growth are expected to improve whereas transplanting soil seed bank can increase regeneration from seed (Table 4).

Melastome massive berry production (Galetti & Stotz 1996; Dalling & Wirth, 1998) can be particularly important for restoration ecology. First, managing seed rain can aid in promoting diversity and wood cover (Table 4). Secondly, transplanted berried-fruited melastomes can surrogate the use of artificial perches, attract a large array of vertebrate fauna and thereby, augment seed rain (Table 4). When crossing disturbed, fragmented areas birds require stepping-stones (Uezu et al., 2005; Martensen et al., 2008) and a high diversity of bird-dispersed seeds can be delivered to nucleated sites, where other species will have improved conditions for recruitment. Finally, disturbed-adapted melastomes can act as nurse-plants and provide conditions for establishment of secondary species (Le-Stradic et al., 2008).

Melastomes have a limited role in forest restoration during advanced successional series. To prevent cycles of pioneer self-replacement at defaunated and fragmented landscapes (Tabarelli et al., 2008), later seedling transplanting should focus on species with life-history traits of secondary and/or late successional woody flora to enhance functional diversity. Current data on ongoing restoration of endangered ironstone outcrops in the Cerrado have shown that inoculation of mycorrhizae and *Rhizobium* promote increased survival and growth of *Tibouchina heteromalla* (D. Don) Cogn., besides improved physical and chemical soil properties (Matias et al., 2009). Because melastomes interact with a wide diversity of pollinators (Renner, 1989), dispersers (see Dispersal Ecology section) and herbivores (Rosumek et al., 2009), the use of melastomes can assist in restoring ecological processes. Ecological restoration is a high-priority issue, and the agenda for conservation of Neotropical hotspots must incorporate science-based techniques that facilitate ecosystem recovery. By introducing melastome species in the early stages of restoration projects, restorationists can take advantage of their life-history traits to approach pre-disturbance levels of diversity, structure and ecological functioning.

CONCLUSIONS

We have presented and analyzed available evidence on the ecophysiology of life cycle transitions of Neotropical melastomes from both forest and savanna. The life-history traits of this pioneer-dominated family are very variable in terms of fruiting phenology, dispersal ecology and seedling establishment. Nevertheless, current data allows acknowledging the following key findings: 1) In response to different selective pressures, capsular-fruited species disperse during the dry-wet transition season, whereas berries are produced year-round; 2) Tanagers, manakins and thrushes are the major seed dispersers, but a broad range of bird species also are important in providing seed cleaning and reducing density-dependent mortality; 3) Ants are dominant secondary seed dispersers, with important benefits arising from their interaction with melastome fruits; 4) The small-sized seeds are light-demanding, non-dormant, long-lived, and accumulate in soil seed banks; 5) Seedling establishment contrasts between Cerrado and Forest sites; 6) Litter abundance, gap size and within-gap microsite can affect regeneration from seed in forests; 7) Global change drivers are expected to have low short-term impact on melastomes, but long-term consequences are expected to be detrimental; 8) Melastomataceae species are key in restoration ecology can assist ecosystem recovery in early-to-mid succesional stages.

CAPÍTULO II

SPECIES-SPECIFIC OUTCOMES OF AVIAN GUT PASSAGE ON GERMINATION OF MELASTOME SEEDS

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ARTIGO SUBMETIDO AO PLANT ECOLOGY & EVOLUTION

Abstract

Background and aims - Frugivory and seed dispersal are of major importance for plant recruitment and distribution. However, few studies have examined the effects of gut passage on seed germination in complex fruit-frugivore interactions involving multiple species. Here, we examined whether gut passage affects seed germination of eight Melastomataceae species from the Brazilian Cerrado (a Neotropical savanna) after gut passage of seven bird species.

Methods – We take advantage of the generalist dispersal system of melastomes in order to compare the dispersal quality among species by contrasting seed germination of hand-cleaned, gut-passed seeds and seeds within intact fruits. We studied gut passage effects on seed germination percentage and mean germination time (MGT) of *Clidemia urceolata* and seven *Miconia* species.

Key results – Less than 4% germinability was observed for seeds within intact fruits across all species, indicating that seed cleaning is required prior to germination. The action of guts on the seeds had non-significant or minor additive effects on germinability compared to hand-cleaned seeds depending on the plant species. Gut treatment had no effect on MGT of two species and minor effects on other three species. However, mechanical/chemical effect significantly decreased MGT of *C. urceolata* and *M. albicans* while it increased MGT of *M. ibaguensis*. There were significant species-dependent effects depending on both bird and plant species, suggesting that species-specific outcomes arise from Neotropical fruit-frugivore interactions in plant with generalist dispersal systems.

Conclusions – Germination enhancement deriving from gut scarification was not observed for all species, but seed cleaning seems to be an important benefit provided by frugivores to Cerrado melastomes. The variable outcomes resulting from complex fruit-frugivore interactions potentially affect the recruitment of Cerrado melastomes.

Key-words - *Clidemia*, dispersal quality, endozoochory, frugivory, Melastomataceae, *Miconia*, scarification, seed cleaning, seed germination, seed dispersal.

Introduction

The consumption of fleshy fruits and the subsequent dispersal of their seeds by frugivores is a key process in plant ecology. Dispersed seeds usually experience lower mortality by natural enemies and competition with siblings, which has favoured the evolution of seed dispersal by natural selection (Janzen 1970). Dispersed seeds also benefit from dispersal because seed deposition in safe sites can increase gene flow and the chances of colonisation of new areas, with implications for population spatial distribution. Moreover, the quality of treatment given by frugivores strongly modifies the probabilities during seed-to-seedling transition stages (Willson & Traveset 2000, Traveset & Verdú 2002, Verdú & Traveset 2004, Schupp et al. 2010).

Since fruit consumption by frugivores does not necessarily implicate successful seed dispersal, the role of vertebrates as potential seed disperses requires a more thorough evaluation that includes germination experiments (Robertson et al. 2006). Studies addressing gut passage effects of a single frugivore species on seed germination do not include possible frugivore-dependent effects on germination and thus, do not cover all potential effects of gut treatment. Changes in the probabilities of seed germination after gut passage comprise an important component of seed dispersal effectiveness and potentially affect the likelihood of seedling establishment (Schupp et al. 2010). In spite of its general importance for plant ecology, the effects of gut passage by multiple frugivore species on seed germination remains relatively unexplored, especially in the Neotropics (but see Jacomassa & Pizo 2010). In this study, we evaluated the effects of gut passage on seed germination of Melastomataceae species from the Brazilian Cerrado (a Neotropical savanna).

Miconieae (Melastomataceae) species evolved generalist dispersal systems (*sensu* McKey 1975). Species with this syndrome often produce abundant, small-sized berries that have fleshy placentas with high water- and sugar-content and enclose numerous and minute seeds. These berries are consumed by a taxonomic diverse community of animals, thus are suitable for comparing the effects of various frugivores on seed germination. Melastome fruits are consumed by a wide range of vertebrate (mainly birds) species and invertebrate species, and these fruits represent a key resource for Neotropical frugivores year-round (Snow 1965, Stiles & Rosselli 1993, Poulin et al. 1999, Lima et al., unpublished data).

Frugivores consuming melastome fruits often overlap in their diet (Silveira 2011), and therefore, the complex system involving melastomes and their frugivores provides an opportunity to enhance our understanding of seed dispersal effectiveness in species-rich communities. Melastomes are dominant species in many Neotropical habitats (Clausing & Renner 2001) and an increased knowledge of their seed biology would provide the basis for their inclusion in restoration ecology programmes.

Since the outcome of fruit-frugivore interactions depend on both intrinsic bird and plant traits, studies covering multiple fruit-frugivore specific interactions are valuable to understand how gut passage affects survival probability of ingested seeds (Traveset et al. 2007, Lehouck et al. 2011). Here, we evaluated the role of avian gut-passage on the seed germination for eight Melastomataceae species from the Cerrado. The species-specific differences of both plants and frugivores were tested here. Our specific goals were: (i) to determine the effects of gut passage on the seed germination; and (ii) to examine whether there are species-specific differences in the outcomes of bird-fruit interaction.

Material and methods

Fruits were collected in two sites in southeastern Brazil, Estação Ecológica de Pirapitinga and Serra do Cipó, Minas Gerais. Both study sites are represented by seasonal, fire-prone Cerrado vegetation (savanna grasslands) establishing on nutrient-poor soils (Giulietti et al. 1997 and Bedetti et al. 2011 give site descriptions). The studied species belong to Miconieae, a group in which the inferior or partly inferior ovaries develop into baccate fruits (hereafter called berries; Michelangeli *et al*. 2008). The plant species studied here are dominant pioneers at both sites that produce small berries which vary in colour, seeds per fruit and dispersal phenology (Tab. 1).

Fruits were offered to seven bird species which are commonly found in both study sites: *Turdus leucomelas* and *Turdus amaurochalinus* (Turdidae), *Thraupis palmarum*, *Thraupis sayaca* and *Schistoclamys ruficapillus* (Thraupidae), *Mimus saturninus* (Mimidae) and *Zonotrichia capensis* (Emberizidae). Most study birds are opportunistic omnivores, except *Z. capensis* which is mainly a granivore (Sick 1997). These bird species are among the wide variety of frugivores consuming melastomes fruits in the Neotropics and all of them have been recorded eating melastome fruits at both sites (F.A.O. Silveira and P.O.

Mafia, pers. obs., T.J. Guerra, Universidade Estadual de Campinas, pers. comm.). We were not able to test all possible bird-fruit combinations because of either bird or fruit availability. We used caged birds in good conditions from a wildlife rehabilitation centre (CETAS) in the city of Belo Horizonte, Minas Gerais. To make sure we included only defecated seeds after bird feeding, we observed fruit ingestion behaviour. *Turdus leucomelas*, *T. amaurochalinus* and *M. saturninus* gulped the whole fruits, whereas *T. palmarum*, *T. sayaca*, *S. ruficapillus* and *Z. capensis* mashed fruits and swallowed small fruit pieces. Sometimes, birds also regurgitated fruits. Regurgitated and non-ingested seeds were omitted from the dataset and not used for statistics.

Ripe berries were collected from at least twenty individuals per species (n=1 for *M. ligustroides*) and offered to birds in the following day. To control for seed source, germination trials of control and gut-passed seeds included seeds collected from the same population. Three experimental treatments were performed. In the first one (hand-cleaning), seeds were removed from the fruits, washed for 5 minutes and dried under shade for 24h. In the second treatment (gut passage), 15--30 fruits for each species were mixed and offered to 1--5 frugivore individual(s) per bird. Fruits were offered during early morning for birds subjected to a regular diet based on a mix of bird ration and fruits (papaya and banana). This regular diet was interrupted 1--2 hr before birds were fed with berries. Recovered seeds were washed for 5 minutes and dried for 24h. After retrieval, four replicates of 25 seeds were set to germinate into Petri dishes layered with double sheet of filter paper moistened with Nistatin solution (2%). For the third treatment, seeds were germinated directly from intact fruit without fruit removal (seed cleaning) or gut passage (gut scarification). For this last treatment four replicates of five fruits per species were used. The dishes were incubated under 25ºC at a 12:12h light: dark cycle to simulate field conditions and germination was monitored at 24-hr intervals for 30 days (Silveira et al. 2012). Data in percentage were arcsine transformed and differences among means were determined through ANOVA followed by Tukey test ($\alpha = 0.05$) or through Kruskal-Wallis when assumptions of the parametric tests were not met.

For all treatments, we calculated germinability (in percentage) and mean germination time (MGT) through the formula (Ranal & Santana 2006):

$$\mathrm{MGT} = \sum_{i=1}^{k} n_i t_i \Big/ \sum_{i=1}^{k} n_i$$

where n_i is the number of seeds germinated in the time i, t_i is the time from the start of the experiment to the *ith* observation, and k is the time of last germination.

Table 1. Growth-form, life-history traits and geographic distribution of *Clidemia urceolata* and *Miconia* species. Means are followed by SD. Seed mass refer to the average weight of 4 replicates of 25 seeds.

Species	Growth -form	Ripe fruit color	Seeds/fruit (n=10 fruits)	Seed mass (mg)	Fruiting phenology*	Geographic range**
C. urceolata	Shrub	Black	168.8 ± 54.2	0.00265	Year-round	Widespread
M. albicans	Shrub	Green	15.6 ± 3.4	0.00743	Mid-wet season	Widespread
M. alborufescens	Shrub	Black	17.5 ± 2.4	0.0125	Early-wet season	Endemic
M. corallina	Shrub	Yellow	3 ± 2.1	0.025	Wet-dry transition	Endemic
M. ferruginata	Tree	Black	28 ± 5.1	0.0274	Dry-wet transition	Widespread
M. ibaguensis	Shrub	Black	80 ± 4.5	0.00298	Mid-wet season	Widespread
M. irwinii	Treelet	Black	3.8 ± 2.3	0.14728	Dry season	Endemic
M. ligustroides	Shrub	Black	13.3 ± 2.8	0.01033	Dry season	Widespread
M. stenostachya	Shrub	Black	58.3 ± 8.2	0.0041	Mid-wet season	Widespread
M. theaezans	Shrub	Purple	34.8 ± 5.1	0.00138	Wet-dry transition	Widespread

* Data are from Silveira et al. (2012) and Lima et al. (unpublished data).

** Endemic species are those restricted to rupestrian fields of eastern Brazil (http://floradobrasil.jbrj.gov.br/2010/)

Results

Germinability of seeds within intact fruits was null (five species) or <4% (three species). Fruits from all species presented extensive signs of fungi infestation after the trials. Gut passage had no significant effect on germinability in three species (*M. albicans*, *M. ibaguensis* and *M. irwinii*; Tab. 2) and had minor effects on germinability in other four species. Gut passage cause interspecific differences among bird species but no-significant differences compared to hand-extracted seeds of *M. stenostachya*. Species-specific decreases in germinability were found in *C. urceolata*, *M. alborufescens*, *M. ligustroides* and *M. stenostachya*. For *M. ferruginata*, gut-passed seeds had lower germinability compared to hand-cleaned seeds irrespective of bird species (Tab. 2).

In regards to germination time, gut treatment had no effect on MGT of *M. irwinii* and *M. ligustroides* but significantly increased MGT in *C. urceolata* and *M. albicans* (Tab. 3). In contrast, gut treatment significantly decreased MGT in *M. ibaguensis* seeds (Tab. 3). Gut passage had species-specific effects on MGT of *M. ferruginata*, *M. alborufescens* and *M. stenostachya* (Tab. 3). Germination enhancement was not observed in any study species (Tab. 2 and 3).

Discussion

Our data provide evidence for potential positive effects of pulp removal on the seed germination of Cerrado melastomes. This study shows minor/neutral effects of gut scarification on the seeds, but rather, points out to a remarkable positive effect of depulping (seed cleaning) for all studied plants. Seed cleaning by birds improves dispersal quality by reducing the influence of the inhibition effect (Samuels & Levey 2005). The inhibition effect arises from high osmotic pressure caused by high sugar content of ripe fruits, light-blocking pigmentation and/or secondary metabolites that inhibit seed germination (Samuels & Levey 2005). Melastomes produce light-demanding seeds (Silveira et al. 2012) embedded in fruits containing germination inhibitors (Amaral & Paulillo 1992). Thus, seed cleaning potentially removes germination inhibitors and allows light to reach the photoblastic seeds (Lima et al., unpublished data). Moreover, seed cleaning decreases the probability of pathogen-induced death to seeds providing these seeds with increased chances of survival and germination.

In this study, the overall probability of germination for ingested and non-ingested melastome seeds differed only slightly. Though birds tend to have highly positive effects on seed germination (Traveset & Verdú 2002), increases in germinability following gut passage is far from universal, because of complexities in how plant and frugivore traits interact to influence seed response to gut passage (Traveset et al. 2001, 2007). In our study, we used generalist birds that also include insects in their diet. Birds that include large amounts of fruits in their diet have a proportionally shorter intestine and a smaller and less muscular gizzard than non-frugivorous birds, which may result in a more gentle treatment of the coats of seeds ingested by specialist frugivores (Traveset et al. 2007). Therefore, future studies are suggested to include specialist birds to determine whether any germination enhancement can be experienced in melastomes seeds following gut passage.

When frugivores accelerate seed germination (as in the case of *M. ibaguensis*), ingested seeds take less time to germinate than uningested seeds (Verdú & Traveset 2004), probably as a result of seed coat scarification (Traveset *et al.* 2001). Our data show bird-mediated differences in germination timing, which have been shown to affect plant fitness (Verdú & Traveset 2005). However, it is still unclear how differences in germination timing could affect seedling establishment. For the study species, a possible benefit plants may derive from accelerated germination is an increase in probabilities of seedling establishment. This may be particularly important in our study system because of two reasons. First, the chances of recruitment for small-sized pioneer plants may be largely affected by seedling emergence time during favourable conditions (Jacomassa & Pizo 2010, Silveira et al. 2012). Therefore, increased germination speed in pioneers may improve recruitment (Jacomassa & Pizo 2010) if early growing seedlings succeed during the intense intra-specific competition that follows germination (Weaver & Cavers 1979). Additionally, reduced germination time may be advantageous in fire-prone habitats (de Luis et al. 2008) such as the Cerrado. In this case, early germinants may have increased chances of growing and reproducing after a major disturbance, such as fire.

The complex nature of fruit-frugivore interactions implies in differences among bird's dispersal quality. Comparative tests of the scarification effect produced by different frugivores feeding on the same plant species often reveal large differences, while the same species of frugivore may have different effects on germination depending upon the species of plant studied (Traveset et al. 2007). Our data show heterogeneity of seed germination responses following gut passage in melastomes from the Cerrado. Similar results were obtained for small-sized rainforest melastomes (Elisson *et al*. 1993; Alves et al. 2007; Gomes et al. 2008). Because gut passage effects on seed germination of melastomes depend on both plant and frugivore species, it may be challenging to accurately establish dispersal-recruitment linkage compared to species with specialized dispersal systems.

In conclusion, Miconieae species potentially benefit from fruit consumption by the bird community exploiting their fruits. Overall, gut scarification had minor effects on seed germination of Cerrado melastomes and the benefits provided by gut passage to melastomes may be restricted to seed cleaning. We do recognize, however, that experimental conditions for seed germination may have affected our results, since germination patterns may differ between natural and controlled conditions (see Rodríguez-Pérez et al. 2005, Robertson et al. 2006). However, studies addressing germination patterns following gut passage under field conditions were mostly conducted for large-seeded species, probably because these trials are extremely difficult to be performed for small-sized seeds such as those of our melastomes. Together with the fertilization effect and the probability of seed deposition in favourable microhabitats, further studies under field conditions will further increase our ability to predict the contributions of bird dispersal to plant recruitment.

Table 2. Germinability (% ± SE) of seeds within intact fruits, hand-extracted seeds and seeds of *Clidemia* and *Miconia* species recovered from bird droppings. Different letters mean statistically significant means. Bold data refers to values of Kruskal-Wallis test. Means refer to four replicates of 25 seeds for each species.

Treatment	*C. urceolata*	*M. albicans*	*M. alborufescens*	*M. ferruginata*	*M. ibaguensis*	*M. irwinii*	*M. ligustroides*	*M. stenostachya*
Intact fruits	0	3.8	0	0	0.25	0	0	3
Hand-extracted	33(4.5)^{ab}	50(6.2)	79(6.8)^{ab}	36(3.3)^{a}	45(5)	81(6.0)	44(10.3)^{a}	41(9.4)^{ab}
T. amaurochalinus	5(2.5)^{c}	52†	83(5.3)^{a}	11.25(4.3)^{b}	48(4)	-	29(6.6)^{ab}	39(4.1)^{ab}
T. leucomelas	30(9.6)^{abc}	71(2.5)	76.5(4.9)^{abc}	9(3.4)^{b}	56(5.9)	76(5.2)	27(7.7)^{ab}	37(1.9)^{ab}
M. saturninus	-	-	-	-	-	-	26(2.6)^{ab}	34(9.1)^{ab}
Z. capensis	-	67(7.9)	64(2.3)^{bc}	-	-	-	-	17(3)^{b}
T. palmarum	-	63(4.1)	76(2.8)^{abc}	-	54(8.1)	-	29(10)^{ab}	40(7.1)^{ab}
T. sayaca	13(5)^{bc}	-	60(9.5)^{c}	17(3.8)^{b}	36(4.3)	81.1(9.8)	59(3)^{a}	-
S. ruficapillus	63(7.7)^{a}	57(5)	84(3.7)^{a}	14(4.8)^{b}	45(5.3)	77.9(3.7)	7(3.4)^{b}	46(7.4)^{a}
Test Statistic	6.4*	1.08	**12.5***	5.7**	1.67	0.38	5.12**	2.62*

*p< 0.05; **p< 0.01.

- interaction not studied due to low sample size.

† data not included in statistical analysis because of lack of replicates.

Table 3. Mean germination time (days ± SE) of hand-extracted seeds and seeds of *Clidemia* and *Miconia* species recovered from bird droppings. Different letters mean statistically significant means. Bold data refers to values of Kruskal-Wallis test. Means refer to four replicates of 25 seeds for each species.

Treatment	*C. urceolata*	*M. albicans*	*M. alborufescens*	*M. ferruginata*	*M. ibaguensis*	*M. irwinii*	*M. ligustroides*	*M. stenostachya*
Hand-extracted	17.8(1.2)^{a}	10.4(0.4)^{a}	9.1(0.6)^{a}	13.5(1.2)^{ab}	20.3(1.1)^{a}	12.1(0.4)	21.5(0.8)	16.6(1.9)^{b}
T. amaurochalinus	27.5(0.7)^{b}	16.8†	9.3(0.3)^{a}	11.1(1.7)^{b}	14.5(1.1)^{b}	-	19.5(1.6)	17.8(0.7)^{ab}
T. leucomelas	26.9(0.5)^{b}	17.8(0.9)^{c}	8.4(0.2)^{a}	15.7(2.9)^{ab}	14(0.3)^{b}	13.8(0.1)	21.1(2.9)	18.8(1.3)^{ab}
M. saturninus	-	-	-	-	-	-	20.5(0.2)	22.5(1.3)^{a}
Z. capensis	-	14.8(0.4)^{b}	11.4(0.6)^{b}	-	-	-	-	17(1.3)^{ab}
T. palmarum	-	17.4(0.8)^{ab}	9.6(0.6)^{a}	-	14.7(0.7)^{b}	-	21.5(1.3)	20.1(0.7)^{ab}
T. sayaca	26.5(0.4)^{b}	-	9.8(0.9)^{a}	20.1(1.6)^{a}	14.8(1.5)^{b}	13.9(1.5)	20.3(0.6)	-
S. ruficapillus	26.8(0.7)^{b}	16.2(0.6)^{ab}	8.6(0.6)^{a}	13.7(0.7)^{ab}	14.5(0.9)^{b}	12.6(0.7)	26.3(1.5)	16.3(0.9)^{b}
Test Statistic	**29.1***	20.7**	3.14*	3.8*	5.49**	**3.91**	1.7	3.41*

*p< 0.05; **p< 0.01.

- interaction not studied due to low sample size.

† data not included in statistical analysis because of lack of replicates.

In conclusion, we argue that recruitment of melastomes potentially depends on fruit consumption by the bird community exploiting its fruits. Overall, gut scarification had minor effects on seed germination of Cerrado melastomes and the benefits provided by gut passage to melastomes may be restricted to seed cleaning. We do recognize, however, that methodological issues may have affected our results (see Rodríguez-Pérez et al. 2005, Robertson et al. 2006). Germination trials under field conditions provide important information the ecological relevance of gut passage, but these trials are extremely difficult to be conducted for small-sized seeds such as those of our melastomes. Therefore, we report data on potential effects of gut passage on seed germination. Together with the fertilization effect and the probability of seed deposition in favourable microhabitats, further studies under field conditions will further increase our ability to predict the contributions of bird dispersal to plant recruitment.

CAPÍTULO III

THE CONTRIBUTION OF GERMINATION TRAITS TO NICHE DIFFERENTIATION IN PLANTS FROM DIFFERENT MICROHABITATS AND DISPERSAL MODES IN NEOTROPICAL SPECIES-RICH COMMUNITIES

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ARTIGO SUBMETIDO AO JOURNAL OF ECOLOGY

Abstract

Ecological correlates with seed and germination traits are poorly known, especially in Neotropical vegetation. Even after decades of research, the role of seed traits in determining species geographic range and ecological breadth remains inconclusive. Here, we studied for the first time whether seed germination requirements are associated with plant distribution at continental (endemics vs. widespreads) and local (xeric vs. mesic sites) scales in a Neotropical vegetation. The germination requirements of 50 sympatric species of Melastomataceae differing in geographic range, microhabitat preference and dispersal modes were examined in the seasonal and fire-prone rupestrian fields, a patchy and species-rich vegetation in southeastern Brazil. All species produced small-sized, photoblastic seeds with <3% dark germination. Under light conditions, there was a great diversity of germination responses to temperature, some of which were influenced by phylogeny. Optimum temperature for 71% species was within 20 and 30^{o}C, with only 10% species germinating at 35^{o}C. Less than 20% of species were physiologically dormant, most belonging to the endemic Microlicieae. Unexpectedly, interspecific variation was not associated with plant geographic distribution but rather, it was related to microhabitat preference. We observed a congruent pattern of distinctive germination responses in species from xeric vs. mesic habitats, with significant lower temperature ranges species from xeric habitats compared to plants from mesic sites. These results are supported by multivariate analyses which clustered species differing in dispersal mode and microhabitat preference, but not in geographic range. This suggests that plants from different functional groups experience opposing selective pressures. Our data provide further support to seed size playing an important role in the evolution of germination traits.

Synthesis. The evolution of accurate mechanisms for temperature and light sensing are important determinants of the regeneration niche, with phylogeny also influencing functional traits and thus niche differentiation. This study provides support for the importance of the regeneration phase determining plant distribution at local scales. Divergences in germination traits in species from different microhabitats and dispersal modes provide a mechanistic explanation for how spatial heterogeneity maintains coexistence in patchy, species-rich tropical plant communities.

Keywords: germination requirements, trait divergence, regeneration niche, temperature range, Cerrado, germination niche, Melastomataceae, plant coexistence, environmental heterogeneity.

Introduction

Understanding the factors that control plant distribution is still a challenging task for plant ecologists. There is a variety of putative evolutionary and ecological explanations for why some species have more extensive geographic range than others. These explanations include environmental variability, competition, niche breadth, latitude, colonisation-extinction dynamics, population density and dispersal ability, among others (Brown, Stevens & Kaufman 1996; Gotelli & McCabe 2002; Lester *et al*. 2007; Hörandl, Cosendai & Temsch 2008; Wang *et al*. 2009; Ranieri *et al*. 2011). Although these factors operate at different ontogenetic stages, the regeneration phase is critical for understanding species distribution, species richness-area curves and evolutionary divergence in plants (the regeneration niche hypothesis; Grubb 1977).

The regeneration phase is linked directly to seeds' traits (Grub 1977) and there are numerous ways by which seed traits can determine plant distribution. Seed and germination traits have been associated with fecundity, local abundance, range size, habitat segregation, seed persistence in the soil and dispersal ability (Thompson, Gaston & Band 1999; Azcárate *et al*. 2002; Fenner & Thompson 2005; Luna & Moreno 2010; Ranieri *et al*. 2011; Thomson *et al*. 2011). If plant distribution depends, at least to some extent, on the frequency of opportunities for regeneration from seed, then species with broad germination niches are expected to have larger ranges. However, current evidence has yielded inconclusive results, with endemics having lower, equal or even higher germination performance compared to their widespread counterparts (Simon & Hay 2003; Oliveira & Garcia 2011; Ranieri *et al*. 2011). The actual evidence for the association between germination requirements of species and their local and continental distribution range is inconsistent (Thompson & Ceriani 2003; Brändle *et al*. 2003), so further studies addressing the role of the regeneration niche in plant distribution are needed (Donohue *et al*. 2010).

In this study, we tested the regeneration niche hypothesis by comparing the seed germination requirements of 50 sympatric Melastomataceae differing in geographic distribution, microhabitat preference and dispersal modes. To the best of our knowledge, this is the first study in a Neotropical vegetation comprising multiple species aiming to test the influence of the regeneration niche on species range. Our focus on Melastomataceae was twofold. First, Melastomataceae is a dominant group in the study area (Conceição & Pirani 2005; Alves & Kolbek 2010) and comprises one of the most morphological, functional and ecological diverse clade in the Neotropics, thus we can examine whether the germination niche is associated with multiple life-history traits. Secondly, several within-family phylogenies are available for the family (see Fritsch et al. 2004, Goldenberg *et al*. 2008) and hence, we were able to implement phylogenetic comparative methods aiming to determine the contribution of historical factors on present-day variation in germination traits.

Our study area is ideal for testing the association of seed traits and plant distribution, the latter used here in a broad sense, encompassing both continental (geographic range) and local (microhabitat occupancy) dimensions. The montane vegetation at the Espinhaço Range called rupestrian fields, though restricted to a small geographic area, includes a large number of species varying in endemism levels. Island-like configuration of the highland rupestrian fields within lowland Cerrado (Neotropical savanna) vegetation promoted *in situ* speciation following reproductive isolation, the putative mechanism for the maintenance of endemic species (Echternacht *et al*. 2011). In addition, the intersection of the Espinhaço Range with lowland savannas, seasonally dry forests and semi-deciduous rainforests may have favoured the arrival and colonisation by species widespread in South America. Another remarkable feature of the rupestrian fields is habitat heterogeneity. This vegetation comprises a considerable diversity of pedoenvironments and associated vegetations mosaics, determined by microenvironmental aspects (Benites *et al*. 2007). Local drainage systems dictated by topography further diversify this environment by creating relatively humid (mesic) and arid (xeric) sites (Alves & Kolbek 2010), and a number of particular plant species-soil relationships (Conceição & Pirani 2005; Benites *et al*. 2007). Therefore, in this species-rich vegetation we were able to test simultaneously the role of the germination niche in influencing species range at both continental and local scales.

We performed multivariate and phylogenetically-controlled analyses aiming to check whether plants with different dispersal modes, local and continental distribution differ in seed germination traits. We expect widespread species to have wider germination niches compared to endemic ones, and anticipate that selection would have favoured the evolution of microhabitat-related germination requirements, so that plants from mesic sites would have lower temperature range for germination compared to plants in xeric ones.

Methods

Study site and study species

Seeds were collected in rupestrian fields vegetation along the Espinhaço Range (19º17′22″ S; 43º35′18″ W; 1260 m asl), a mountain chain spanning 1000km in length in eastern Brazil with altitudes varying between 900 and 1700m (Giulietti, Pirani & Harley 1997). These rupestrian fields occur primarily in areas above 900m asl where plant communities establish on shallow, acidic, nutrient-poor, and excessively drained quartzite-derived or ironstone soils (Giulietti, Pirani & Harley 1997; Benites *et al*. 2007). The Espinhaço Range was designated as an UNESCO Biosphere Reserve (UNESCO 2011), because of high levels of plant biodiversity and endemism, which are threatened by increasing human pressures (Giulietti *et al.* 1997). Plants often experience strong winds, high sun exposition, and water shortage during the dry winters. The distribution of rainfall is strongly seasonal, with a rainy season from October to April and a dry season from May to September (Madeira & Fernandes 1999). Fire is a recurrent phenomenon in the study site during the dry season.

We collected seeds from 50 species of Melastomataceae belonging to 17 genera and four tribes. Species had broad variation in geographic distribution, habitat and dispersal mode (Table S1). Unfortunately, precise data on the geographic range of most Neotropical species is not available as they are for European species (Thompson & Ceriani 2003; Brändle *et al*. 2003; Luna & Moreno 2010). Consequently, we decided to categorize species into the two extreme categories of a *continuum* of plant distribution: endemic species from the rupestrian fields and widespread species (those whose ranges extend beyond the rupestrian fields vegetation and/or in other Brazilian states or countries). The fruits of Melastomataceae are structurally diverse and dispersal mode varies as a function of fruit morphology (Clausing et al., 2000). Fruits of Miconieae are fleshy berries consumed by a wide diversity of frugivores, but mainly birds (Goldenberg & Shepherd 1998; Silveira 2011, Lima *et al*. 2012). Germination of Miconieae is slightly changed after passage through bird guts (Cap. 2), so cross-species comparisons are not biased. On the other hand, species in Merianieae and in the clade Melastome-Microlicieae produce dry capsules, which are abiotically dispersed (Cap. 1). Species assignments to microhabitats (xeric vs. mesic), dispersal mode and geographic range were independent (Table S1).

Seed collection and germination experiments

Mature fruits were collected during the peak of fruiting phenology during the 2008-2010 period (Silveira *et al*. 2012). Seeds were collected from at least 20 individuals per species occurring in their natural habitats and immediately set to germinate. In the case of species with low local abundance (*A. punctatissima*, *L. pulcherrima*, *L. coriacea*, *L. cordatum*, *M. sipolisii*, *M. ligustroides*, *M. rubiginosa*, *P. alpestris* and *T. candolleana*) the number of sampled individuals was lower (3-15).

The effects of light and temperature on seed germination were determined under controlled conditions for 34 species. We focused on light and temperature because these two factors are among the most important ones controlling seed germination (Fenner & Thompson 2005). For the other 16 species, which we lack enough seeds for conducting all experiments, we only report data for seeds exposed to 25ºC. This was shown to be the optimum temperature for germination for most species (see Results). Seeds were set to germinate in 9-mm Petri dishes layered with double sheet of filter paper moistened with Nistatin solution (2%) to avoid fungi growth. The Petri dishes were incubated under constant temperatures of 15, 20, 25, 30 and 35ºC at a 12:12h light: dark cycle (photosynthetic photon flux density = 25.86 µmol m^{-2} s^{-1}) and continuous dark for 30 days. This temperature range was selected because it covers the variation experienced by most species from rupestrian fields. The darkness condition was obtained by wrapping up the Petri dishes with double sheet of aluminium paper and germination was checked after 30 days. Seed germination was monitored at daily intervals and seeds were considered germinated when radicle emergence was observed.

For each replicate, we calculated final germination percentage (germinability), mean germination time (MGT; Ranal & Santana 2006), germination synchrony ($\overline{E}$; Ranal & Santana 2006) and light requirement index (LRI; Milberg, Andersson & Thompson 2000).

$$\mathrm{MGT} = \sum_{\mathrm{i}=1}^{\mathrm{k}} \mathrm{n_i t_i} \Big/ \sum_{\mathrm{i}=1}^{\mathrm{k}} \mathrm{n_i}$$

where n_i is the number of seeds germinated in the time i, t_i is the time from the start of the experiment to the *ith* observation, and k is the time of last germination.

$$\overline{E} = -\sum_{i=1}^{k} f_i \log_2 f_i \quad \text{being} \quad f_i = n_i \Big/ \sum_{i=1}^{k} n_i$$

where f_i is the relative frequency of germination, n_i is the number of seeds germinated in the day i and k is the time of last germination. Low $\overline{E}$ values indicate more synchronized germination whereas values approaching zero denote perfect synchrony.

$$LRI = Gl/(Gd + Gl)$$

where Gl = the germination percentage in light, and Gd = the germination percentage in darkness. LRI ranges from zero (germination occurring only under dark conditions) to one (total light requirement). Values near 0.5 indicate aphotoblastism.

Soil temperature was measured in both mesic and xeric sites using a thermistor probe NTC (Incoterm, Brazil). Superficial soil temperature was measured at monthly intervals at sites where the study species naturally occur between September and April (the establishment season). The probe was introduced in parallel to the soil surface at a 1-cm depth in three substrate types: rocky outcrops, sandy grasslands and in streamside vegetation soil. Soils near watercourses are hydromorphic and waterlogged during the rainy season. Soils associated with rocky outcrops have high levels of fibric organic material and accumulate organic matter. Grassy fields soils, in turn, are mostly made of coarse sand derived from histosoils (Benites *et al*. 2007). The latter two substrates were considered as xeric sites and the former was considered as mesic sites. Measurements (n=3 per substrate) were obtained at 06:00 and 13:00 in the same day to account for maximum daily variation. We have followed the same procedures to measure soil moisture with the aid of a TDR (Soil Moisture, Trase System I 6050X1).

Statistical analysis

For each treatment, four replicates of 50 seeds (25 seeds for *Miconia* and *Leandra*) were mounted. Data in percentage were squared root arcsine transformed and significant differences in germinability and MGT for each species were determined through one-way ANOVA followed by Tukey test ($\alpha = 0.05$; Zar 1996). When data did not meet the assumptions of the parametric tests, statistical differences among temperatures were determined through Kruskal-Wallis followed by Conover test (Conover 1980). Optimum temperature ranges were defined as the temperature(s) allowing maximum germinability in the least amount of time.

Maximum temperature range (MTR) was defined as the thermal amplitude allowing which ≥ 5% seeds germinated germination. MTR was calculated by subtracting the lower temperature from maximum temperature, thus varied from 20ºC to 5ºC. Species with wide MTR (15-20ºC) were considered eurythermic, and species with narrow MTR (5-10ºC) were assigned as stenothermic. Chi-square tests were used to determine differences in the frequency of stenothermic and eurythermic species among plant functional types. T test for independent samples were used to compare MTR from species belonging to different categories of geographic range, microhabitat preference and dispersal mode.

A principal component analysis (PCA) was carried out based on the correlation matrix of four germination traits: germinability, MGT, $\overline{E}$ and temperature range. LRI was excluded from the analyses because there is a conserved trait (Silveira *et al*., unpublished data) and thus, showed low among-species variance. A second PCA was run based on the correlation matrix germinability under five temperatures. Data were log-transformed for a better fitting. A cluster analysis was run based on the single linkage method with the Morisita-Horn index for quantitative traits.

Phylogenetic-controlled statistics

Briefly, we reconstructed a pruned phylogenetic tree of the studied species in the Phylomatic (http://www.phylodiversity.net/phylomatic/), randomly resolved the polytomies (using the software Mesquite) and dated branch length in Phylocom. We implemented analyses of phylogenetic signal and divergence/convergences of temperature range in nodes along the phylogenetic tree (see Supplementary material for further details on phylogenetic-controlled statistics). A significant divergence means that trait divergence between sister groups was higher than expected by chance, whereas a significant convergence means that the divergences in a given trait was lower than expected by chance in two lineages arising from a given node.

Results

The substrates of the microhabitats from the rupestrian fields exhibited contrasting environmental conditions. Soil moisture in mesic sites ranged from 16.6% to 35.5% at the end of the rainy season. In contrast, soil moisture in more xeric sites was lower ranging from 2.8% to 31.5% in rocky outcrops and 5.4% to 25.2% in sandy soils. A decrease in soil moisture was observed at all sites in February, but values remained higher for moist sites year-round (Fig. 1). Minimum monthly temperature was quite similar for all sites and was nearly 20°C year-round. Maximum monthly soil temperature in xeric sites ranged from 27 to 34°C, whereas in mesic soils, variation was between 23-31°C. Mean daily soil temperature variation was higher (between 8-12°C) for xeric sites compared to soils associated with mesic ones (4-8°C). For all sites, daily soil fluctuations were lower at the onset of the rainy season, increased at mid-rainy season and then decreased again (Fig. 1).

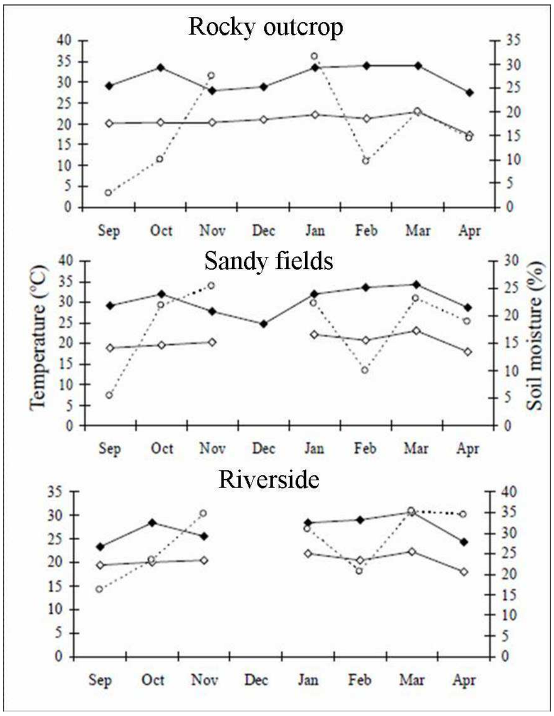


Figure 1. Mean minimal and maximum monthly temperature (diamonds) and soil moisture (circles) in three types of substrates in the rupestrian fields of Serra do Cipó (south of Espinhaço Range) during the establishment season.

All 50 species produced small-sized, photoblastic seeds with <3% dark germination (Table S1). Under light conditions, however, there was a great interspecific variation in germination responses to temperature. Germinability was skewed towards values lower than 20% regardless of the temperature (Fig. 2A). Germinability above 60% was never observed at 15 and 35^{o}C. Only five species (*A. alsinaefolia, L. confertiflora, M. ibaguensis, M. stenostachya* and *R. cordata*), four of them from mesic sites, were able to germinate to >5% at 35^{o}C, but values never reached percentages >40% under this temperature (Fig. 2A, Fig. 3). A very different frequency distribution was observed for MGT. Most seeds germinated at intermediate values (between 6 and 18 days) under 20 and 25^{o}C. Fast germination (MGT <6 days) was never observed under 15 and 35^{o}C (Fig. 2B). In general, species from mesic sites had lower MGT under optimum temperatures compared to species from xeric sites (Fig. S1).

Eight out of 34 species showed physiologically dormant seeds. Hence, the following results apply for the remaining 26 species. For those species, nine were eurythermic and 17 were stenothermic (Fig. 3). Although a larger proportion of eurythermic species were found in mesic sites (6 species), this proportion was not significant (χ^2=0.47; p=0.49). In contrast, more stenothermic species were found in xeric sites than would be expected by chance (χ^2=8.07; p=0.004). With regards to the geographic range, half of the endemic species were stenothermic and half were eurythermic. For widespread species, no significant differences were detected between the frequency of eurythermic and stenothermic species (χ^2=3; p=0.08). Average MTR for plants in mesic microhabitats (12.92± 1.42, SE) was significantly higher than plants in xeric ones (8.61±1.06; W=55, p= 0.02) but no significant difference was found in MTR between endemic (10.35±1.25) and widespread species (10.3±1.23; W=111, 0.98). Non-significant differences were also observed between average MTR in species with biotic (10±1.1) and abiotic dispersal (11.8±1.3; t= 1.07; p= 0.29).

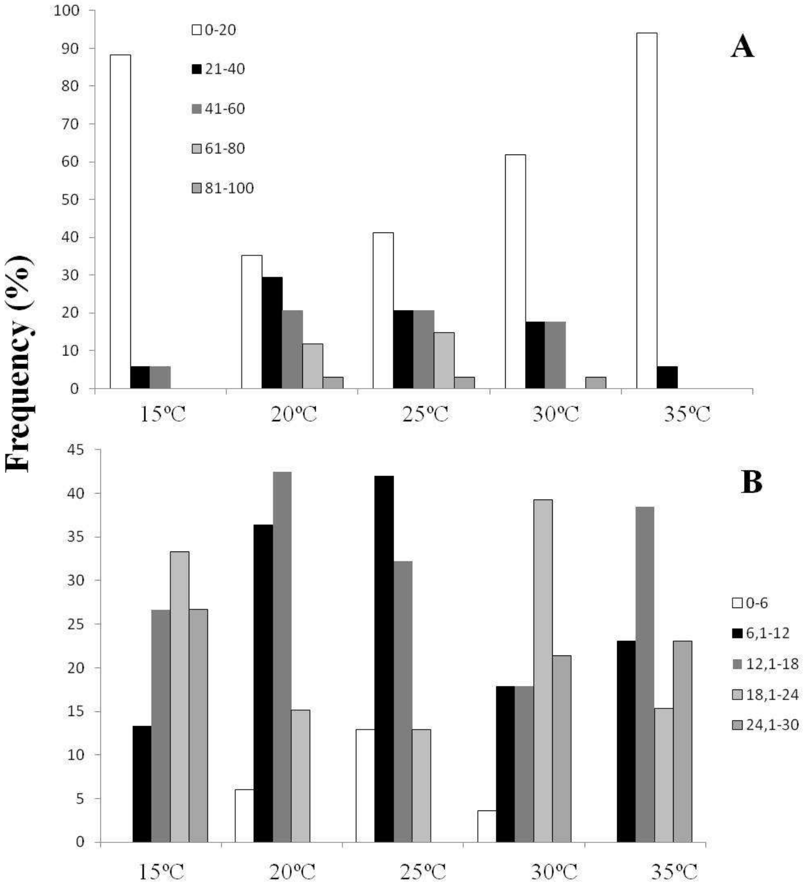


Figure 2. Frequency distribution pattern of germinability (A, percentage of germination) and mean germination time (B, in days) of 34 Melastomataceae species under controlled conditions of temperature. The numbers inside the figures refer values of germination percentage (A) and days (B), respectively.

Optimum temperature range for germination was quite similar for all species. Among the studied species, 15 species had optimum temperature range between 20-25^{o}C. Wider temperature ranges were observed for *R. cordata* and *L. subulata* (15-30^{o}C) and for *M. stenostachya* (20-30^{o}C). Optimum temperature range of 20-30^{o}C was observed for *C. sessilis, L. aurea, M. albicans* and *M. elegans*, while optimum range of 25-30^{o}C was observed for *L. campos-portoana* and *M. cipoensis* (Fig. 3). The narrowest optimum temperature for germination of 20^{o}C and 25^{o}C were shown by *L. confertiflora* and *M. ferruginata*, respectively.

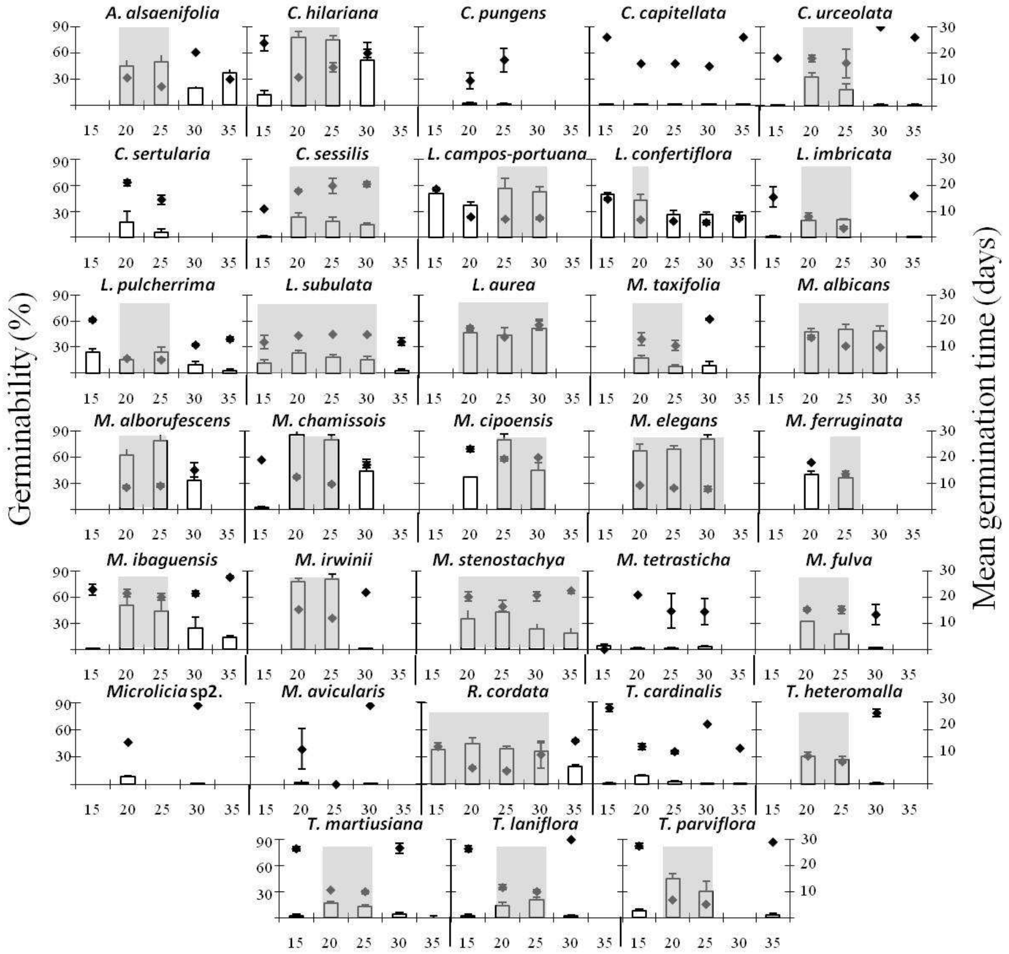


Figure 3. Average (±SE) germinability (bar) and mean germination time (circles) of 33 species of Melastomataceae under different temperatures. Shaded areas represent optimum conditions for germination and are not shown for dormant species.

Optimum temperature range for germination was quite similar for all species. Among the studied species, 15 species had optimum temperature range between 20-25^{o}C. A wider temperature range was observed for *R. cordata* and *L. subulata* (15-30^{o}C) and for *M. stenostachya* (20-30^{o}C). Optimum temperature range of 20-30^{o}C was observed for *C. sessilis, L. aurea, M. albicans* and *M. elegans,* while optimum range of 25-30^{o}C was observed for *L. campos-portoana* and *M. cipoensis* (Fig. 3). Optimum temperature for germination was 20^{o}C and 25^{o}C for *L. confertiflora* and *M. ferruginata*, respectively.

We observed three groups of species according to germination responses to temperature (Fig. 4). The first group (I) was formed by physiologically dormant species, mainly in the Microlicieae tribe (Fig. 4) and had lower germinability under all conditions. The second and larger group was formed by two subgroups with eurythermic germination: the first comprised phylogenetically unrelated species of eurythermics in mesic sites (II-A) and the second subgroup consisted of a mix of plants in mesic and xeric sites (II-B) including most species of *Miconia.* The third group (III) consisted mostly of stenothermic, unrelated in xeric sites. The distribution of species of same genus in distinct groups point out that the cluster based on temperature responses of germination was mostly independent of phylogenetic affiliation (Fig. 4).

In the phylogenetic tree (Fig. 5), we observed a significant divergence between Miconieae and the clade Microliceae-Melastomeae. The Miconiae clade presented a narrow temperature range of 5 to 15ºC, with 64% of studied species presenting a temperature range of 10ºC. In the Microliceae-Melastomae clade the temperature range was wider (5-20ºC) and more heterogeneous among species. There was no relationship between the phylogenetic relatedness and microhabitat preference of geographic range (Fig. 5).

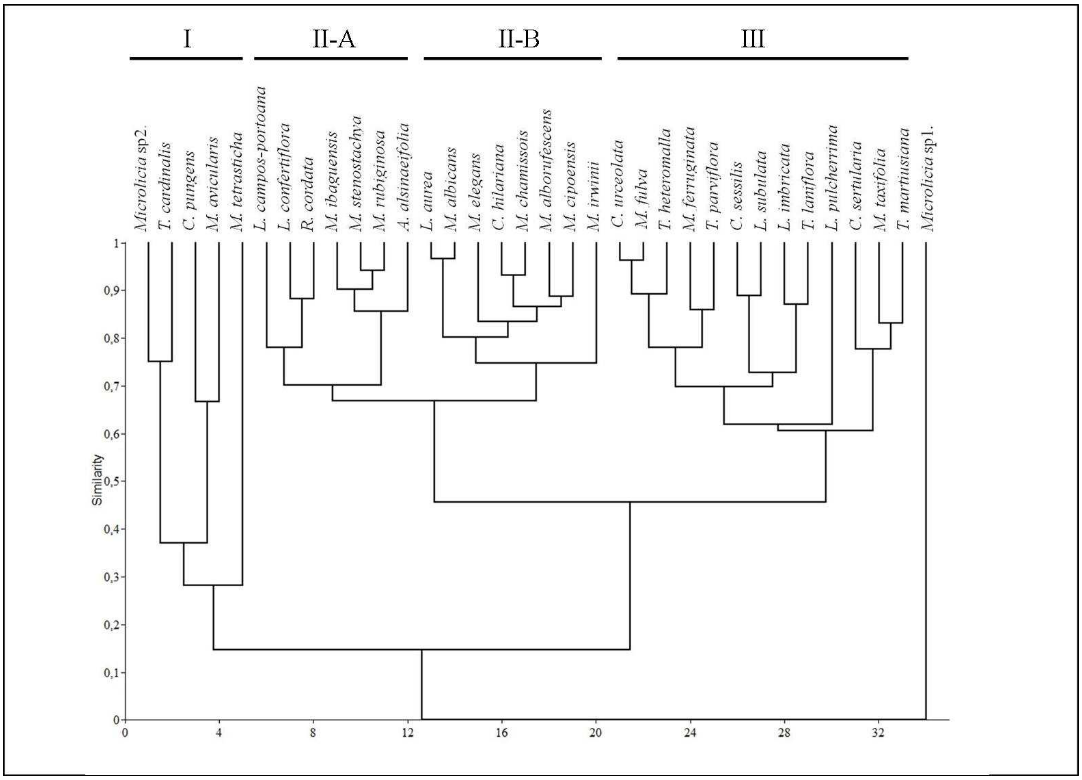


Figure 4. Dendrogram showing the relationship among 34 species of Melastomataceae based on seed germinability (percentage) under constant temperatures of 15, 20, 25, 30 and 35°C.

We did not find any specific association of geographic distribution range and germination traits. Endemic and widespread species were evenly distributed in the PCA with high overlapping between species in the two functional groups (Fig. 6A). However, species with biotic dispersal were more associated with higher germinability and asynchronous germination, whereas species with abiotic dispersal were associated with slower germination time and higher temperature range (Fig. 6B). The PCA separated species according to microhabitat. Most of the species from mesic habitats were associated with higher germinability, asynchronous germination and higher temperature range, whereas most species from more xeric habitats were associated with slower germination time (Fig. 6C). The PC1 and PC2 explained 54.3% and 26.4% of total variance, respectively. Species with different ranges were not segregated in the PCA built with the matrix of temperatures. In contrast, species in different microhabitats, and to a higher extent, with distinct dispersal modes formed relatively differentiated groups (Fig. 2S).

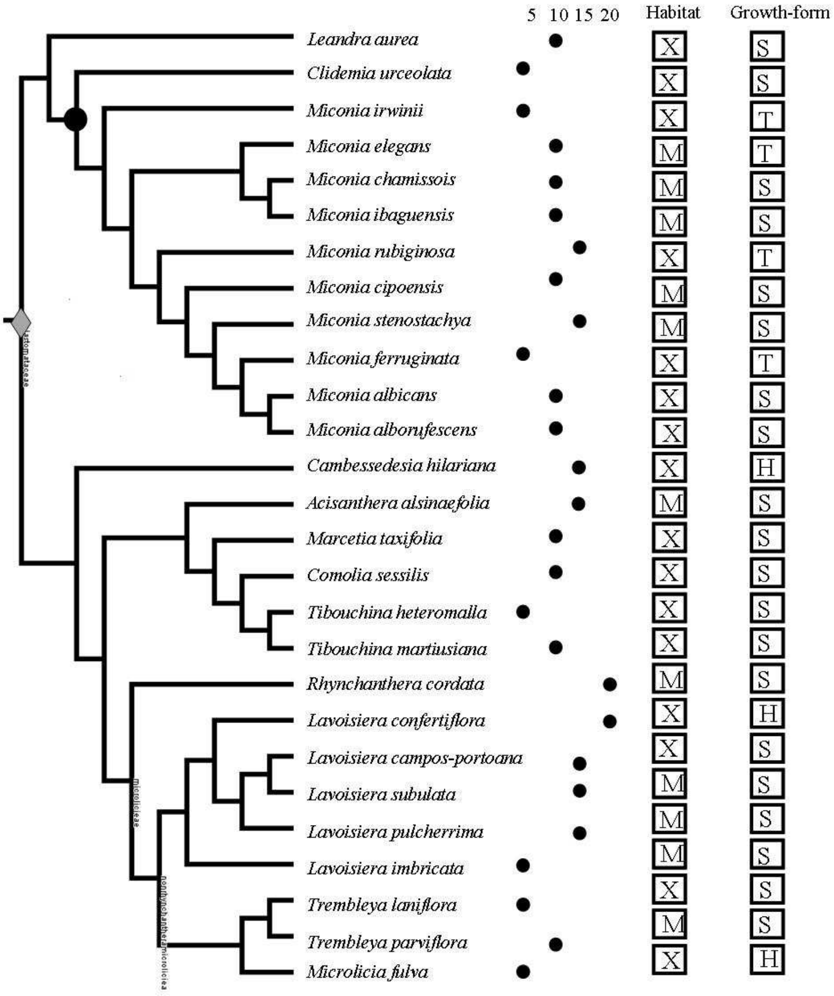


Figure 5. Traitgram showing the topology of the phylogenetic tree of 27 Melastomataceae species showing the location of divergences (gray diamonds) and convergences (filled circles) in temperature range for germination. Temperature range variation is seen in the mid panel and plant life-histories are shown in the right panel. M- mesic site, X- xeric site, T- tree, S-shrub, H-subshrub.

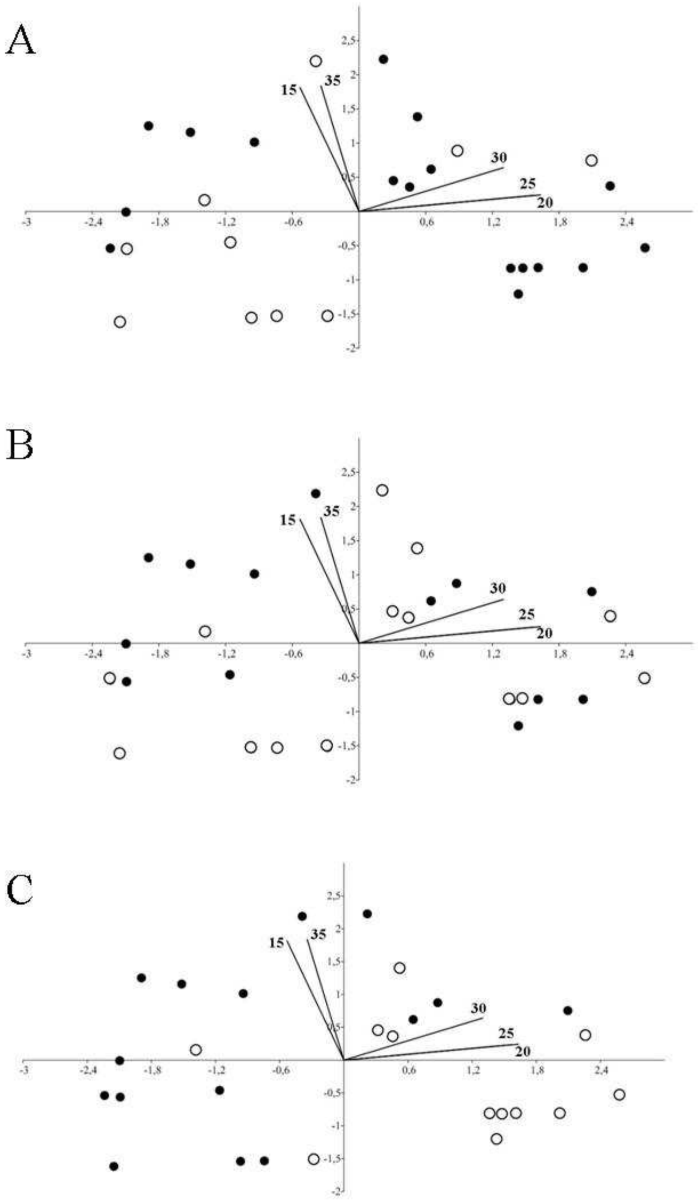


Figure 6. Biplot representation of trait scores of the first two axes of the principal component analysis (PCA) four germination traits (germinability, germ; temperature range, temp; synchrony, sync; mean germination time, MGT) in 34 species of Melastomataceae from different functional groups. Functional groups are represented by microhabitats (A), geographic range (B) and dispersal mode (C). Open circles represent plant in xeric sites, widespread species and with biotic dispersal mode. Full circles represent plant in mesic sites, endemic species and with abiotic dispersal mode.

Discussion

Plants have evolved accurate mechanisms for temperature and light sensing (Pearson *et al*. 2003; Penfield 2008), and their remarkable responses to small changes in variation of these cues has been associated with strategies to ensure germination under favourable conditions for seedling establishment (Kos & Postchold 2007). All Melastomataceae species studied here presented a homogeneous pattern of photoblastic positive response. Light-induced germination is typical of many small-seeded pioneer species (Pearson *et al*. 2003) and may have evolved to prevent emergence during unfavourable conditions, such as burial (Fenner & Thompson 2005). Small-seeded species are dependent on light for germination because they produce seedlings lacking enough reserves to reach soil surfaces following dark germination (Milberg, Andersson & Thompson 2000). For the small-sized species studied here, both seed size and the photoblastic response are conserved traits (Silveira *et al*., unpublished data), indicating evolutionary stasis on these traits along their history. The strong phylogenetic signal in the photoblastic response implies in null or few evolutionary changes in this trait (Silveira *et al*., unpublished data) and suggests that light response is homogeneous across species. Therefore, seed responses to light do not play an important role in germination niche differentiation in Melastomataceae from the rupestrian fields.

Germination responses to temperature, in turn, were very heterogeneous among species and had great potential for creating niche differentiation. Still, our data do not support the allegedly association between species germination requirements and their geographic distribution range. The evidence suggesting that widespread species have wider germination niches compared to endemic counterparts is inconsistent, at best (Donohue *et al*. 2010). Many explanations have been proposed to explain the lack of association between germination niche and adult niche. The most straight-forward is that mature plant traits are more important than seedling emergence in determining plant ranges (Thompson & Ceriani 2003). Other explanations include edaphic isolation, lower competitive ability, low phenotypic plasticity and climatic changes in the Quaternary, dispersal limitation and environmental filtering (Bevill & Louda 1999; Lloyd, Lee & Wilson 2002; Simon & Hay 2003; Thompson & Ceriani 2003; Negreiros *et al*. 2009; Donohue *et al*. 2010). For the Melastomataceae, unusual high levels of apomictic species have been reported, especially in the Miconieae (Goldenberg & Shepherd 1998). Apomictic species seem to have larger distribution ranges compared to their sister groups (Hörandl, Cosendai & Temsch 2008), and this appears to be the case for apomictic and widespread species such as *M. albicans*, *M. stenostachya* and *M. rubiginosa*.

To date, the association of germination patterns and species geographic distribution and microhabitat preference have not been determined unambiguously (Gomes *et al*. 2001; Simon & Hay 2003; Ranieri *et al*. 2003; Garcia, Jacobi & Ribeiro 2007; Oliveira & Garcia 2011; Ranieri *et al*. 2011). We have not found associations between germination requirements and geographic range of species, but rather find strong evidence for the association of species germination traits and local distribution (microhabitat preference). All data were consistent in showing that species from mesophytic sites had wider temperature ranges compared to species from xeric sites. Seeds from mesic sites are also heavier, germinate to higher percentages and take less time to germinate compared to species from xeric microsites (Silveira *et al*., unpublished data). Our results are consistent with studies developed in temperate habitats, which actually show distinctive seed and germination traits in species from humid and dry microsites, but not for species differing in geographic ranges (Leyer & Pross 2009; Wang *et al*. 2009). In addition, we show that species in Miconieae had more similar MTR than would be expected by chance, indicating the role of phylogeny determining temperature-dependent germination and thus, the ecological niche.

The divergence in germination traits between dry-fruited and fleshy-fruited species shown here suggests that germination traits differ among species with different dispersal modes. Distinct germination behaviour in species with different dispersal modes may be caused by differences in seed size. The association between the evolution of fleshy fruits and increasing seed size is expected either because the reduced dispersal capability following an increase in seed mass may be counterbalanced by evolution of traits mediating seed dispersal by animals, or because increasing availability and capabilities of frugivores may promote the evolution of fleshy fruits and allow an increase in seed size (Bolmgren & Eriksson 2010). Seeds from fleshy-fruited study species are heavier compared to seeds from dry-fruited species (Silveira *et al*., unpublished data), which agrees with the study of Wang *et al*. (2009) who also found distinctive germination strategies in plants with different dispersal modes. Our data suggest that the correlated evolution of seed size and fruit morphology (see also Bolmgrem & Eriksson 2010; Silveira *et al*., unpublished data) is accompanied by changes in germination traits. However, this result must be interpreted with caution. Because all fleshy fruits species studied here belong to Miconieae, it is impossible to separate the effects of phylogeny from fruit type. Indeed, the positive cross-species correlation between seed size and germinability is not significant after phylogeny is accounted for (Silveira *et al*., unpublished data). As a result, additional comparative data are required to shed light in the correlated evolution of seed size, fruit morphology and germination traits.

With regards to phylogeny effects, we show that MTR was considered a conserved trait in the Miconieae, meaning that few and small divergences occurred along the evolutionary history and in the occupation of particular favourable regeneration niches (Losos 2008). The patchy distribution of microhabitats at the rupestrian field implies that the spatial structure of populations of Miconieae species depends on the likelihood of seeds reaching a safe site (with suitable temperature conditions) following biotic dispersal. The likelihood of establishment depends on whether birds and ants transport seeds to suitable sites with adequate conditions for germination and establishment (Silveira 2011; Lima *et al*. 2012). The shorter dispersal distances in the dry-fruited species is due to abiotic dispersal and lower plant heights (Thomson *et al*. 2011). Many species in this group, particularly in Microlicieae, have gravity dispersal (Fritsch *et al*. 2004), so most seeds just fall beneath the parent plant where seeds have a higher probability of finding similar conditions for regeneration as their mother-plants. However, the chances of establishment and survival are lower beneath the parentals (Janzen 1970), and the evolution of seed dormancy in those species (Silveira *et al*. 2012, unpublished data) may help spreading germination in space thus, potentially affecting population spatial structure.

The chances of recruitment for small-sized species are largely affected by seedling emergence time (Pearson et al. 2003). That optimum temperature ranges for germination overlapped for most studied species suggest intense interspecific competition by seedlings emerging at the onset of the rainy season. However, the plethora of germination strategies of Melastomataceae, determined in part from trait displacement, in part from historical effects, potentially favours species coexistence. On the one hand, natural selection may favour rapid germination if early-growing seedlings obtain competitive advantages under suitable conditions (Weaver & Cavers 1979). This could be the case for species in mesic sites. On the other hand, seed dormancy and delayed germination may have been selected as strategies to avoid competition and increased plant fitness under conditions of relaxed competition (see Silveira *et al*. 2012). This strategy would otherwise have evolved in species from more xeric sites. The large dynamism in germination strategies shown here may be even more complex when disturbance regimes are considered. In the fire-prone and nutrient-poor vegetation, early and fast-growing seedlings are expected to derive great fitness benefits from rapid germination after fires (de Luis et al. 2008). So, the increased frequency of man-made fires may pose strong environmental filtering selecting for faster germination.

Under the predicted scenarios of global warming, increasing soil and air temperatures are altering environmental cues for germination (mainly temperature and water; Walck *et al*. 2011), resulting in dramatic changes in recruitment from seed (Ooi, Auld & Denham 2009). In this study, only five species germinated at 35ºC and other 13 species had reduced germinability at 30ºC. Therefore, we anticipate suboptimal germination and/or reduced regeneration from seed in most Melastomataceae species under warming conditions because of the negative and detrimental effects of high temperatures on seed germination. This can especially threaten the endemic species with restricted distribution ranges. Reduced regeneration from seed is expected to have ramifying consequences at population level (Walck *et al*. 2011), what might ultimately change the community structure of the rupestrian fields vegetation.

In conclusion, our study suggests that the germination niche is not related with plant distribution at continental scales and thus, germination breadth may not be an important factor controlling plant species range in rupestrian fields. Germination niche differentiation between species in xeric and mesic sites underpins the idea that germination traits are related to plant distribution at local scales. The evolution of microhabitat-specific germination requirements helps providing a mechanistic explanation on how spatial heterogeneity maintains plant coexistence in these species-rich communities. Rapid evolutionary changes in germination niche may have contributed to the colonisation dynamics of spatially heterogeneous Neotropical habitats. Germination niche differentiation between species with different dispersal modes indicates that evolutionary transitions in fruit morphology are accompanied not only in differences of seed size, but also by changes in germination traits. Our data have important implications for conservation of the threatened rupestrian fields and can lead to better management of endemic species and more effective restoration practices. Studies with unrelated taxa are encouraged to confirm the generality of the patterns found here.

Electronic supplementary material

Silveira et al. – The contribution of germination traits to niche differentiation in plants from different microhabitats and dispersal modes in Neotropical species-rich communities

Contents:

1) Table S1
2) Phylogenetic comparative analyses
3) Figure S1
4) References

Table 1. Life-history traits and distribution of 50 Melastomataceae species from Neotropical montane grasslands in Brazil.

Species	Habitat	Geographic distribution	Seed size (mg)	LRI
Acisanthera alsinaefolia	Mesic	Endemic	0.00093	0.99
Acisanthera punctatissima	Xeric	Endemic	-	1
Cambessedesia hilariana	Xeric	Endemic	-	1
Chaetostoma armatum	Xeric	Widespread	0.0006	1
Clidemia capitellata	Xeric	Widespread	0.0017	1
Clidemia urceolata	Xeric	Widespread	0.00265	1
Comolia sertularia	Xeric	Endemic	0.01155	0.971
Comolia sessilis	Xeric	Endemic	-	1
Lavoisiera campos-portoana	Xeric	Endemic	0.0027	1
Lavoisiera caryophyllea	Mesic	Endemic	0.0025	1
Lavoisiera confertiflora	Xeric	Endemic	0.0023	1
Lavoisiera cordata	Xeric	Endemic	0.00995	1
Lavoisiera imbricata	Mesic	Endemic	0.0024	1
Lavoisiera pulcherrima	Mesic	Endemic	-	0.98
Lavoisiera subulata	Mesic	Endemic	0.00275	1
Leandra aurea	Xeric	Widespread	-	1
Leandra coriacea	Xeric	Widespread	-	1

Lithobium cordatum	Xeric	Endemic	-	1
Macairea radula	Mesic	Widespread	-	1
Marcetia taxifolia	Xeric	Widespread	0.0009	1
Merianthera sipolisii	Xeric	Endemic	-	1
Miconia albicans	Xeric	Widespread	0.007425	1
Miconia alborufescens	Xeric	Endemic	0.01245	1
Miconia cipoensis	Xeric	Endemic	0.027725	0.987
Miconia chamissois	Mesic	Widespread	-	1
Miconia corallina	Xeric	Widespread	0.024	1
Miconia elegans	Mesic	Widespread	-	1
Miconia ferruginata	Xeric	Widespread	0.013675	1
Miconia ibaguensis	Mesic	Widespread	0.0012	1
Miconia irwinii	Xeric	Endemic	0.0736375	0.975
Miconia ligustroides	Xeric	Widespread	0.010325	1
Miconia stenostachya	Xeric	Widespread	0.0041	1
Miconia rubiginosa	Xeric	Widespread	-	1
Miconia theaezans	Mesic	Widespread	0.00138	1
Microlicia avicularis	Xeric	Endemic	-	1
Microlicia fulva	Xeric	Widespread	0.00035	0.973
Microlicia graveolens	Mesic	Endemic	-	1
Microlicia sp.	Xeric	Endemic	0.00095	1
Microlicia sp2	Xeric	Endemic	0.00045	1
Microlicia tetrasticha	Xeric	Endemic	0.00043	1
Pterolepis alpestris	Mesic	Endemic	-	1
Rhynchanthera cordata	Mesic	Widespread	0.00255	1
Rhynchanthera grandiflora	Mesic	Widespread	0.0011875	1
Tibouchina candolleana	Xeric	Widespread	-	1
Tibouchina cardinalis	Xeric	Endemic	0.002825	1
Tibouchina heteromalla	Xeric	Widespread	0.002775	1
Tibouchina martiusiana	Xeric	Widespread	0.0008	0.791
Tibouchina sp.	Xeric	Endemic	0.0009	1

Trembleya laniflora	Xeric	Endemic	0.0005	1
Trembleya parviflora	Mesic	Endemic	-	0.989

Phylogenetic comparative analyses

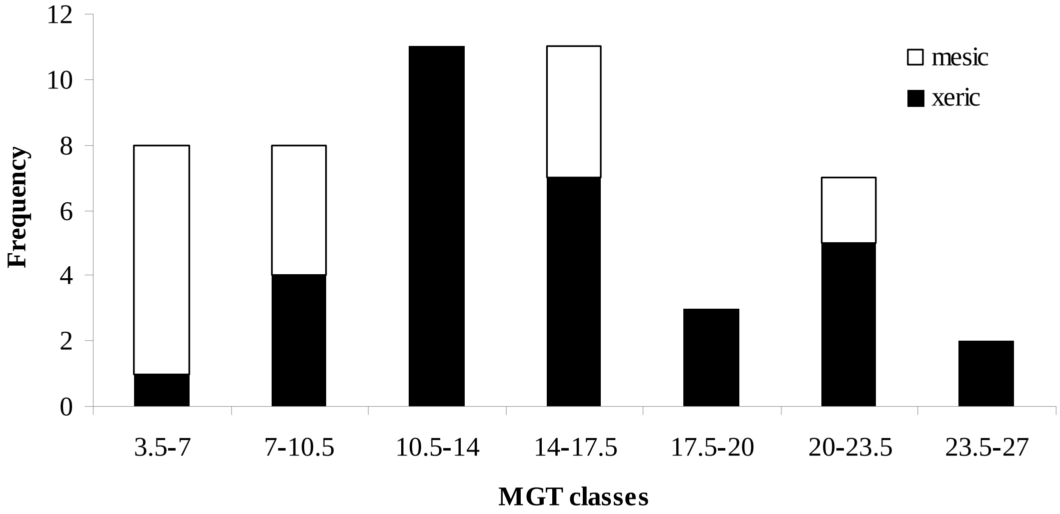
To understand the evolution of seed traits in Melastomataceae, an ultrametric phylogenetic tree showing relationships among the studied taxa was built. A pruned tree was built with the study species as terminal tips using the maximally resolved tree with the use of Phylomatic (http://www.phylodiversity.net/phylomatic/).

The occurrence of several polytomies in our tree (Fig. S1) may prevent the correct interpretation of the evolution of seed dormancy. However, most polytomies were in terminal taxa and thus, considered soft polytomies (Webb *et al.* 2006), which are likely to cause low interference in interpretation (Ackerly 2009). Polytomies in the tree were resolved by randomly generating 100 fully resolved trees using Mesquite (Maddison & Maddison 2008). Species relationships were improved and polytomies were resolved based on available and published data for the taxa relationships within the family (Renner 1993, Clausing & Renner 2001, Fritsch et al. 2004, Goldenberg et al. 2008, Michelangeli et al. 2008). Unpublished theses were also used to determine relationships at generic and specific levels.

Dating branch length is important to avoid bias on estimates of phylogenetic distance. Based on estimates on clade age for Melastomataceae, we calibrated the tree by dating know nodes with the Branch Length ADJustment (BLADJ) algorithm of Phylocom (Webb *et al.* 2006). We were able to perform node dating by including node age estimates for five nodes, including the root node (known estimated ages for the root node are a prerequisite for BLADJ. Even with only a few nodes dated, the resulting phylogenetic distances can be a marked improvement on simply using the number of intervening nodes as a phylogenetic distance (Webb et al. 2006). Age estimates were extracted from Renner & Meyer (2001), Renner et al. (2001), Fritsch et al. (2004) and Rutschmann et al. (2007). Undated nodes were assigned equal branch lengths between nodes for which age estimates are available, minimizing tree-wise variance in branch length (Moles et al. 2005, Milla 2009).

We obtained the phylogenetic signal and calculated divergence and convergence across nodes in the phylogenetic tree for temperature range. A significant divergence means that trait divergence between sister groups is higher than expected by chance whereas a significant convergence means that the divergences in a given trait is lower than expected by chance in two lineages arising from a given node. Then, we obtained tree-wise phylogenetic signal for each of the five seed traits (Blomberg et al. 2003). Trait values were permuted across the tips of the tree 19,999 times to test whether the observed values are significantly different than would be expected by chance (Moles et al. 2005, Milla 2009). All analyses were implemented with Analysis of Traits (AOT) package of Phylocom (Webb et al. 2006).

Figure S1. Frequency distribution of Mean Germination Time (MGT) classes for seeds of 34 Melastomataceae species from rupestrian fields in Southeastern Brazil under optimum temperatures.



CAPÍTULO IV

EVOLUTION OF PHYSIOLOGICAL DORMANCY MULTIPLE TIMES IN MELASTOMATACEAE FROM NEOTROPICAL MONTANE VEGETATION

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ARTIGO PUBLICADO NO SEED SCIENCE RESEARCH
DOI:10.1017/S0960258511000286

Abstract

We investigated seed dormancy among species of Melastomataceae from Neotropical montane vegetation of Brazil. Four out of 50 studied species had dormant seeds: *Miconia corallina* (Miconieae), *Tibouchina cardinalis* (Melastomeae), *Comolia sertularia* (Melastomeae) and *Chaetostoma armatum* (Microlicieae). For these four species, germinability of seeds collected in different years was always <10%, and the percentages of embryoless seeds and non-viable embryos were both insufficient to explain low or null germinability. This is the first unequivocal report of seed dormancy in tropical Melastomataceae. The production of seeds with permeable seed coats and fully-developed, differentiated embryos indicates the occurrence of physiological dormancy. The reconstructed phylogenetic tree of the 50 species suggests that physiological dormancy evolved multiple times during the evolutionary history of Melastomataceae in this vegetation. Physiological dormancy evolved in species and populations associated with xeric microhabitats, where seeds are dispersed in unfavourable conditions for establishment. Therefore, drought-induced mortality may have been a strong selective pressure favouring the evolution of physiological dormancy in Melastomataceae. We argue that dormancy may have been independently selected in other lineages of Cerrado plants colonizing xeric microhabitats and dispersing seeds at the end of the rainy season. The contributions of our data to the understanding of seed dormancy in tropical montane vegetation are discussed.

Keywords: Cerrado, convergent evolution, drought-induced mortality, Espinhaço Range, seasonality, rupestrian fields.

Introduction

The seed habit contributed decisively to the evolutionary success of gymnosperms and angiosperms. Seeds provided plants with large ecological and evolutionary advantages, including extended survival periods after dispersal, increased resistance to adverse environmental conditions, increased dispersal distance and, subsequently, increased gene flow (Linkies et al., 2010). Another remarkable event in the life history of seed plants was the evolution of dormancy. Among the numerous definitions of dormancy (Harper, 1977; Baskin & Baskin, 2004; Finch-Savage & Leuber-Metzger, 2006; Finkelstein et al., 2008; Linkies et al., 2010), a dormant seed can be defined as one that does not have the capacity to germinate in a specified period of time under any combination of normal physical environmental factors that otherwise are favourable for its germination (Baskin & Baskin, 2004). Because germination is an irreversible process, selection should favour strategies that prevent seeds from germinating under unfavourable conditions for seedling establishment (Jurado & Moles, 2002).

Here, we report the occurrence of dormancy in Melastomataceae from Neotropical montane vegetation (rupestrian fields) and reconstruct a phylogenetic tree to obtain a better understanding of the evolutionary history of seed dormancy in this family. The rupestrian fields belong to the Cerrado biome, which is the most species-rich savanna in the world with more than 12,000 plant species (Mendonça et al., 2008). The climate is seasonal with dry winters from April-September and rainy summers (Oliveira-Filho & Ratter, 2002), and the typical landscape of the Cerrado consists of a complex mosaic of savanna physiognomies on the well-drained interfluves with riparian forests along the watercourses (Ratter et al., 1997).

Melastomataceae is the fourth most speciose family in the Cerrado with 521 taxa (Mendonça et al., 2008). This pantropical family is comprised of shrubs, woody climbers, herbs, epiphytes and trees that colonise montane to lowland forests, savannas and disturbed vegetation (Clausing & Renner, 2001). The family has about 5,000 species arranged into two subfamilies and nine tribes and is ranked as the eight largest plant family (Renner, 2004; Angiosperm Phylogeny Website 2011).

Despite its higher diversity in the Neotropics (Renner et al. 2001), reports of seed dormancy in melastomes are available only for the temperate herb *Rhexia mariana*

(Rhexieae). Physiological dormancy (PD) evolved in this species as an adaptation to the climatic conditions in temperate eastern North America (Baskin et al., 1999). Neotropical melastomes evolved different life-history strategies and colonised a broad diversity of habitats (Renner et al., 2001), and thus we predict that dormancy may have evolved in other lineages of the family, particularly in seasonal habitats (Salazar et al., 2011). Understanding the evolution of seed dormancy in melastomes is important not only to obtain insight into reproductive ecology of tropical plants, but also because several melastomes (such as *Miconia calvescens* and *Clidemia hirta*) have become devastatingly invasive species (Meyer & Florence, 1996; Medeiros et al., 1997).

In this study, we: 1) screened for dormant seeds among 50 Melastomataceae species from Neotropical montane vegetation in Brazil; 2) determined the class of seed dormancy (*sensu* Baskin & Baskin, 2004) in the dormant species; 3) examined the processes driving the evolution of seed dormancy; and 4) discussed the phylogenetic and biogeographical implications of our findings for the general understanding of seed dormancy in montane vegetations.

Material and methods

Study site and species

This study was conducted in four sites in southeastern Brazil. Plant material was collected in a particular vegetation of Cerrado known as rupestrian fields (rocky outcrop montane vegetation) in Minas Gerais, southeastern Brazil. The rupestrian fields occur in quartzite-derived or ironstone soils above 900m above sea level and harbour high levels of plant biodiversity and endemism (Alves & Kolbek, 2010). In this vegetation, plant communities are established on shallow, acidic, nutrient-poor, and excessively drained soils (Giulietti *et al.*, 1997; Jacobi *et al.*, 2007), where plants often experience strong winds, high irradiance exposure, frequent fires, high daily thermal amplitudes and water shortage during the dry season (Giulietti *et al.*, 1997; Madeira & Fernandes, 1999). Local drainage systems dictated by topography diversify this environment by creating relatively humid (mesic) or arid (xeric) sites, often separated from each other by a few centimeters (Alves & Kolbek, 2010). Because of increasing human pressures (Giulietti *et al.*, 1997; Jacobi *et al.*, 2007), several native species of this vegetation are under the threat of extinction.

Seeds of 48 species were collected at Serra do Cipó (19º17' S, 43º35' W), located at the southern portion of the Espinhaço Range. Seeds of *Lithobium cordatum* and *Merianthera sipolisii* were collected in the Diamantina plateau (18°07'S and 44°20'W). Seeds of *Comolia sertularia* also were collected at Serra do Cabral (17°03'S-18°13'S and 44°05'W-44°52'W) and those of *Miconia corallina* also at the Parque Estadual do Ibitipoca (PEI) (21º40'-21º44'S e 43º52'- 43º55'W). The dominant vegetation in all studied sites consists of rupestrian fields, but sites differ in climate and in surrounding vegetation. Serra do Cipó and Diamantina are located in a transition zone between the Cerrado and the Atlantic Forest. However, their western side (were seeds of this study where collected) and the Serra do Cabral are both associated with the Brazilian Cerrado, whereas the dense rupestrian fields of PEI are enclosed by the more humid semideciduous Atlantic Forest (Chiavegatto 2005, Alves & Kolbek, 2010). All sites are under the influence of seasonal climates with a 4-6 month dry and cold season, but relatively higher annual rainfall occurs at PEI compared to the other sites (Madeira & Fernandes, 1999; Chiavegatto 2005; Hatschbach et al., 2006).

We investigated seed dormancy in 50 species of Melastomataceae belonging to 17 genera and four tribes (Table S1). These species represent nearly 10% of Melastomataceae from the Cerrado. Species selection was oriented towards maximizing life-history strategies, phylogenetic diversity and microhabitat occupancy. Germination experiments were conducted over a wide range of temperature and light conditions for these species (data not shown) and focus was given to four species showing germinability of fresh seeds <10%. Our species selection was phylogenetically-oriented and included species in the main lineages (Tribes) within the family. Figure 1 shows the reconstructed phylogeny of the 50 species (see supplemental material for phylogenetic methods).

Tibouchina cardinalis (Melastomeae) and *Comolia sertularia* (Melastomeae) are endemic shrubs from the rupestrian fields of Minas Gerais, *Chaetostoma armatum* (Microlicieae) a common subshrub occurring in southeastern and south of Brazil and *Miconia corallina* (Miconieae) a common plant in the rupestrian fields in southeastern Brazil. *Miconia* produces biotically-dispersed berries, whereas the other species produce dry capsules that allow for abiotic dispersal. *Miconia*, *Comolia* and *Tibouchina* establish on rocky outcrops, whereas *Chaetostoma* establishes on dry grasslands. Soil moisture decreases significantly at the beginning of the dry season in these xeric microhabitats (Silveira, 2011). These species disperse seeds at the transition between rainy and dry seasons at Serra do Cipó and Serra do Cabral. *Miconia*, however, disperses fruits during mid rainy season at PEI and colonises a shrubby and relatively denser rupestrian field (Chiavegatto, 2005).

Seed collection and germination experiments

Ripe fruits of each *Tibouchina*, *Chaetostoma* and *Comolia* were collected at Serra do Cipó between April and June 2008, 2009 and 2010. For *Miconia*, seeds were collected in April 2009, April 2010 and May 2010. Germination was conducted separately for *Miconia* seeds collected in 2010. Fruits of *Comolia* were also collected in July 2008 at Serra do Cabral and fruits of *Miconia* were collected in March 2010 at PEI.

Morphometric analyses (seed length and width) were performed on 50 seeds of each four species. Seeds were photographed with a stereomicroscope (Zeiss Stemi 2000-C), and variables were measured using Zeiss AxioVision Rel 4.7 software. Seed viability was assessed on these four species by means of seed dissection and the tetrazolium test. Four replicates of 25 seeds were cut under a stereomicroscope and checked for embryo presence/absence. Further, the tetrazolium test was conducted for four replicates of 25 seeds with embryos. Cut seeds were soaked in Petri dishes containing a 1% solution of tetrazolium. The Petri dishes were wrapped in double sheet of aluminium foil and incubated at 30ºC. For all four species, staining required 24-48h. After this period, seeds containing white or light red coloration were considered non-viable and seeds containing red or dark red coloration were considered viable.

Four replicates of 50 (25 for *Miconia*) seeds were set per treatment. Seeds were set to germinate in 9-mm Petri dishes on a double sheet of filter paper moistened with Nistatin solution (2%) to prevent fungi growth. The Petri dishes were incubated under constant temperatures of 15, 20, 25, 30 and 35°C at a 12:12 hour light: dark cycle (PPFD = 26 μmol m^{-2} s^{-1}). For *Tibouchina*, *Chaetostoma* and *Comolia* seeds collected in 2008, experiments were carried out at all temperatures. In 2009 and 2010, experiments were repeated under the optimum temperature for each species. Experiments were performed only at 25^{o}C for *Comolia* seeds collected at Serra do Cabral and for *Miconia* seeds collected in both years and sites. All melastome species studied to date are photoblastic (Silveira, 2011), so germination data for dark conditions are not shown here. Seed germination was monitored at 24 hour intervals for 30 days, and seeds were considered germinated when radicle emergence was observed. Since our focus is on the dormant species, the results for the other 46 species will be presented in an accompanying paper.

For each treatment, mean germination time (MGT) was calculated by the formula:

$$\mathrm{MGT} = \sum_{i=1}^{k} n_i t_i \Big/ \sum_{i=1}^{k} n_i$$

where n_i is the number of seeds germinated in the time i, t_i is the time from the start of the experiment to the *ith* observation and k is the time of last germination (Ranal & Santana, 2006).

Data in percentage were arcsin transformed, and tests for normality and variance equality were run prior to hypothesis-testing analysis. Whenever data met the assumptions of parametric statistics, differences among means were determined by ANOVA followed by Tukey test ($\alpha = 0.05$) for multiple comparisons. When data did not meet the assumptions of the parametric tests, statistical differences among means were determined by Kruskal-Wallis followed by Conover test. The same procedures were carried on for MGT data.

Seed coat permeability

Five to 10 replicates of recently-collected seeds (25 each for *Comolia* and *Miconia* and 50 each for *Chaetostoma* and *Tibouchina*) were weighed on a digital balance. Seeds were soaked in tap water for 72h at room temperature and reweighed. Seed permeability was determined by the increase in seed mass and differences in percentage increase between seed mass of dried and soaked seeds were determined by paired T-test or Wilcoxon test.

Seed anatomy and histochemistry

Seeds of the four species were submitted to routine anatomical procedures. Fresh seeds were fixed, washed, dehydrated in ethanol series and infiltrated according to Paiva *et al.* (2011). Sections were stained, mounted and submitted to histochemical tests (see supplemental material).

Results

Seed biometry, viability and germination

Seeds of the four species are small, seed length ranged from 0.63 mm to 1.64 mm and width from 0.35 mm to 1.36 mm. Dry seed mass ranged from 0.0013 mg in *Chaetostoma* to 0.023 mg in *Miconia* (Table 1).

Germinability of all four species was low irrespective of year of collection (Fig. 2). However, germinability of *Miconia* seeds from PEI was significantly higher than that of seeds from Serra do Cipó (Fig. 2). For most experimental conditions, germination was not observed. These conditions were represented by extreme temperatures (15^{o}C and 35^{o}C) in *Comolia* and *Chaetostoma* and also in *Chaetostoma* seeds collected in 2009. Across all experiments, only a single seed germinated (mean germinability = 0.33%) for *Chaetostoma* 2010, *Comolia* 2009 and 2008 and *Tibouchina* 2010. For *Tibouchina* 2009, germinability was 16.7%.

Seed coat permeability

We found significant increases in seed mass following a 72-hour imbibition period for all species (Table 2). Percentage increase in seed mass ranged from 46 to 75%; thus, all species produce seeds with permeable seed coats.

Ecological association of seed dormancy

We found uneven distribution of dormant species regarding microhabitat and dispersal phenology (Table 3). There were significant associations of dormant species and xeric microhabitats (χ^2= 5.02; p= 0,024) and of non-dormant species with other phenologies (χ^2= 16.37; p< 0,001). Other phonologies include seed dispersal during the dry and rainy season, and during dry-rainy transitions (Silveira, 2011).Thus, seed dormancy was strongly related to both microhabitat and life-history.

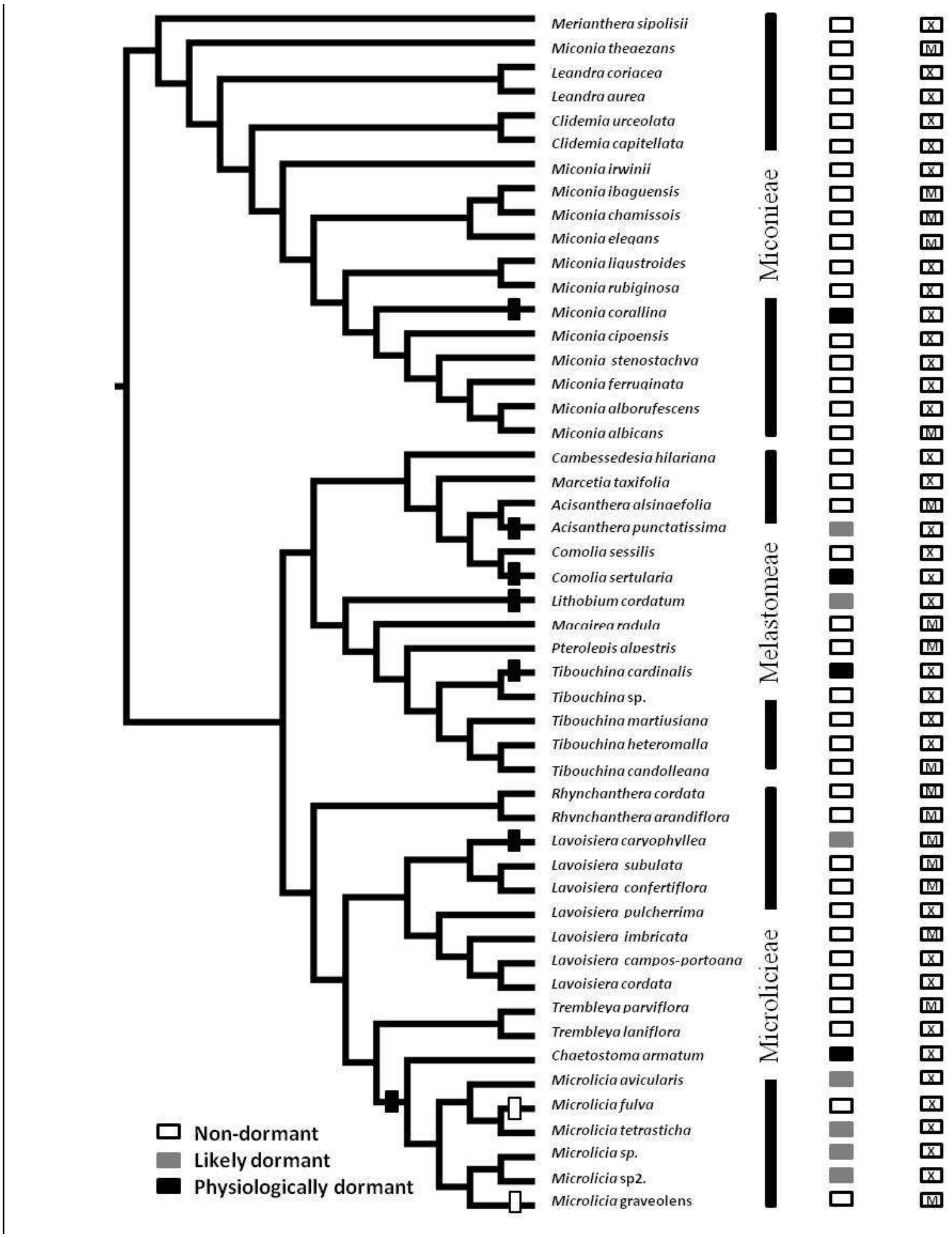

Figure 1. Reconstruction of the phylogenetic tree of 50 Melastomataceae species showing non-dormant, likely-dormant and dormant species. Filled dashes indicate the transition between non-dormant to dormant states and empty dashes indicate transition between dormant to non-dormant states. Species microhabitats are also shown as xeric (X) and mesic (M).

Seed anatomy and histochemistry

Mature seeds are exarillate and exalbuminous. In all species, the embryos are well developed, filling the whole seed cavity and showing a conspicuous embryo axis and two fleshy cotyledons. In *Chaetostoma*, *Comolia* and *Tibouchina*, the embryo is curved, following the shape of the seeds, while the embryo of *Miconia* is folded, with cotyledons parallel to the embryo axis (see supplemental material).

Phylogenetic distribution of physiological dormancy

Based on the phylogenetic study, non-dormant seeds are assumed to be the ancestral condition and physiological dormancy (PD) is a derived character that was selected at least four times (once in Miconieae, once in Microlicieae and twice in Melastomeae; Fig. 1). Our data also suggest that seven additional species produce dormant seeds (Fig. 1).

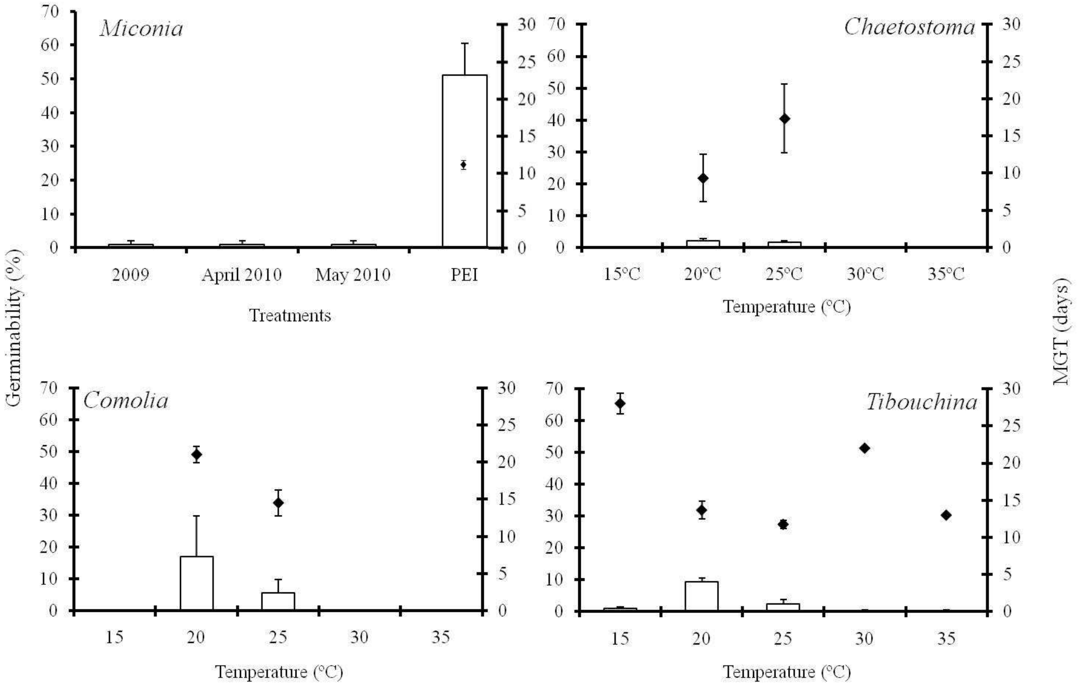

Figure 2. Mean (±SE) of germinability (%, bars) and mean germination time (MGT, days; diamonds) of fresh seeds of *Miconia corallina*, *Tibouchina cardinalis*, *Comolia sertularia* and *Chaetostoma armatum* collected at Serra do Cipó and Parque Estadual do Ibitipoca (PEI).

Table 1. Mean seed length and seed width (±SD), percentage of embryoless seeds, viable embryos, non-dormant and dormant seeds in four Melastomataceae species from Neotropical montane savannas in Southeastern Brazil.

Species	Seed length (mm)	Seed width (mm)	Embryoless seeds (%)	Viable embryos (%)	Non-dormant (%)	Dormant seeds (%)
Chaetostoma armatum	0.63 ± 0.08	0.35 ± 0.05	58	48	1.3	41
Comolia sertularia	0.85 ± 0.1	0.71 ± 0.07	50	73.4	4.5	45
Miconia corallina	1.64 ± 0.12	1.36 ± 0.18	41	28.8	1	54
Tibouchina cardinalis	0.72 ± 0.1	0.46 ± 0.08	61	52.3	9.5	30

Table 2. Seed mass before and after soaking in tap water for 72h for four Melastomataceae species from Neotropical montane savannas in Southeastern Brazil. Statistics refer to Wilcoxon test for *Comolia* and paired T-test for the other species.

Species	Seed mass (mg) before incubation	Seed mass (mg) after incubation	Statistics	Mean % increase
Chaetostoma armatum	0.0013	0.0019	7.6*	46.1
Comolia sertularia	0.00349	0.00509	45*	48.6
Miconia corallina	0.02322	0.03482	4.67*	55.4
Tibouchina cardinalis	0.00245	0.00428	13.87**	75.1

* p> 0.01; ** p> 0.001

Table 3. Frequency of dormant and non-dormant species in mesic and xeric sites in species shedding seeds during the rainy-dry transition and in other periods of the year.

Dormancy status	Microhabitat		Phenology	
	Mesic	Xeric	Rainy-dry	Others
Dormant	1	11	9	3
Non-dormant	13	25	3	35

Discussion

We have presented data showing that PD evolved at least four times during the history of Melastomataceae in the rupestrian fields. Although seed dormancy is thought to have evolved in melastomes from rainforest (Elisson et al. 1993) and Cerrado (Pereira-Diniz, 2003; Mendes-Rodrigues et al., 2010), these previous studies do not present enough evidence to support the evolution of dormancy in tropical Melastomataceae. Firstly, none of these studies included viability tests, so lack of germination may have been due to nonviable instead of dormant seeds. Moreover, neither of these two studies evaluated the frequency of embryoless seeds, which can be confounded with dormant seeds when seeds do not germinate. In addition, seeds of *Clidemia hirta* germinated to high percentages in a few days without any dormancy-breaking treatments (Pereira-Diniz, 2003), and low germinability in *M. ferruginata* is best explained by high percentages of embryoless seeds, rather than physical dormancy (seed coats were shown to be permeable; Mendes-Rodrigues et al., 2010). Finally, dormancy-breaking treatments and germination promoters failed to increase germinability for those species for those species. Our study represents the first unequivocal report on seed dormancy in tropical Melastomataceae.

PD is the most common type of dormancy in the major clades of Angiosperms and across vegetations types in Earth (Baskin & Baskin, 2005). The gain and loss of PD quite likely occurred many times during the evolution of seed plants (Linkies et al., 2010), and our data suggest convergent evolution of PD in Melastomataceae. Nonetheless, we also present evidence for divergent evolution of dormancy in melastomes. Phylogeny-dependent evolution of seed dormancy is widely recognised (Baskin & Baskin, 2005) and may explain dormancy evolution. Dormancy appears to have evolved early in *Chaetostoma-Microlicia*, and for most *Microlicia* species reported here dormancy appears to be an ancestral (plesiomorphic) seed trait. The loss of dormancy in *M. graveolens* may have occurred prior or after the transition of this species to mesic microhabitats and, thus should be interpreted as a derived character.

Dormant and likely-dormant melastomes were associated with colonisation of xeric microhabitats and with rainy-to-dry-season dispersal. Soil moisture is still high during the rainy-to-dry transition in xeric microhabitats of the rupestrian fields, but it significantly drops as the dry season approaches (Coelho et al., 2006; Silveira, 2011). Small- rooted seedlings of these species would have to survive a forthcoming 4-5-month dry period before the perennial rains begin in October (Madeira & Fernandes, 1999). Therefore, we would expect high seedling mortality under these unfavourable (dry) conditions to be a strong selective pressure driving the evolution of seed dormancy. Drought-induced mortality is argued to be a strong and directional pressure favouring the evolution of PD in melastomes from the rupestrian fields. Therefore, avoiding germination at the rainy-to-dry transition can be an adaptive strategy delaying germination until the following rainy season.

Although we lack data to confidently assign dormancy for seven species, we assume they produce dormant seeds because these seeds are in the right place and time where dormancy evolved (Table S1, Table 3) and because embryoless seeds and nonviable embryos are insufficient to explain low germinability (Silveira, 2011). To be conservative, we assign seeds of these seven species to the likely dormant seed category. If future studies confirm seed dormancy in these seven species, three additional non-dormant-to-dormant transition events would be added.

Our data have important eco-evolutionary implications. Firstly, they help us understand the biogeographical and phylogenetic distribution of seed dormancy across the global types of vegetations. Current estimates are that nearly 60% of tropical montane species have dormant seeds (Baskin & Baskin, 2005). We found 8% of the surveyed species had dormant seeds (percentage increases to 22% if dormancy is assigned in the other seven likely dormant species by future studies). Together with recent reports of seed dormancy in species from tropical montane vegetation (Gomes et al., 2001; Silveira & Fernandes, 2006; Garcia, Q.S., pers. comm.), the data reported here suggest that seed dormancy in tropical montane vegetation was overestimated.

Secondly, our data provide key and practical information for predicting seed dormancy evolution in the Cerrado flora. Previous models suggest that most seeds dispersed in the dry season are dormant, while most seeds dispersed in the wet season are non-dormant (Salazar et al., 2011). We, on the other hand, have identified that the rainy-to-dry transition is the critical period for establishment in xeric microhabitats in the Cerrado and that seed dormancy can be a central strategy to avoid germination when conditions are unlikely to remain favourable long enough for seedling establishment. However, microhabitat and phenology alone are not powerful predictors of seed dormancy. There are numerous species in xeric microhabitats in which dormancy has not evolved (Table 1). On the other hand, species with rainy-to-dry transition dispersal are not necessarily dormant (see the case of the mesophyte *Miconia theaezans*). Therefore, the combination of phenology and microhabitat is a strong, but not universal, predictor of the evolution of seed dormancy in melastomes.

Another interesting example supporting our conceptual framework for the evolution of seed dormancy in tropical melastomes comes from among-population variation in seed dormancy in *M. corallina*. Individuals of *M. corallina* at PEI disperse their non-dormant seeds under favourable conditions for establishment. At Serra do Cipó, however, individuals dispersing seeds under unfavourable conditions produce dormant seeds. It is commonly accepted that among-population variation in seed dormancy and germination (Anderssen & Milberg, 1998; Lacerda et al., 2004) is related to climatic conditions (Wagner & Simons, 2009). If this variation is genetically-based (Lacerda, et al. 2004), microevolutionary changes would account for the evolution of locally-adapted ecotypes. On the other hand, variation can also be the result of plastic responses mediated by maternal effects.

In summary, we have presented evidence for convergent evolution of PD in melastomes from Neotropical montane savannas. There is no reason to assume that multiple origins of seed dormancy would not explain the evolution of dormancy in other Cerrado lineages. In contrast to tropical rainforest, the upper soil layers of seasonal vegetation have very negative soil water potential during the dry season (Coelho et al., 2006; Salazar et al., 2011), creating a suitable scenario for the evolution of seed dormancy. Therefore, we expect dormancy to have evolved in other taxa sharing similar microhabitats and life-histories as those of dormant melastomes. Improving the predictability of presence of seed dormancy has important implications for plant conservation, seed technology and restoration ecology.

Electronic supplementary material

Silveira et al. – Evolution of physiological dormancy multiple times in Melastomataceae from Neotropical montane vegetation

Contents:

1) Table S1
2) Phylogenetic reconstruction method
3) Seed anatomy and histochemistry
4) Figure S1
5) References

Table S1. Habitat and life-history traits of the 50 Melastomataceae species screened for the evolution of seed dormancy.

Tribe	Species	Growth-form	Habitat	Dispersal	Phenology	Geographic distribution
Melastomeae	*Acisanthera alsinaefolia*	Shrub	Mesic	abiotic	Continuous	Endemic
Melastomeae	*Acisanthera punctatissima***	Sub-shrub	Xeric	abiotic	Rainy-Dry	Endemic
Melastomeae	*Cambessedesia hilariana*	Sub-Shrub	Xeric	abiotic	Continuous	Endemic
Microlicieae	*Chaetostoma armatum**	Sub-Shrub	Xeric	abiotic	Rainy-dry	Widespread
Miconieae	*Clidemia capitellata*	Shrub	Xeric	biotic	Rainy	Widespread
Miconieae	*Clidemia urceolata*	Shrub	Xeric	biotic	Continuous	Widespread
Melastomeae	*Comolia sertularia**	Shrub	Xeric	abiotic	Rainy-dry	Endemic
Melastomeae	*Comolia sessilis*	Shrub	Xeric	abiotic	Dry	Endemic
Microlicieae	*Lavoisiera campos-portoana*†	Shrub	Xeric	abiotic	Dry	Endemic
Microlicieae	*Lavoisiera caryophyllea***†	Sub-shrub	Mesic	abiotic	Rainy-dry	Endemic

Microlicieae	*Lavoisiera confertiflora*	Sub-Shrub	Xeric	abiotic	Rainy	Endemic
Microlicieae	*Lavoisiera cordata*†	Shrub	Xeric	abiotic	Dry	Endemic
Microlicieae	*Lavoisiera imbricata*	Shrub	Mesic	abiotic	Rainy	Endemic
Microlicieae	*Lavoisiera pulcherrima*	Shrub	Mesic	abiotic	Dry	Endemic
Microlicieae	*Lavoisiera subulata*†	Sub-Shrub	Mesic	abiotic	Dry	Endemic
Miconieae	*Leandra aurea*	Shrub	Xeric	biotic	Rainy	Widespread
Miconieae	*Leandra coriacea*	Shrub	Xeric	Biotic	Rainy	Widespread
Microlicieae	*Lithobium cordatum***†	Sub-Shrub	Xeric	abiotic	Rainy-dry	Endemic
Melastomeae	*Macairea radula*	Shrub	Mesic	abiotic	Rainy	Widespread
Melastomeae	*Marcetia taxifolia*	Shrub	Xeric	abiotic	Dry	Widespread
Merianeae	*Merianthera sipolisii*	Tree	Xeric	abiotic	Rainy-dry	Endemic
Miconieae	*Miconia albicans*	Shrub	Xeric	biotic	Rainy	Widespread
Miconieae	*Miconia alborufescens*	Shrub	Xeric	biotic	Rainy	Endemic
Miconieae	*Miconia cipoensis*†	Shrub	Xeric	biotic	Rainy	Endemic
Miconieae	*Miconia chamissois*	Shrub	Mesic	biotic	Rainy	Widespread
Miconieae	*Miconia corallina**	Shrub	Xeric	biotic	Rainy-dry	Widespread
Miconieae	*Miconia elegans*	Tree	Mesic	biotic	Rainy	Widespread
Miconieae	*Miconia ferruginata*	Tree	Xeric	biotic	Dry-Rainy	Widespread
Miconieae	*Miconia ibaguensis*	Shrub	Mesic	biotic	Rainy	Widespread
Miconieae	*Miconia irwinii*	Tree	Xeric	biotic	Dry	Endemic
Miconieae	*Miconia ligustroides*	Shrub	Xeric	biotic	Rainy-dry	Widespread
Miconieae	*Miconia stenostachya*	Shrub	Xeric	biotic	Rainy	Widespread

Miconieae	*Miconia rubiginosa*	Tree	Xeric	biotic	Rainy	Widespread
Miconieae	*Miconia theaezans*	Shrub	Mesic	biotic	Rainy-dry	Widespread
Microlicieae	*Microlicia avicularis***	Sub-Shrub	Xeric	abiotic	Dry	Endemic
Microlicieae	*Microlicia fulva*	Sub-Shrub	Xeric	abiotic	Dry	Widespread
Microlicieae	*Microlicia graveolens*	Sub-Shrub	Mesic	abiotic	Dry	Endemic
Microlicieae	*Microlicia* sp**	Sub-Shrub	Xeric	abiotic	Rainy-Dry	Endemic
Microlicieae	*Microlicia* sp2**	Sub-Shrub	Xeric	abiotic	Dry	Endemic
Microlicieae	*Microlicia tetrasticha***	Sub-Shrub	Xeric	abiotic	Rainy-Dry	Endemic
Melastomeae	*Pterolepis alpestris*	Sub-Shrub	Mesic	abiotic	Dry-Rainy	Endemic
Microlicieae	*Rhynchanthera cordata*	Shrub	Mesic	abiotic	Dry-Rainy	Widespread
Microlicieae	*Rhynchanthera grandiflora*	Shrub	Mesic	abiotic	Rainy	Widespread
Melastomeae	*Tibouchina candolleana*	Tree	Mesic	abiotic	Rainy	Widespread
Melastomeae	*Tibouchina cardinalis**	Shrub	Xeric	abiotic	Rainy-dry	Endemic
Melastomeae	*Tibouchina heteromalla*	Shrub	Xeric	abiotic	Continuous	Widespread
Melastomeae	*Tibouchina martiusiana*	Shrub	Xeric	abiotic	Dry-Rainy	Widespread
Melastomeae	*Tibouchina* sp.	Tree	Xeric	abiotic	Dry	Endemic
Microlicieae	*Trembleya laniflora*	Shrub	Xeric	abiotic	Dry-Rainy	Endemic
Microlicieae	*Trembleya parviflora*	Shrub	Mesic	abiotic	Rainy	Endemic

* Denotes species with confirmed seed dormancy.

** Denotes likely dormant species.

† Included in the list of rare plants of Brazil or threatened by extinction by red lists.

Phylogenetic reconstruction method

For understanding the evolution of seed dormancy in Melastomataceae, a phylogenetic tree showing relationships among the studied taxa was built. Nomenclature was checked against the Missouri Botanical Garden's nomenclatural database (http://www.tropicos.org/Home.aspx) and pruned tree was built with the study species as terminal tips using the maximally resolved tree with the use of Phylomatic (http://www.phylodiversity.net/phylomatic/).

The occurrence of several polytomies in our tree may prevent the correct interpretation of the evolution of seed dormancy. However, most polytomies were in terminal taxa and thus, considered soft polytomies (Webb *et al.* 2006), which are likely to cause low interference in interpretation. Polytomies in the tree were resolved by randomly generating 50 fully resolved trees using Mesquite (Maddison & Maddison 2008). Species relationships were improved and polytomies were resolved based on available and published data for the taxa relationships in the family (Renner 1993, Clausing & Renner 2001, Fritsch et al. 2004, Goldenberg et al. 2008, Michelangeli et al. 2008). Unpublished theses were also used to determine relationships at generic and specific levels.

Seed anatomy and histochemistry

Seeds of the four species were fixed in formalin-acetic acid-50% ethanol (Johansen 1940) for 48 hours, including 24 hours, with a vacuum passage. Seeds were kept in Franklin solution (glacial acetic acid and hydrogen peroxide 30%, 1:1 v/v) for 4 to 10 hours at 60ºC until clearing (Franklin, 1965, modified). The samples were washed in running tap water to remove the solution, and seeds were dehydrated in an increasing ethanol series (10, 20, 30, 40, 50, 60, 70%) with 1% glycerine. Seeds were stored in ethanol 70% + 1% glycerine for three days and then another ethanol series (80, 90 and 95%) was carried out. The seeds were infiltrated in the refrigerator at 4ºC for a week and embedded in 2(hydroxy-ethyl)-methacrylate (Leica™). The samples were kept in the embedding solution for another week under freezer (-20ºC), according to Paiva *et al.* (2011).

Longitudinal and transversal sections with 6µm thickness were prepared with a rotary microtome (Jung Biocut mod. 2035). The sections were stained with toluidine blue 0.05%, pH 4.7 (O'Brien *et al.*, 1964, modified) and mounted in Entellan™.

Histochemical tests were carried out in order to detect phenolics in the seed coat of the studied species. Tests were prepared on the 6µm sections by using 10% aqueous ferric chloride plus a little sodium carbonate, or by fixing in aqueous solution formalin and 10% ferrous sulfate (Johansen, 1940), and toluidine blue (Briggs *et al.* 2005).

Mature seeds are exarillate and exalbuminous (Fig. S1A). In all species, the embryos are well developed, filling the whole seed and showing a conspicuous embryo axis and two fleshy cotyledons. In *Chaetostoma* (Fig. S1B), *Comolia* (Fig. S1C) and *Tibouchina* (Fig. S1D), the embryo is curved, following the shape of the seeds, while the embryo of *Miconia* (Fig. S1A) is folded, with cotyledons parallel to the embryo axis.

The cotyledons are symmetrical and well developed, and the hypocotyl-radicle axis varies from one (*Tibouchina*) to three (*Chaetostoma*) times the length of the cotyledons. The embryo has uniseriate protoderm (Fig. S1E), with small cells, cuboidal in longitudinal section. These cells have pectic-celulosic walls, dense cytoplasm and evident nuclei. The ground meristem is composed by approximately-isodiametric cells larger than the protoderm. The procambium has slightly longitudinally elongated cells in a standard organization (Fig. S1D). The radicle is short, and hardly distinguishable from the hypocotyl (Fig. S1A-D). Its apex has standard organization. The cotyledon node is relatively large and the epicotyl is indistinct. The plumule is undifferentiated, consisting of only the shoot meristem (Fig. S1D).

In the seed coat of all four species, the epidermis is uniseriate (*Chaetostoma*, *Comolia* and *Tibouchina*) or multiseriate (*Miconia*). The epidermis in the seed coat contains dead cells with walls that are and thin in *Chaetostoma* (Fig. S1B, E-F), irregularly and thick in *Comolia* (Fig. S1C) and homogeneous and thick in *Miconia* (Fig. S1A, H) and *Tibouchina* (Fig. S1D, I). Phenolic compounds were observed in all seed coats, in cell walls (Fig. S1E-F, H) or in the vacuoles (Fig. S1G, I). Parenchymatic layers occur in the inner epidermis, which can be distinguishable in *Miconia* and *Tibouchina* by the presence of protoplast remains. In the other species, the parenchyma in the seed coat is completely compressed. In the raphe, the parenchyma is multi-layered (Fig. S1B).

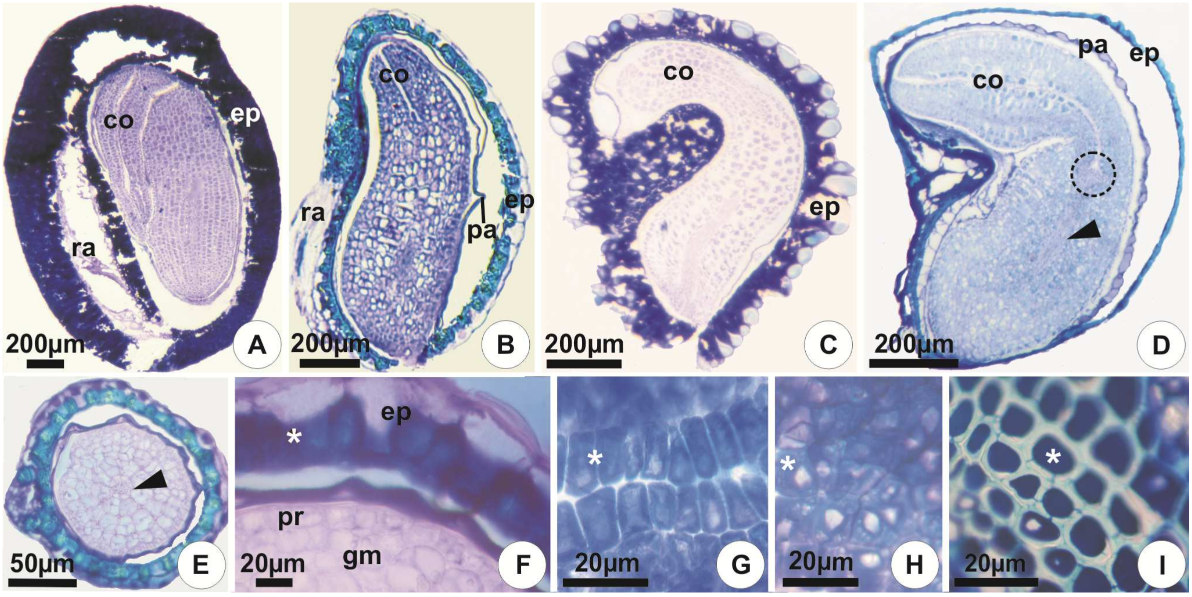

Figure S1. Longitudinal (A-D, G-I) and transversal (E-F) sections of dormant seeds. (A, H). *Miconia corallina*. (B, E-F). *Chaetostoma armatum*. (C, G). *Comolia sertularia*. (D, I). *Tibouchina cardinalis* (circle, shoot meristem). Asterisk, phenolic compounds; arrowhead, procambium; co, cotyledon; ep, epidermis; gm, ground meristem; pa, parenchyma; pr, protoderm; ra, raphe.

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CAPÍTULO V

COMPARATIVE ECOPHYSIOLOGY OF SEED DORMANCY AND GERMINATION IN NEOTROPICAL MONTANE SAVANNAS

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Abstract

In this study, we have investigated the evolution of seed size and germination traits in 50 perennial species in Neotropical montane savannas. We focused on Melastomataceae because this family comprises one of the most functionally distinctive, ecologically diverse and species-rich clade of flowering plants. We asked how is the variation in seed traits distributed among plant functional types by means of phylogenetic comparative methods. Variation in ecophysiological and morphological seed traits was associated with plant functional types and distribution, in more complex ways than previously thought. Species with seed dispersal during rainy-dry transition had lower germinability and delayed germination, but germination traits were not related to geographic distribution. Trait-trait correlations along the phylogenetic tree of Melastomataceae were significant for germinability and germination synchrony, but not for seed size neither light requirement, because these two traits were conserved traits along the phylogenetic tree. Germinability, germination time and synchrony showed similar patterns of evolution. We found a consistent pattern of divergences in germination traits between Merianieae and Miconieae and within the Miconieae whereas coordinated patters of convergences in germination traits were found between Melastomeae and Microlicieae and within Microlicieae. These patterns indicate 1) different selective pressures driving the evolution of seed strategies in those groups and 2) coordinated evolution of dispersal mode and growth-form. Competition avoidance is argued to be the evolutionary mechanism explaining divergences in berry-fruited tall plants. On the other hand, convergent evolution is put forward as a selective force driving the evolution of germination traits in capsule-fruited, short plants. Character displacement in germination traits as a result of disruptive selection may help species coexistence in species-rich communities.

Keywords: Melastomataceae, phylogenetic signal, trait-trait association, seed traits, trait-environment association.

Introduction

Plant biologists have long recognized the importance of seeds for general understanding of plant ecology and evolution. The multiple benefits provided by seeds have played a central role in the rise and dominance of seed plants (Linkies et al. 2010). Because germination is an irreversible process, it should be fine-tuned with optimum conditions for establishment and germination traits are expected to be under strong selective pressures (Jurado & Moles 2002, Norden et al. 2009). Early life-history traits are of primary significance for reproductive success (Bu et al. 2008) and are under strong selective pressure because without successful establishment following germination, no postgermination phenotype can ever be expressed (Donohue 2005).

Plant of different functional groups and plants occurring in different vegetations are subjected to a multitude of selective pressures operating in different directions (Rowe & Speck 2005, Lavorel et al. 2007, Bu et al. 2008). Therefore, it is of interest to find ecological and phylogenetic correlates with seed traits across clades and vegetations in order to illuminate the evolution of seed traits. Phylogenetic and ecological correlates with seed traits across contrasting biomes provide the basis for the study of adaption in seed traits. Studies spanning vegetations in several continents (Hodkinson et al. 1998, Milberg et al. 2000, Brändle et al. 2003, Thompson et al. 2003, Bu et al. 2008, Leyer & Press 2009, Wang et al. 2009, Luna & Moreno 2009, 2010) employed the comparative method (though not always based on phylogenies) and shed light on the association of seed traits (size, germination and persistence) with life-history traits, ecological variables and phylogeny. These studies are useful for general the understanding of seed evolution (Moles et al. 2005, Ackerly 2009b) and also because the patterns arising from those studies have implications for the understanding of population-based processes that drive plant interactions in community-level (Azcárate et al. 2002, Donohue et al. 2010).

In spite significant advances promoted by these studies, they were all carried out in temperate areas, and many angiosperm lineages have been overlooked. Because variation in seed traits is dependent upon phylogeny (Moles et al. 2005), community-level studies addressing the evolution seed traits in temperate areas are limited in fostering the understanding of seed evolution in species-rich communities. Therefore, studies capturing the variation in seed traits in tropical vegetations are called for.

In this study, we aimed to understand the evolution of germination strategies in a species-rich Neotropical rocky montane vegetation. We asked how is the variation in seed traits distributed among plant functional types by means of phylogenetic comparative methods. Incorporating the phylogenetic information of the species is important to understand how evolutionary history underlies present-day morphological and ecophysiological patterns (Ackerly 2009a). Moreover, the use of phylogenetic comparative methods allows us to separate ecological and evolutionary effects on seed traits (Ackerly 2009a). Our focus was on species of Melastomataceae because this plant family is not only dominant in the Neotropics but also because it constitutes one of the most functionally distinctive, ecologically diverse and species rich clade of flowering plants (Angiosperm Phylogeny Website 2011). Reasonable phylogenetic hypothesis are being made available for the family, which allows us to implement the phylogenetic comparative methods.

Material and methods

Study area and study species

Seeds were collected along the Espinhaço Range, a mountain chain spanning 1000km in length in south-eastern Brazil. The Espinhaço Range was designated as an UNESCO Biosphere Reserve (UNESCO 2011), in part because they harbor high levels of plant biodiversity and endemism (Alves & Kolbek 2010), and in part, because increasing human pressures are leading native species to the threat of extinction (Giulietti *et al.* 1997, Jacobi *et al.* 2007). The main vegetation in the study site is a montane rocky grassland called rupestrian fields (one vegetation type among the various of the Brazilian Cerrado, Fig. S1). The rupestrian fields occur in areas above 900m a.s.l. where plant communities establish on shallow, acidic, nutrient-poor, and excessively drained quartzite-derived or ironstone soils (Giulietti *et al.* 1997, Benites et al. 2007, Jacobi *et al.* 2007). Plants in these communities experience strong winds, high sun exposition, frequent fires, high daily thermal amplitudes, and water shortage during the dry season (Giulietti *et al.* 1997, Madeira & Fernandes 1999).

Island-like configuration of highland rupestrian fields among lowland Cerrado vegetation promoted *in situ* speciation and might be responsible for the maintenance of endemic species (Alves & Kolbek 2010, Echternacht et al. 2011). The intersection of the Espinhaço Range among savannas, seasonally dry forests and semi-deciduous rainforests favored the colonization by South American widespread species. Another remarkable feature of the rupestrian fields is habitat heterogeneity. In rocky outcrop vegetation, resource heterogeneity results in a plethora of microhabitats with particular species-soil relationships (Conceição & Pirani 2005) in a way that some species are restricted to isolated outcrop environments and others are restricted to the matrix of zonal herbaceous vegetation (Alves & Kolbek 2010). Local drainage systems dictated by topography further diversify this environment by creating relatively humid or arid sites, which are found practically side-by-side (Alves & Kolbek 2010). The coarse-textured soils of this vegetation have low water retention capacity (Benites et al. 2007, Negreiros et al. 2009) and create seasonally xeric habitats during the dry season (Coelho et al. 2006, Oliveira & Garcia 2011). In contrast, mesic sites occur near watercourses and are capable of maintain relatively higher water content than xeric sites for long periods of time (Cap. 3). Humid streamside vegetation includes dense, low stands in which the shrub and herb layer have many peculiar hygrophilous species of Melastomataceae (Alves & Kolbek 2010).

Melastomataceae consists of morphologically diverse plants that occur throughout different habitats in the Neotropical region (Clausing & Renner 2001, Fig. S1). This pantropical family comprises nearly 5000 species arranged into nine tribes and 150-166 genera (Renner 2004), and attains highest diversity in the Neotropics (Renner et al. 2001). With nearly 521 taxa (Mendonça et al. 2008), the Melastomataceae is the fourth most speciose family in the Brazilian Cerrado.

Our sampled comprised 50 perennial species of melastomes (~10% of the species from the biome) into 17 genera and four tribes (Table S1). Representative taxa in our sample include Miconieae, Microlicieae and Melastomeae. Miconieae is a monophyletic group characterized by small flowers with inferior or partly inferior ovaries that develop into baccate fruits (Michelangeli et al. 2008). Nearly 90% of the 275-300 species of Microlicieae are endemic to the rupestrian fields, where it originated (Fritsch et al. 2004). Species in this Tribe and in its sister-group, the Melastomeae, produce dry capsules whose seeds are abiotically dispersed (Fig. S1).

Seed collection and germination experiments

Mature fruits were collected during the peak of seed dispersal (F. A. O. Silveira, unpublished data) from 2008 to 2010. Seeds were collected from individuals occurring in their natural habitats from at least 20 individuals per species. For nine species (*A. punctatissima*, *L. pulcherrima*, *L. coriacea*, *L. cordatum*, *M. sipolisii*, *M. ligustroides*, *M. rubiginosa*, *P. alpestris* and *T. candolleana*) the number of sampled individuals was 3-15. Dry seed mass was determined in 33 species by weighting four replicates of 100 seeds on a digital scale after drying at 70ºC for 5 consecutive days. Seeds were set to germinate under light and dark conditions at controlled temperature conditions (Cap. 3) and germination was monitored at daily intervals for 30 days. For each replicate, we calculated final germination (germinability), mean germination time (MGT; Ranal & Santana 2006), germination synchrony (Ranal & Santana 2006) and light requirement index (LRI; Milberg et al. 2000).

$$\mathrm{MGT} = \sum_{\mathrm{i}=1}^{\mathrm{k}} \mathrm{n_i t_i} / \sum_{\mathrm{i}=1}^{\mathrm{k}} \mathrm{n_i}$$

where n_i is the number of seeds germinated in the time *i*, t_i is the time from the start of the experiment to the *ith* observation, and *k* is the time of last germination.

$$\overline{E} = -\sum_{i=1}^{k} f_i \log_2 f_i \quad \text{being} \quad f_i = n_i / \sum_{i=1}^{k} n_i$$

where f_i is the relative frequency of germination, n_i is the number of seeds germinated in the day *i* and k is the time of last germination. Low $\overline{E}$ values indicate more synchronized germination whereas values approaching zero denote perfect synchrony.

$$LRI = Gl/(Gd + Gl)$$

where Gl = the germination percentage in light, and Gd = the germination percentage in darkness. LRI ranges from zero (germination occurring only under dark conditions) to one (total light requirement). Values near 0.5 indicate aphotoblastism.

Ordinary statistical analyses

To carry out the analyses, species were grouped according to functional groups and distribution. Plant functional types are used here in a broad concept. Plant functional types are defined as a group of plants that, irrespective of phylogeny, are similar in given set of traits and similar in their association to certain variables (*sensu* Pillar & Sosinski-Jr 2003). In this study, plant functional types comprise growth-form (trees, shrubs or sub-shrubs), dispersal mode (biotic vs. abiotic) and dispersal phenology (continuous, dry, dry-rainy, rainy or rainy-dry). As defined here, plant distribution encompasses plant microhabitat (xeric or mesic) and geographic range (endemic of rupestrian fields or widespread in Brazil and/or South America). All 50 species were assigned to only one category for each plant functional types and distribution based on our previous knowledge on their natural history (F. A. O. Silveira, unpublished data, Cap. 3) and species description.

Four replicates of 50 seeds (25 seeds for *Miconia* and *Leandra*) were mounted. Data in percentage were arcsin transformed to meet the assumptions of the parametric analysis (Santana & Ranal 2006). Firstly, we ran one-way ANOVAs for each functional group separately (y-variables were germinability and MGT). One-way ANOVAs measured the effect of each factor across all variables. Then, we ran two-way and three-way ANOVAs in order to detect significant interactions among functional groups in response variables (Wang et al. 2009). The Tukey test was used for post-hoc in all multiple comparisons (Zar 1996).

Correlations among germinability, MGT, $\overline{E}$, LRI and seed size were determined through Pearson correlation test. These correlations were carried out for the 33 species for which we measured seed size (Zar 1996). In all analyses, we established α values of 0.05.

Finally, we calculated the coefficient of variation (CV) in germinability and in MGT in order to get insight into the amount of variance exhibited by those traits.

Phylogenetic-based statistics

To explore the evolutionary patterns of seed traits in Melastomataceae, an ultrametric phylogenetic tree showing relationships among the studied taxa was built. A pruned tree was built with the study species as terminal tips using the maximally resolved tree with the use of Phylomatic (http://www.phylodiversity.net/phylomatic/).

The occurrence of several polytomies in our tree (Fig. S2) may prevent the correct interpretation of the evolution of seed dormancy. However, most polytomies were in terminal taxa and thus, considered soft polytomies (Webb *et al.* 2006), which are likely to cause low interference in interpretation (Ackerly 2009a). Polytomies in the tree were resolved by randomly generating 100 fully resolved trees using Mesquite (Maddison & Maddison 2008). Species relationships were improved and polytomies were resolved based on available and published data for the taxa relationships in the family (Renner 1993, Clausing & Renner 2001, Fritsch et al. 2004, Goldenberg et al. 2008, Michelangeli et al. 2008). Unpublished theses were also used to determine relationships at generic and specific levels.

Dating branch length is important to avoid bias on estimates of phylogenetic distance. Based on estimates on clade age for Melastomataceae, we calibrated the tree by dating know nodes with the Branch Length ADJustment function (BLADJ) algorithm of the Phylocom (Webb *et al.* 2006). We were able to perform node dating by including node age estimates for five nodes, including the root node (known estimated ages for the root node are a prerequisite for BLADJ. Even with only a few nodes dated, the resulting phylogenetic distances can be a marked improvement on simply using the number of intervening nodes as a phylogenetic distance (Webb et al. 2006). Age estimates were extracted from Renner & Meyer (2001), Renner et al. (2001), Fritsch et al. (2004) and Rutschmann et al. (2007). Undated nodes were assigned equal branch lengths between nodes for which age estimates are available, minimizing tree-wise variance in branch length (Moles et al. 2005, Milla 2009).

Our approach included four phylogenetically-based analyses. All of them were implemented with Analysis of Traits (AOT) package for Phylocom (Webb et al. 2006). Firstly, we calculated the PICs for each seed trait. For all nodes the contrast size is the estimated trait value at one daughter node, minus the estimated trait value of the second daughter node, divided by the square root of the total branch length between the two nodes (Felsenstein 1985). The second analysis was the calculations of divergence and convergence across nodes in the phylogenetic tree for five seed traits: germinability, MGT, $\overline{E}$, LRI and seed size. A significant divergence means that trait divergence between sister groups is higher than expected by chance whereas a significant convergence means that the divergences in a given trait is lower than expected by chance in two lineages arising from a given node. Then, we obtained tree-wise phylogenetic signal for each of the five seed traits (Blomberg et al. 2003). Trait values were permuted across the tips of the tree 19,999 times to test whether the observed values are significantly different than would be expected by chance (Moles et al. 2005, Milla 2009).

Finally, we calculated the contribution index which ranks divergences according to the amount of present-day variation in seed mass they explain (Moles et al. 2005). Expected values for the internal nodes of the tree were multiplied by the amount of the total variation (across the whole tree) that lies within the focal clade. This method gives high scores to divergences that are large and/or involve clades with large numbers of descendent species (Moles et al. 2005).

Correlations among PICs in germinability, MGT, $\overline{E}$, LRI and seed size were determined through Pearson correlation test for the same 33 species used in ordinary statistics. A principal component analysis (PCA) was carried out based on the correlation matrix of all PICs.

Results

Ordinary statistical analyses

We have found significant effects of distribution and plant functional types on both seed germinability and MGT. Except for geographic range, all explanatory variables were highly correlated with y-variables (Table 1). Sub-shrubs had lower germinability and shrubs had lower MGT compared to the other growth-forms (Fig. 1A). Seeds of species from mesic sites germinated faster and to higher percentages compared to seeds from species occurring in seasonally xeric sites (Fig. 1C). Seeds from berries germinated to higher percentage compared to seeds from capsules but the former took longer to complete germination compared to the latter (Fig. 1D). Remarkable differences were also found for germinability and MGT in species differing in seed dispersal seasons. Plants dispersing seeds during the rainy-dry transition had the lowest germinability and highest germination time (Fig. 1E).

We also found significant interactions among explanatory variables. Nine out of ten interactions were significant for germinability and seven out of ten interactions were significant for MGT (Table 2). In general, two-level interactions were characterized by lower germinability and higher MGT of biotically-dispersed species, lack of differences between species in xeric and mesic habitats and varied responses between widespread and endemic species (Fig. S3).

Table 1. Summary of one-way ANOVA for the effects of plant functional types and distribution on germinability and mean germination time of 50 Melastomataceae species from a Neotropical montane savanna.

Source of Variation	**DF**	**Germinability**		**Mean germination time**	
		F	**p**	**F**	**p**
Growth-form	2	24.555	0.000	5.898	0.003
Microhabitat	1	5.218	0.023	18.555	0.000
Dispersal	1	25.747	0.000	19.379	0.000
Phenology	4	28.520	0.000	6.445	0.000
Geographic range	1	2.595	0.109	0.136	0.713

Finally, we have detected significant three-level interactions for both variables. All interactions were significant for germinability and seven out of ten were significant for MGT (Table 3). The variance in germinability was considerably higher than that of MGT. The relative frequency of CV for MGT was skewed toward low values, whereas for germinability, the relative frequency of CV values was more evenly distributed despite higher frequency of low values (Fig. 4).

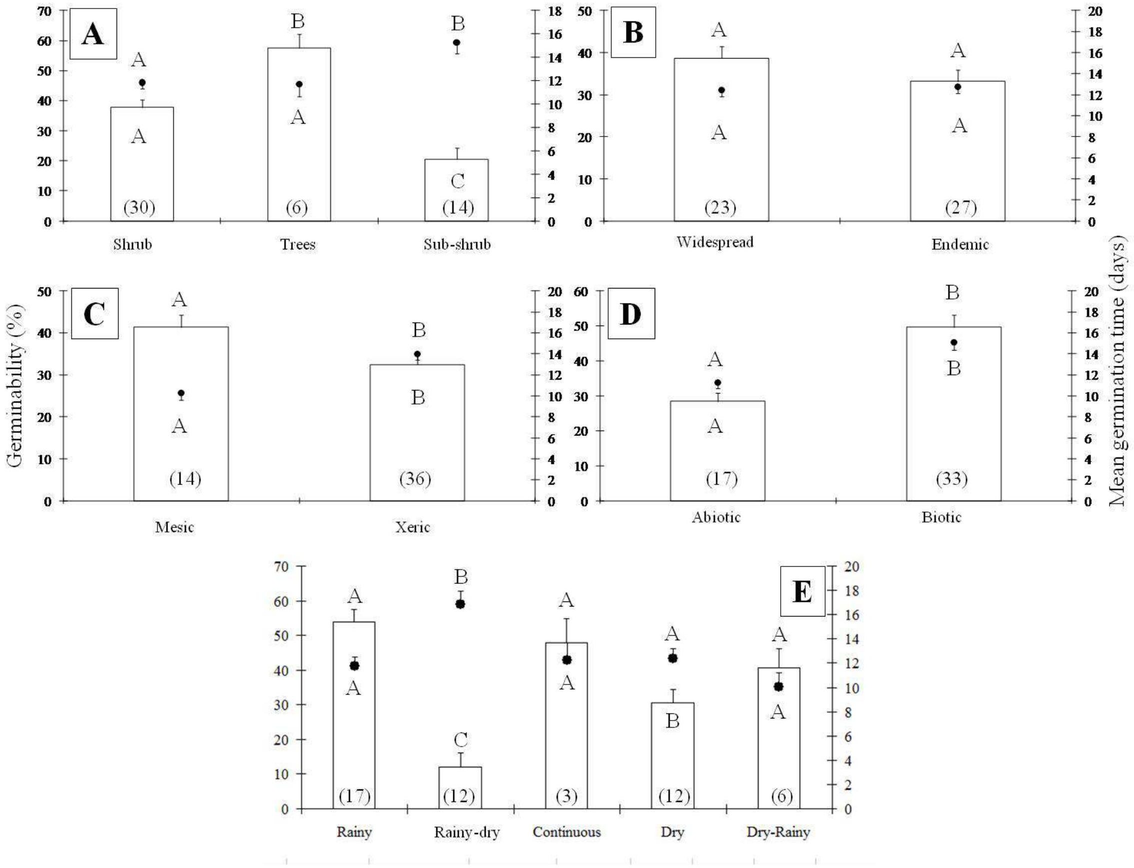


Figure 1. Average (±SE) germinability (bars) and mean germination time (filled circles) of 50 Melastomataceae species as a function of growth-form (A), geographic range (B), microhabitat (C), dispersal mode (D) and phenology (E). Numbers in brackets refer to sample size in each category.

Phylogenetic-based statistics

We found significant tree-wise phylogenetic signal for LRI (p= 0.017) and seed size (p=0.027), but not for MGT (p= 0.81), germinability (p= 0.63) and $\overline{E}$ (p= 0.43). We then, analyzed trait conservatism by node along the phylogenetic tree.

For germinability we found six divergences and four convergences. One divergence occurred between Merianieae and Miconieae and the remaining divergences all occurred within the Miconieae. One of them occurred between *Leandra* and the rest of Miconieae and the other four among *Miconia* species (Fig. 2A). A convergence was found between Melastomeae and Microlicieae and three within Microlicieae (Fig 2A).

A similar pattern was found for MGT. Divergences occurred between Merianieae and Miconieae and also in Miconieae. A single divergence occurred among *Microlicia* species (Fig. 2B). Divergences occurred between Melastomeae and Microlicieae, between *Rhynchanthera* and among *Lavoisiera* (Fig. 2B).

Table 2. Summary of the two-way ANOVA interactions in germinability and mean germination time of 50 Melastomataceae species from rupestrian fields. Growth-form (GF), microhabitat (MH), dispersal (D), phenology (P) and geographic range (GR).

Source of Variation	**DF**	**Germinability**		**Mean germination time**	
		F	**p**	**F**	**p**
GF x MH	4	2.406	0.093	2.594	0.078
GF x P	8	14.613	0.000	6.168	0.000
GF x D	2	5.876	0.003	29.514	0.000
GF x GR	4	4.098	0.018	3.593	0.030
MH x D	1	17.424	0.000	1.974	0.162
MH x GR	1	6.355	0.012	7.535	0.007
MH x P	4	8.718	0.000	5.165	0.001
D x GR	1	17.444	0.000	15.162	0.000
D x P	4	11.414	0.000	7.587	0.000
GR x P	4	5.661	0.000	2.101	0.083

The patterns found for the evolution of $\overline{E}$ were very similar to those found for germinability. Again, divergences were observed between Merianieae and Miconieae and within Miconieae, whereas convergences were observed between Melastomeae and Microlicieae and within Microlicieae (Fig. 2C). For LRI, a single convergence occurred between *Trembleya* species and no significant divergences were observed (Fig. 2D).

Five convergences and no divergences were observed for seed size along the phylogenetic tree. Consistent to previous patterns, convergences were observed between Melastomeae and Microlicieae and within Microlicieae, but also occurred in *Tibouchina* (Fig. 2E). We have found a strong pattern of coordinated evolution in divergences and convergences along the phylogenetic tree of Melastomataceae (Table 3).

Cross-species comparisons revealed significant positive correlation between germinability and synchrony and between germinability and seed size. A negative significant correlation was found between germinability and MGT (Table 4). Phylogenetic-controlled correlations revealed significant correlations between germinability and synchrony and a negative correlation between synchrony and MGT. However, the other two significant correlations in ordinary-cross species comparisons ceased to be significant when phylogeny is taken into account (Table 4).

Table 4. Pearson correlation coefficient among seed traits of 33 species of Melastomataceae from rupestrian fields, southeastern Brazil. Values below diagonal refer to classical cross-species comparisons. Values above diagonal refer to phylogenetic-controlled statistics. Germination traits are mean germination time (MGT), germination synchrony ($\overline{E}$) and light requirement index (LRI).

	Germinability	MGT	$\overline{E}$	LRI	Size
Germinability	0	-0.279	0.581***	0.0613	-0.0260
MGT	-0.373*	0	-0.773***	-0.0180	0.0989
$\overline{E}$	0.722***	-0.255	0	0.0112	-0.0733
LRI	0.0682	0.0945	-0.158	0	-0.0399
Size	0.457**	0.0823	0.242	-0.0511	0

* $P > 0.05$; ** $P > 0.01$; *** $P > 0.001$.

Table 5. Correlations among the divergences making the 20 largest contributions to present-day variation in germination traits of 50 Melastomataceae species. All LRI correlations were non-significant. Germination traits are mean germination time (MGT) and germination synchrony ($\overline{E}$).

	MGT	Germinability
MGT	-	
Germinability	0.98***	-
$\overline{E}$	0.97***	0.98***

*** $P > 0.001$.

The first two axes of the PCA using PICs accounted for 67.1% of total variance (Table S2). Contrasts in MGT were positively associated with PC1, whereas contrasts in germinability and synchrony were negatively associated with PCI. Contrasts in seed size and in LRI were correlated with PC2, but in opposite directions (Fig. 3, Table S2).

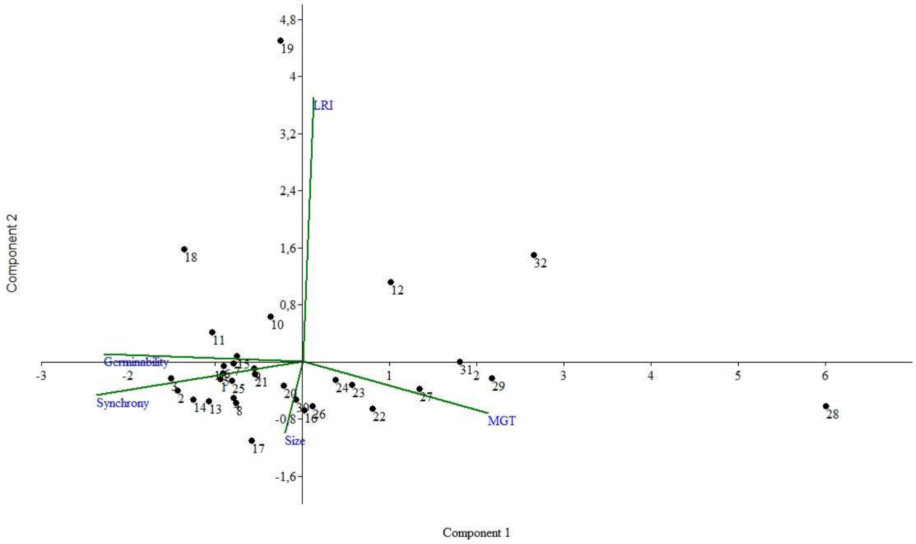


Figure 3. Biplot representation of trait scores of the first two axes of the principal component analysis (PCA) of PICs in seed traits of 33 species of Melastomataceae from Neotropical montane grasslands. Numbers indicate the nodes from where PICs were calculated. MGT= mean germination time and LRI= light requirement index.

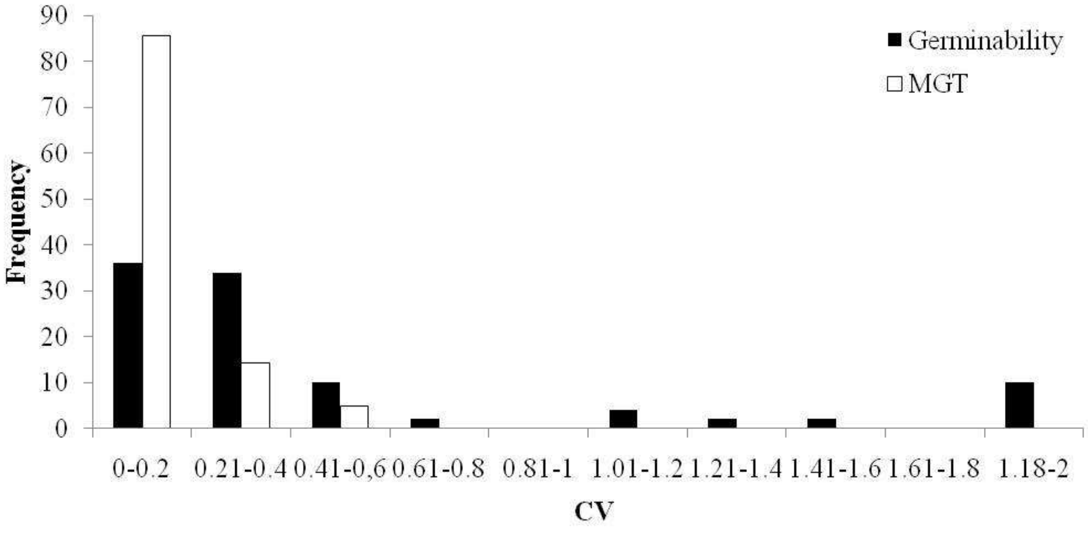


Figure 4. Relative frequency distribution of values of coefficient of variance (CV) in germinability and mean germination time (MGT) in 50 species of Melastomataceae from Neotropical montane grasslands.

Discussion

In our cross-species comparisons, we were able to show how variation in ecophysiological and morphological seed traits is associated with plant functional types and distribution. Association of seed germination with plant functional types and phylogeny has been found in arid and alpine meadows (Bu et al. 2008), semi-arid vegetations (Wang et al. 2009) and Mediterranean ecosystems (Luna & Moreno 2009) but our study is unique in that it shows a germination-phenology linkage. Species with seed dispersal during rainy-dry transition (unfavourable conditions for establishment, Cap. 4) had lower germinability and delayed germination compared to the other phenological groups. The patterns held even considering the interactions with other life-history attributes.

The fact that most significant two-way and three-way were significant suggests that germination and ecological traits are associated in more complex ways than previously thought. Multiple selective pressures operating in opposite directions may drive the evolution of germination traits and it is more likely that present-day patterns result from the balance among the strength in each selective pressure (Moles et al. 2005, Bu et al. 2008). Irrespective of phylogenetic position, germination traits evolved in similar ways in plants under the same combination of interacting selective forces. A good example of convergent evolution is seed dormancy (Cap. 4).

Our data provides low support for the association between germination and plant distribution (Donohue et al. 2010). We did not observe significant differences in germination traits between widespread and endemic species. As a matter of fact, the lack of association between germination traits and adult plant distribution is increasingly being documented in the rupestrian fields (Silveira et al. 2011, Cap. 3). Therefore, we argue that factors controlling seedling establishment, rather than seed germination, may account for restricted geographic distribution of endemic species.

We have found strong and consistent patterns in the evolution of germination traits along the phylogenetic tree of Melastomataceae. Germinability, MGT and $\overline{E}$ lacked a tree-wise phylogenetic signal, but showed similar patterns of evolution when trait conservatism is examined by node. The consistent pattern of divergences Merianieae and Miconieae and within the Miconieae is only rivalled by the coordinated patters of convergences between Melastomeae and Microlicieae and within Microlicieae. These patterns indicate different selective pressures driving the evolution of seed strategies in those groups. For the Miconieae, data suggests strong competition whereas for Microlicieae, germination traits independently evolved in responses to similar environmental pressures.

Divergences in seed traits are often associated with shifts in dispersal mode and growth-form (Moles et al. 2005). In our case, the early divergence between Merianieae and Miconieae ~53 million years ago (Ma) was associated with a shift in dispersal mode from abiotic to biotic. Further divergences within the Miconieae suggest competition avoidance in these berry-fruited species. Species of *Miconia* often show minor overlaps in fruiting seasons, so that selection may have favoured temporal displacement in dispersal seasons as well (Snow 1965, Poulin et al. 1999, F. A. O. Silveira, unpublished data). It is appealing to argue that divergences in germination traits are also a product of character displacement resulting from disruptive selection, but additional studies are needed before invoking competition avoidance as drivers of present-day patterns. As similar as for phenological strategies, assuming competition as an selective force driving the evolution of germination syndromes would imply in accepting that divergence in germination traits may also help plant coexistence in these species-rich grasslands (Grubb 1977).

Melastomeae and Microlicieae diverged ~24.5 Ma, but in contrast to putative competition experienced by berry-fruited species, germination traits in these abiotically-dispersed species were more similar than would be expected by chance. This functional convergence in germination is not only associated with the dry-capsule habit, but also to shifts in growth-form and geographic distribution. Microlicieae comprise shrubs or sub-shrubs and virtually all species are endemic of the rupestrian fields (Fritsch et al. 2004), where they originated and adapted (Simon et al. 2009).

The divergence of *Rhyncanthera* and the rest of Microlicieae ~3.7 Ma was also followed by a convergence in the clade including *Microlicia*, *Lavoisiera*, *Chaetostoma* and *Trembleya*. Two main ecological shifts were associated with this divergence. First, the hygrophilous *Rhynchanthera* species are associated with humid streamside vegetation (Alves & Kolbek 2010) and whereas the remaining genera are primarily associated with seasonally xeric sites. In addition, *Rhynchantera* is a widespread genus whereas the other genera are nearly endemic to the rupestrian fields. Hence, this late convergence is considered a physiological adaptation to this environment.

But what particular set of traits distinguish the germination of Microlicieae? Apart from *Rhynchantera* and few *Lavoisiera*, seeds in this tribe show physiological dormancy, delayed germination and low germinability (Cap. 3, Cap. 4). Moreover, these species, as low-stature plants do in general (Thomson et al. 2011), share poor dispersal distances (apex-to-base dehiscence in *Trembleya, Microlicia* and *Chaetosoma* and base-to-apex dehiscence in *Lavoisiera*, Fritsch et al. 2004). As a result of poor dispersal distance by these capsules, plants in these genera often show clumped distribution (see Fig. S1 K-P). Seeds of poorly-dispersed species are more likely to experience sibling competition and often show delayed germination and/or dormancy (Bu et al. 2008).

Ecological and evolutionary meaning of the functional convergence in Microlicieae seeds will only be understood when we establish trait-environment association (Martins 2000), i.e. when we know enough on the dormancy breaking mechanisms in the field. Fire-mediated dormancy release is a good example of functional convergence in fire-prone habitats (Keeley & Bond 1997), and is also expected in the flora of the rupestrian fields.

Some cross-species correlations were not significant when phylogeny was accounted for. Different outcomes in cross-species and phylogenetic-controlled correlations indicate that trait correlations among extant species do not provide a reliable estimate of the historical patterns of correlated evolutionary changes that have occurred along the branches of the phylogeny (Ackerly 2009a). Therefore, there was no consistent evolutionary trend for correlated changes between germinability and MGT and between germinability and seed size. Our data strongly supports the idea that germinability and germination synchrony evolved in a coordinated manner.

We expected significant phylogenetic correlations between seed size and LRI (Milberg et al. 2000) and seed size and germination time (Norden et al. 2009, Bu et al. 2008), because these traits have been shown to have co-evolved (Milberg et al. 2000, Norden et al. 2009). A possible explanation for the lack of significant in phylogenetic correlation is the small seed size amplitude used here. Despite variation in seed size was 155-fold among the studied species (Cap. 3), seed size in Melastomataceae is a conserved trait.

Contrary to seed size which is a phylogenetically conserved trait (Moles et al. 2005, Norden et al. 2009), we found numerous events of divergence and convergence in germination traits along the phylogenetic tree of Melastomataceae. In this study, we documented phylogenetic signals for seed size and LRI, which are interpreted as niche conservatism (Losos 2008, but see Ackerly 2009b). Therefore, for the studied species, light requirement for germination shall not be interpreted as an adaptation to open environments as suggested for other small-sized species (Garcia & Oliveira 2007). Rather, photoblastism in this set of species is a plesiomorphic character and should be considered as a constraint to the small seed size.

Small seed size is often associated with mesic sites whereas xeric sites are related to larger seed size (Leyer & Press 2009). The lack of divergences in seed mass in daughter nodes shifting in microhabitat does not necessarily means the lack of the pattern. These divergences would be expected in terminal tips of the tree, where resolution is poor. The polytomies in *Lavoisiera* and *Miconia* (two genera where habitat divergence were recurrent) may have prevented the detection of late divergences.

Concluding, morphological and ecophysiological seed traits of Melastomataceae were affected by phylogeny and ecological factors. Our data shed light on the evolution of seed traits in a Neotropical montane savanna. Moreover, we have shown a great dynamism in seed germination strategies in a relative small set of species. The implications of our findings are twofold: first, character displacement in germination traits as a result of disruptive selection may help promoted species coexistence in species-rich communities. Secondly, our data suggest coordinated evolution of dispersal mode and growth-form. Although Melastomataceae is a suitable model for the study of seed evolution, we advise caution before extrapolating our results for other taxa because seed size is highly conserved in the family. We encourage further studies employing the phylogenetic comparative methods in species-rich savannas to include species belonging to clades with greater variation in seed size. These studies will help understand the role of seed in structuring plant communities.

Table 3. Summary of the three-way ANOVA interactions in germinability and mean germination time of 50 Melastomataceae species from rupestrian fields. Growth-form (GF), microhabitat (MH), dispersal (D), phenology (P) and geographic range (GR).

Source of Variation	DF	Germinability		Mean germination time	
		F	**p**	**F**	**P**
GF x MH x DM	2	3.048	0.050	5.963	0.003
GF x MH x GR	2	5.022	0.007	4.381	0.014
GF x MH x P	8	8.092	0.000	4.381	0.014
GF x D x GR	2	5.116	0.007	7.293	0.000
GF x D x P	8	11.720	0.000	2.119	0.123
GF x GD x P	8	9.448	0.000	2.933	0.004
MH x D x GR	1	5.910	0.016	0.816	0.367
MH x D x P	4	3.789	0.005	0.303	0.876
MH x GR x P	4	4.425	0.002	6.538	0.000
D x GR x P	4	9.953	0.000	3.268	0.013

Figure 2. Topology of the phylogenetic tree of 50 Melastomataceae species showing the location of divergences (gray diamonds) and convergences (filled circles) in seed germinability (A), mean germination time (B), germination synchrony (C), light requirement index (D) and seed mass (E). Data for E is restricted to only 33 species.

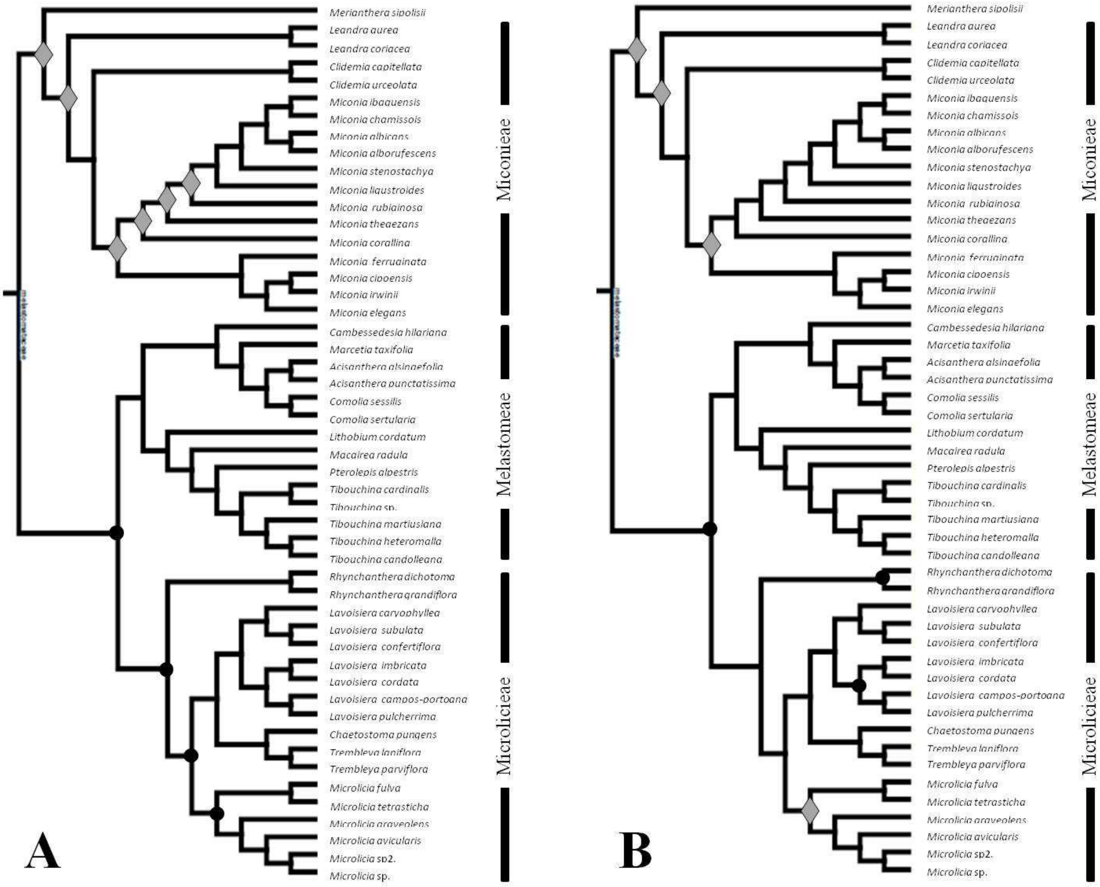
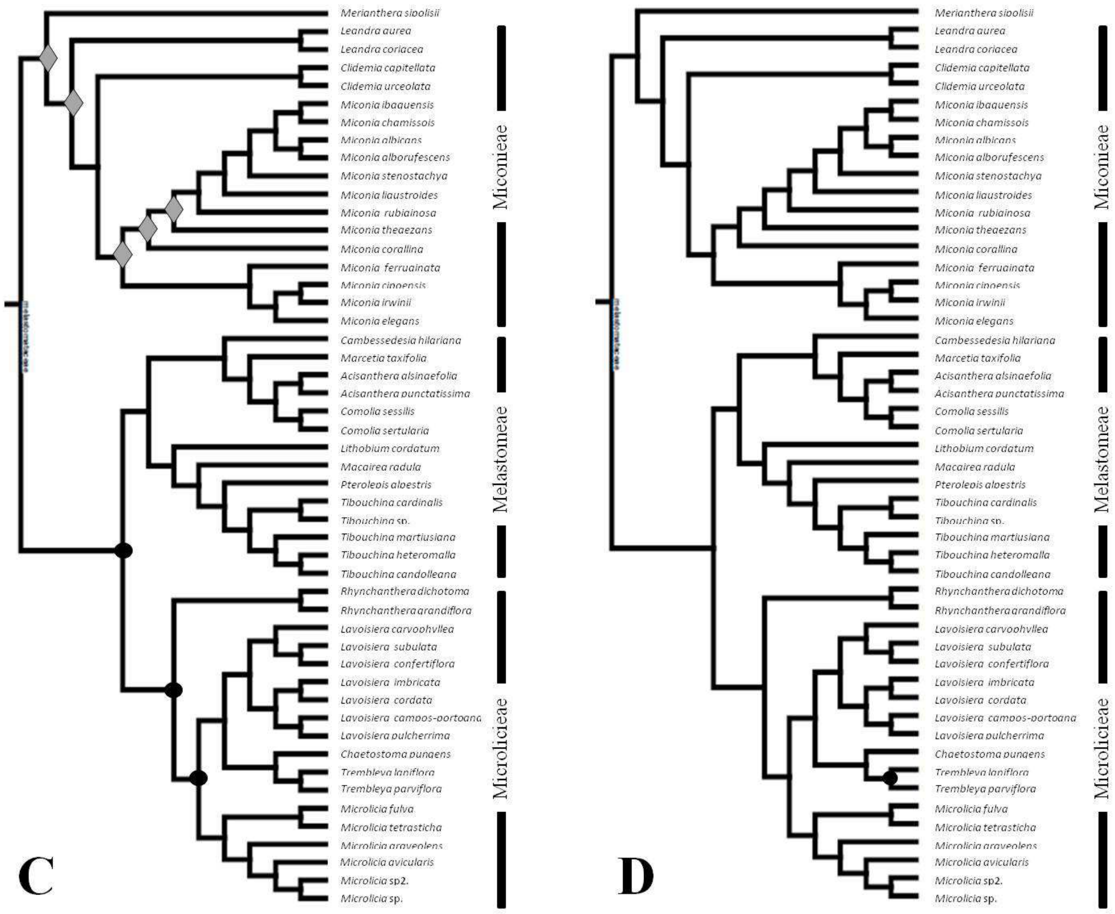


Figure 2


Merianthera sipolisii
Leandra aurea
Leandra coriacea
Clidemia capitellata
Clidemia urceolata
Miconia ibaguensis
Miconia chamissois
Miconia albicans
Miconia alborufescens
Miconia stenostachya
Miconia ligustroides
Miconia rubiginosa
Miconia theaezans
Miconia corallina
Miconia ferruginata
Miconia cipoensis
Miconia irwinii
Miconia elegans
Cambessedesia hilariana
Marcetia taxifolia
Acisanthera alsinaefolia
Acisanthera punctatissima
Comolia sessilis
Comolia sertularia
Lithobium cordatum
Macairea radula
Pterolepis alpestris
Tibouchina cardinalis
Tibouchina sp.
Tibouchina martiusiana
Tibouchina heteromalla
Tibouchina candolleana
Rhynchanthera dichotoma
Rhynchanthera grandiflora
Lavoisiera caryophyllea
Lavoisiera subulata
Lavoisiera confertiflora
Lavoisiera imbricata
Lavoisiera cordata
Lavoisiera campos-portoana
Lavoisiera pulcherrima
Chaetostoma pungens
Trembleya laniflora
Trembleya parviflora
Microlicia fulva
Microlicia tetrasticha
Microlicia graveolens
Microlicia avicularis
Microlicia sp2.
Microlicia sp.
Miconieae
Melastomeae
Microlicieae
C
Merianthera sipolisii
Leandra aurea
Leandra coriacea
Clidemia capitellata
Clidemia urceolata
Miconia ibaguensis
Miconia chamissois
Miconia albicans
Miconia alborufescens
Miconia stenostachya
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Miconia rubiginosa
Miconia theaezans
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Pterolepis alpestris
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Rhynchanthera grandiflora
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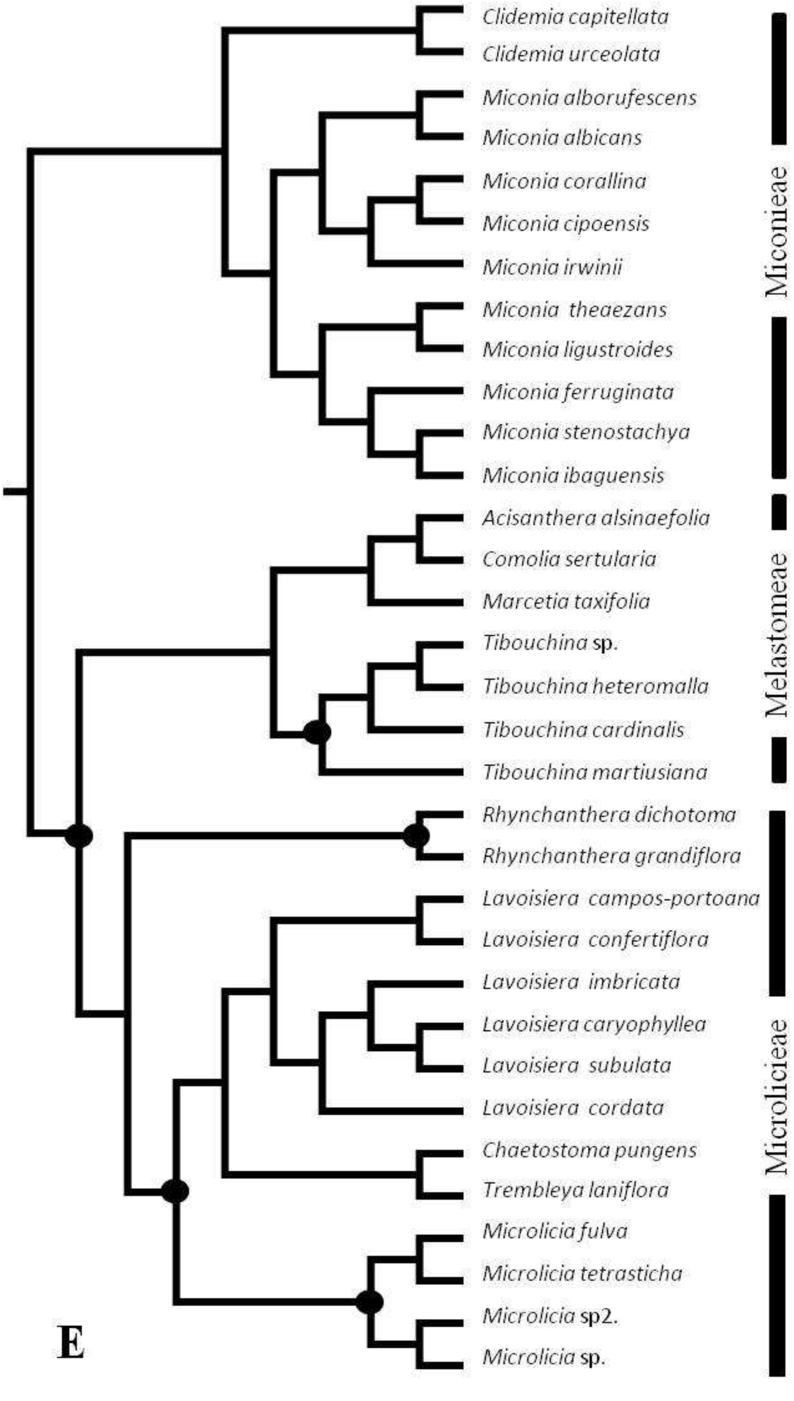
Lavoisiera subulata
Lavoisiera confertiflora
Lavoisiera imbricata
Lavoisiera cordata
Lavoisiera campos-portoana
Lavoisiera pulcherrima
Chaetostoma pungens
Trembleya laniflora
Trembleya parviflora
Microlicia fulva
Microlicia tetrasticha
Microlicia graveolens
Microlicia avicularis
Microlicia sp2.
Microlicia sp.
Miconieae
Melastomeae
Microlicieae
D

Figure 2


Clidemia capitellata
Clidemia urceolata
Miconia alborufescens
Miconia albicans
Miconia corallina
Miconia cipoensis
Miconia irwinii
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Miconia ferruginata
Miconia stenostachya
Miconia ibaguensis
Acisanthera alsinaefolia
Comolia sertularia
Marcetia taxifolia
Tibouchina sp.
Tibouchina heteromalla
Tibouchina cardinalis
Tibouchina martiusiana
Rhynchanthera dichotoma
Rhynchanthera grandiflora
Lavoisiera campos-portoana
Lavoisiera confertiflora
Lavoisiera imbricata
Lavoisiera caryophyllea
Lavoisiera subulata
Lavoisiera cordata
Chaetostoma pungens
Trembleya laniflora
Microlicia fulva
Microlicia tetrasticha
Microlicia sp2.
Microlicia sp.
Miconieae
Melastomeae
Microliciaeae
E

Figure 2

Electronic supplementary material

Silveira et al. – Comparative ecophysiology of seed dormancy and germination in Neotropical montane savannas

Contents:

1) Table S1
2) Figure S1
3) Figure S2
4) Figure S3
5) Table S2

Table S1. Life-history traits and geographic range of 50 Melastomataceae species from Neotropical montane grasslands in Brazil.

Tribe	**Species**	**Growth-form**	**Habitat**	**Dispersal**	**Dispersal phenology**	**Geographic distribution**
Melastomeae	*Acisanthera alsinaefolia*	Shrub	Mesic	Abiotic	Continuous	Endemic
Melastomeae	*Acisanthera punctatissima**	Sub-shrub	Xeric	Abiotic	Rainy-Dry	Endemic
Melastomeae	*Cambessedesia hilariana*	Sub-Shrub	Xeric	Abiotic	Continuous	Endemic
Microlicieae	*Chaetostoma armatum**	Sub-Shrub	Xeric	Abiotic	Rainy-dry	Widespread
Miconieae	*Clidemia capitellata*	Shrub	Xeric	Biotic	Rainy	Widespread
Miconieae	*Clidemia urceolata*	Shrub	Xeric	Biotic	Dry-Rainy	Widespread
Melastomeae	*Comolia sertularia**	Shrub	Xeric	Abiotic	Rainy-dry	Endemic
Melastomeae	*Comolia sessilis*	Shrub	Xeric	Abiotic	Dry	Endemic
Microlicieae	*Lavoisiera campos-portoana*†	Shrub	Xeric	Abiotic	Dry	Endemic

Microlicieae	*Lavoisiera caryophyllea**†	Sub-shrub	Mesic	Abiotic	Rainy-dry	Endemic
Microlicieae	*Lavoisiera confertiflora*	Sub-Shrub	Xeric	Abiotic	Rainy	Endemic
Microlicieae	*Lavoisiera cordata*†	Shrub	Xeric	Abiotic	Dry	Endemic
Microlicieae	*Lavoisiera imbricata*	Shrub	Xeric	Abiotic	Rainy	Endemic
Microlicieae	*Lavoisiera pulcherrima*	Shrub	Mesic	Abiotic	Dry	Endemic
Microlicieae	*Lavoisiera subulata*†	Sub-Shrub	Mesic	Abiotic	Dry	Endemic
Miconieae	*Leandra aurea*	Shrub	Xeric	Biotic	Rainy	Widespread
Miconieae	*Leandra coriacea*	Shrub	Xeric	Biotic	Rainy	Widespread
Microlicieae	*Lithobium cordatum**†	Sub-Shrub	Xeric	Abiotic	Rainy-dry	Endemic
Melastomeae	*Macairea radula*	Shrub	Mesic	Abiotic	Rainy	Widespread
Melastomeae	*Marcetia taxifolia*	Shrub	Xeric	Abiotic	Dry	Widespread
Merianeae	*Merianthera sipolisii*	Tree	Xeric	Abiotic	Rainy-dry	Endemic
Miconieae	*Miconia albicans*	Shrub	Xeric	Biotic	Rainy	Widespread
Miconieae	*Miconia alborufescens*	Shrub	Xeric	Biotic	Rainy	Endemic
Miconieae	*Miconia cipoensis*†	Shrub	Xeric	Biotic	Rainy	Endemic
Miconieae	*Miconia chamissois*	Shrub	Mesic	Biotic	Rainy	Widespread
Miconieae	*Miconia corallina**	Shrub	Xeric	Biotic	Rainy-dry	Widespread
Miconieae	*Miconia elegans*	Shrub	Mesic	Biotic	Rainy	Widespread
Miconieae	*Miconia ferruginata*	Tree	Xeric	Biotic	Dry-Rainy	Widespread
Miconieae	*Miconia ibaguensis*	Shrub	Mesic	Biotic	Rainy	Widespread
Miconieae	*Miconia irwinii*	Tree	Xeric	Biotic	Dry	Endemic
Miconieae	*Miconia ligustroides*	Shrub	Xeric	Biotic	Rainy-dry	Widespread

Miconieae	*Miconia stenostachya*	Shrub	Xeric	Biotic	Rainy	Widespread
Miconieae	*Miconia rubiginosa*	Tree	Xeric	Biotic	Rainy	Widespread
Miconieae	*Miconia theaezans*	Shrub	Mesic	Biotic	Rainy-dry	Widespread
Microlicieae	*Microlicia avicularis**	Sub-Shrub	Xeric	Abiotic	Dry	Endemic
Microlicieae	*Microlicia fulva*	Sub-Shrub	Xeric	Abiotic	Dry	Widespread
Microlicieae	*Microlicia graveolens*	Sub-Shrub	Mesic	Abiotic	Dry	Endemic
Microlicieae	*Microlicia* sp*	Sub-Shrub	Xeric	Abiotic	Rainy-Dry	Endemic
Microlicieae	*Microlicia* sp2*	Sub-Shrub	Xeric	Abiotic	Dry	Endemic
Microlicieae	*Microlicia tetrasticha**	Sub-Shrub	Xeric	Abiotic	Rainy-Dry	Endemic
Melastomeae	*Pterolepis alpestris*	Sub-Shrub	Mesic	Abiotic	Dry-Rainy	Endemic
Microlicieae	*Rhynchanthera cordata*	Shrub	Mesic	Abiotic	Dry-Rainy	Widespread
Microlicieae	*Rhynchanthera grandiflora*	Shrub	Mesic	Abiotic	Rainy	Widespread
Melastomeae	*Tibouchina candolleana*	Tree	Xeric	Abiotic	Rainy	Widespread
Melastomeae	*Tibouchina cardinalis**	Shrub	Xeric	Abiotic	Rainy-dry	Endemic
Melastomeae	*Tibouchina heteromalla*	Shrub	Xeric	Abiotic	Continuous	Widespread
Melastomeae	*Tibouchina martiusiana*	Shrub	Xeric	Abiotic	Dry-Rainy	Widespread
Melastomeae	*Tibouchina* sp.	Tree	Xeric	Abiotic	Dry	Endemic
Microlicieae	*Trembleya laniflora*	Shrub	Xeric	Abiotic	Dry-Rainy	Endemic
Microlicieae	*Trembleya parviflora*	Shrub	Mesic	Abiotic	Rainy	Endemic

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* Denotes species with dormant seeds.

† Included in the list of rare plants of Brazil or threatened by extinction by red lists.

Figure S1. Representative landscape and species of three melastome subfamilies. (A) Typical grassland showing a rocky outcrop in the left part. (B) General aspect of a rocky outcrop. (C) *T. cardinalis* established on rocky outcrops. (D) *C. hilariana* in xeric grasslands. (E) *L. caryophyllea* in mesic grasslands. (F) *M. chamissois* in streamside vegetation. (G) Fruit dehiscence of *C. sertularia*. (H) blooming of *M. rubiginosa* and a gallery forest in the back. (I-J) Berries of *M. stenostachya* and *M. ferruginata*. (K) Clumped distribution of *M. tetrasticha* (fruiting; fruit details in the inset) and *Microlicia* sp1. (flowering). (L) Clumped distribution of *M. taxifolia*. (M) Fruit dehiscence of *L. cordata*. (N) Clumped distribution of *T. parviflora*. (O) Fruit dehiscence of *L. subulata*. (P) Fruit dehiscence of *L. campos-portoana* (Q) Flowering of *T. laniflora*. All photos by F. A. O. Silveira, except K and N by D. Negreiros.

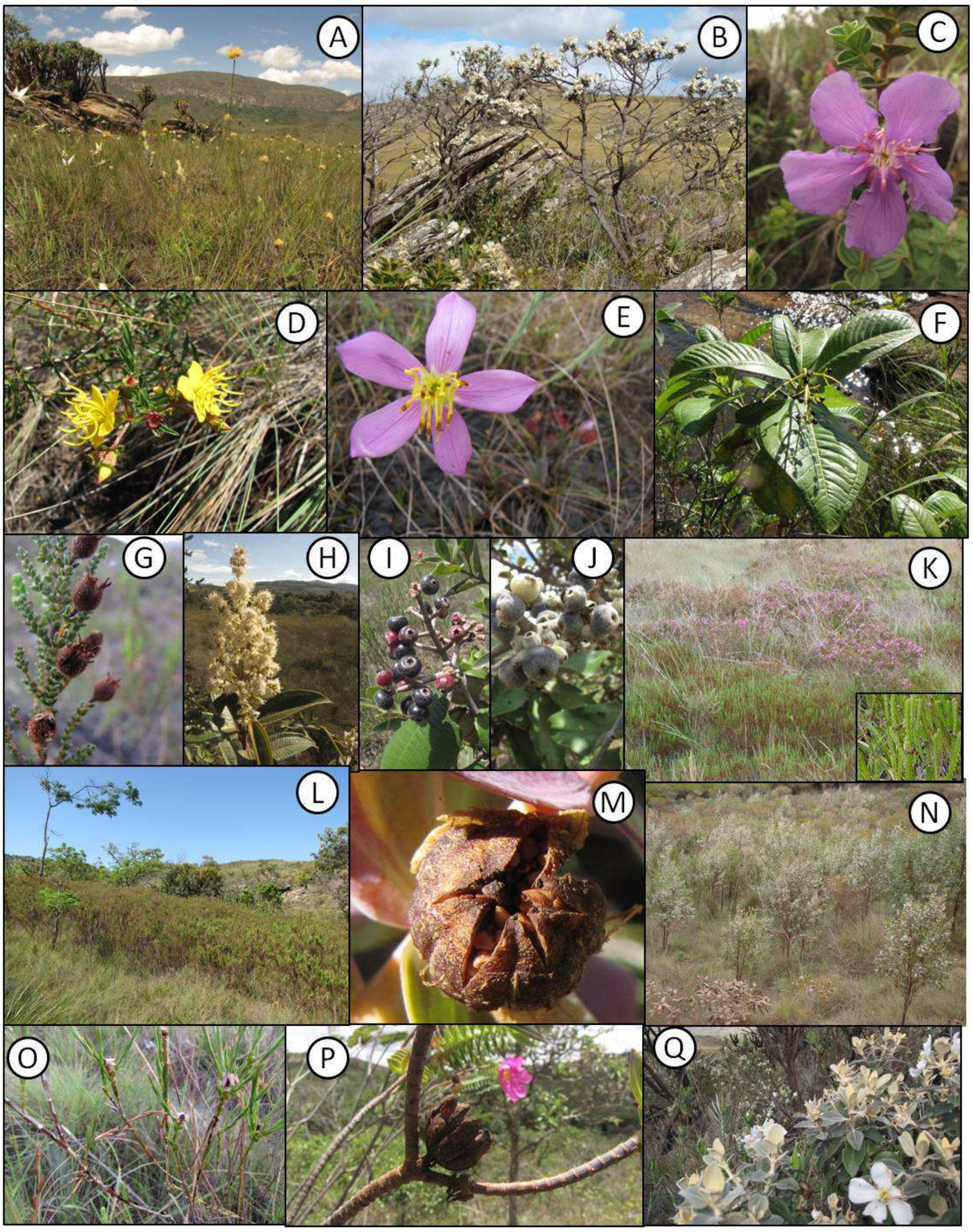

Figure S2. The reconstructed phylogeny of the 50 species of Melastomataceae showing soft polytomies indicated by arrows.

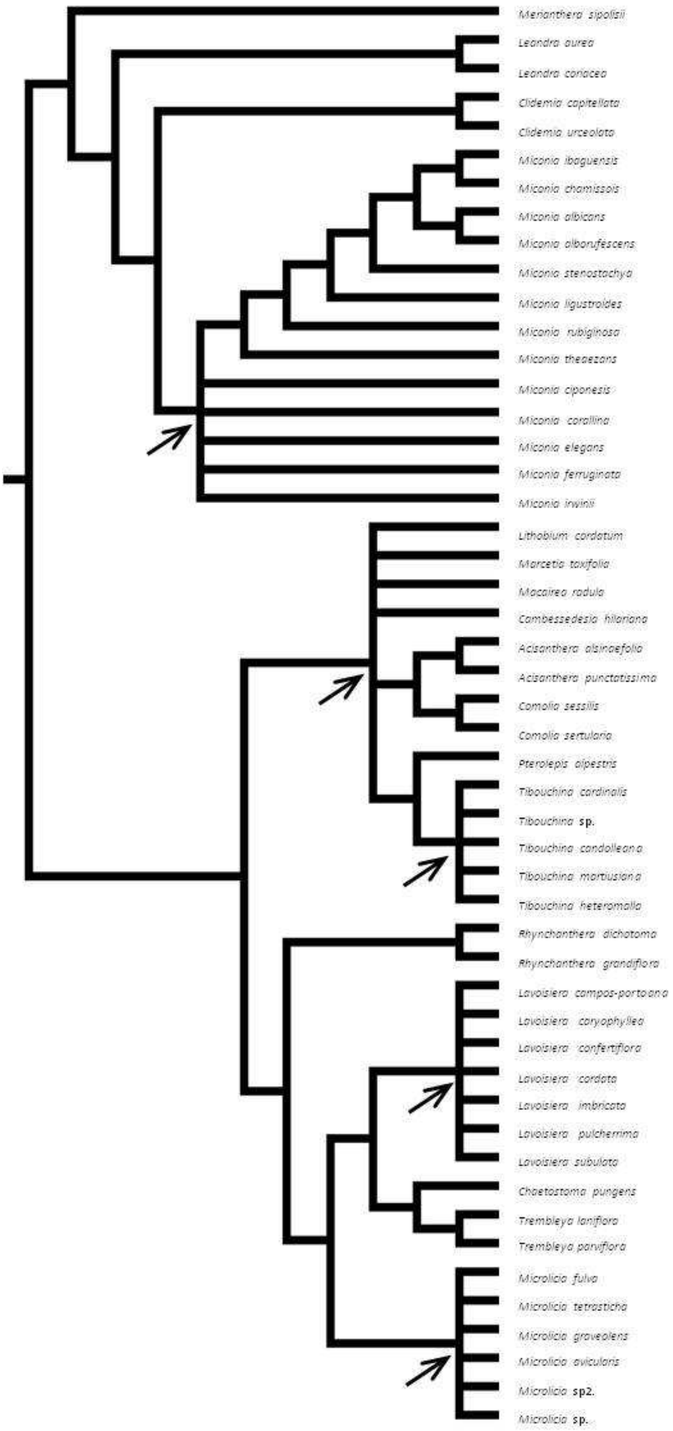

Figure S3. Average (±SE) germinability and mean germination time of 50 Melastomataceae species as a function of growth-form, geographic distribution, microhabitat, dispersal mode and phenology.

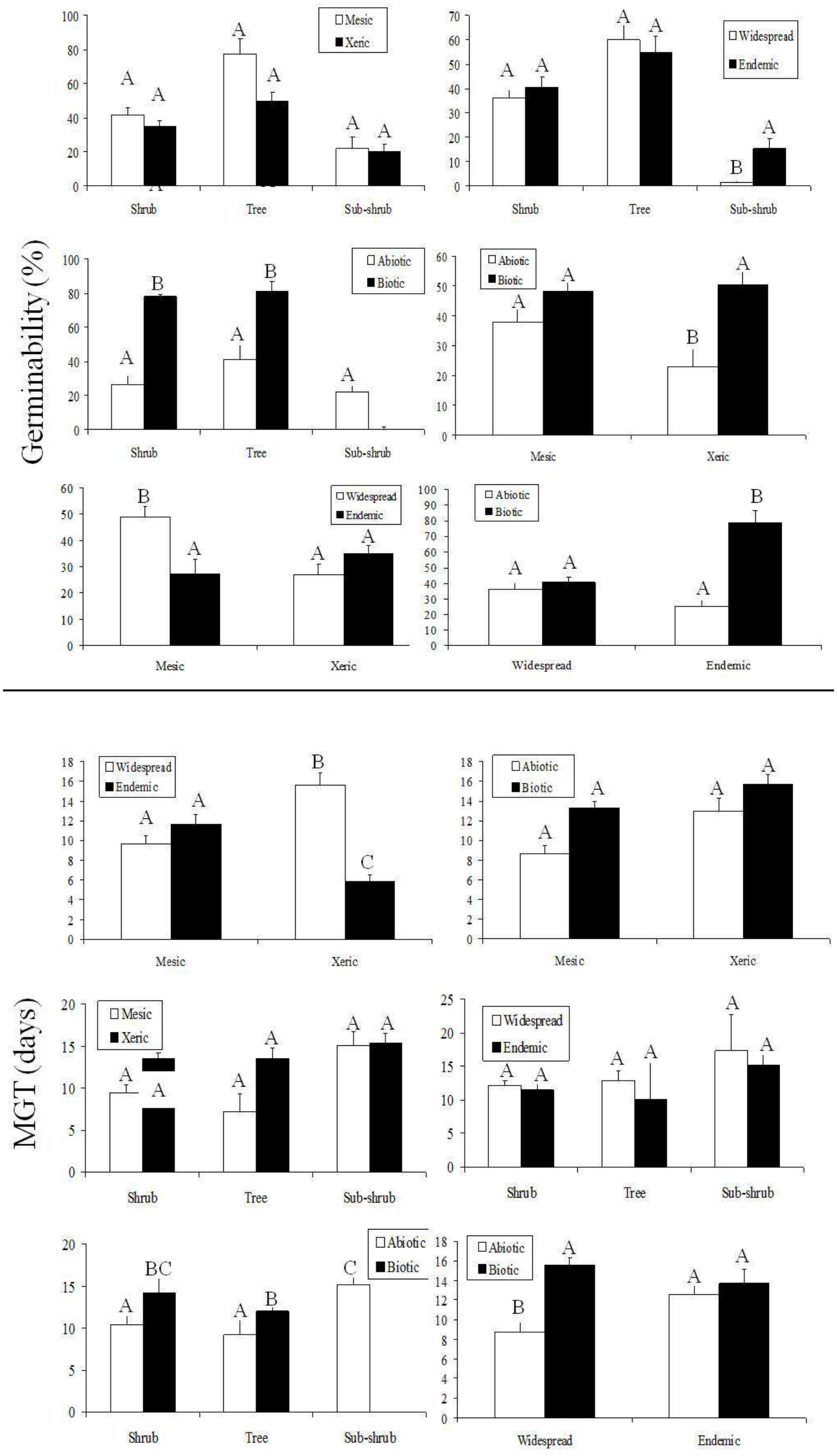

Figure S3

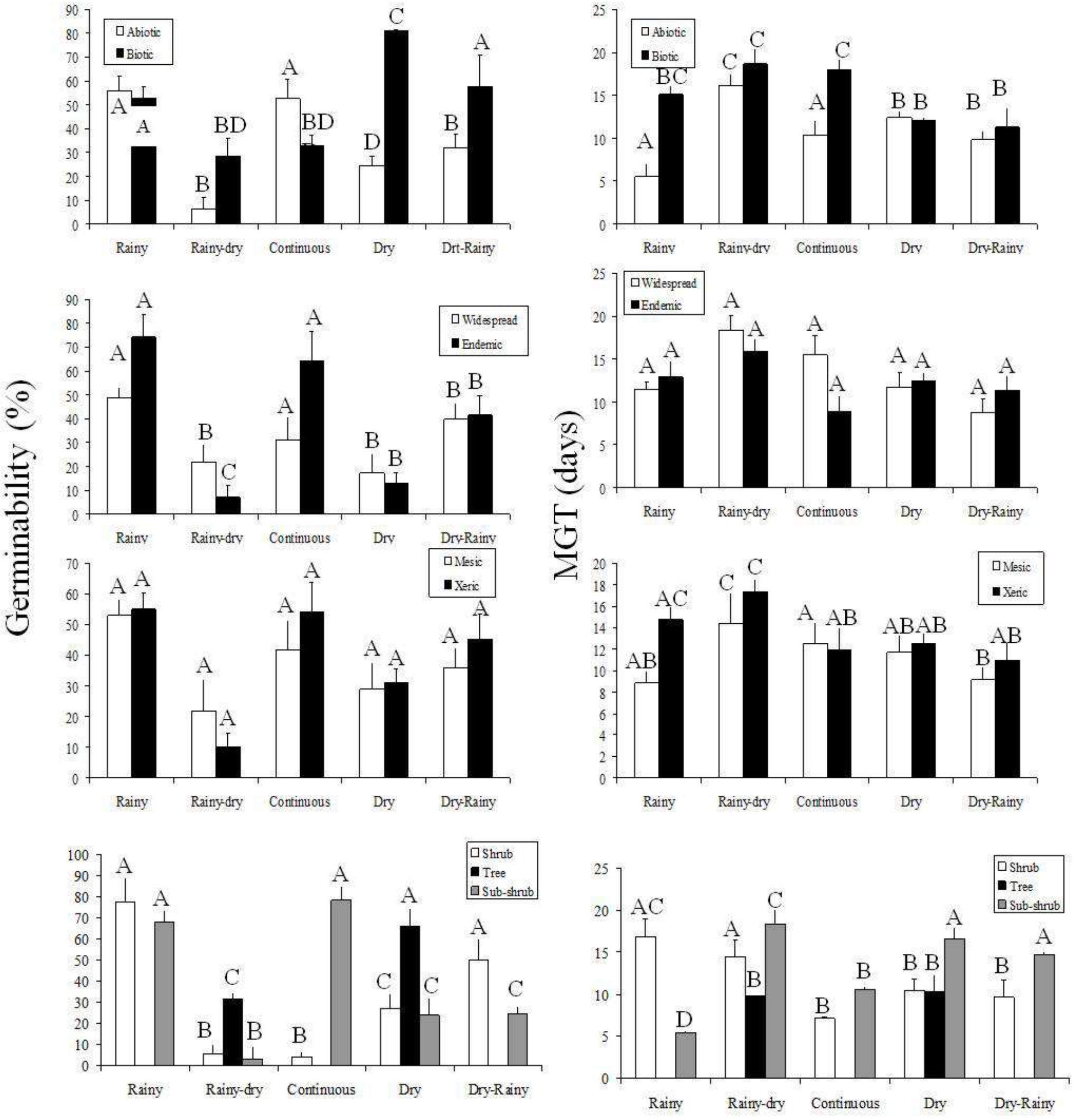
Germinability (%)
MGT (days)
Abiotic
Biotic
Widespread
Endemic
Mesic
Xeric
Shrub
Tree
Sub-shrub
Rainy
Rainy-dry
Continuous
Dry
Drt-Rainy
Dry-Rainy

Figure S3

Table S2. Principal components 1 (PC1) and 2 (PC2) obtained from the matrix of correlation for contrasts in seed traits in 50 species of Melastomataceae. LRI= light requirement index

Contrasts in seed traits	Axis 1	Axis 2
MGT	0.5429	-0.1848
Germinability	-0.5817	0.02605
Synchrony	-0.6026	-0.1189
LRI	0.03237	0.9412
Size	-0.05244	-0.2553
Variance explained (%)	46.35	20.7

CAPÍTULO VI

DINÂMICA DE BANCOS DE SEMENTES DE MELASTOMATACEAE EM HABITATS HETEROGÊNEOS

Fernando A. O. Silveira, Natália C. Robles, Natália M. Sales, G. Wilson Fernandes & José P. Lemos-Filho

Resumo

Bancos de sementes são importantes para a manutenção e regeneração de populações e comunidades vegetais. Neste estudo avaliamos o comportamento de sementes de 18 espécies de Melastomataceae enterradas em solos de vegetações de alta heterogeneidade ambiental. Sacos com sementes foram enterrados e exumados em períodos favoráveis e desfavoráveis para o estabelecimento em solos associados aos afloramentos rochosos e aos campos graminosos. Os sacos contendo sementes foram exumados em intervalos regulares de seis meses até um período de 12 (sete espécies) a 18 meses (11 espécies). As sementes das espécies estudadas apresentaram complexo padrão de dinâmica nos bancos, com espécies variando em longevidade, indução de dormência secundária e capacidade de detecção de heterogeneidade ambiental. As espécies estudadas evoluíram vários traços nas sementes que favorecem a sobrevivência e persistência no solo. Ciclos anuais de dormência são documentados pela primeira vez na família e provavelmente evoluíram como resposta à mortalidade de plântulas induzidas pela seca. A similaridade nas condições de temperatura e umidade do solo nas condições favoráveis e desfavoráveis deve impedir o reconhecimento dos locais e momentos apropriados para o estabelecimento. Nossos dados sugerem a existência de limitação de dispersão e filtros ecológicos durante o estabelecimento de plântulas. A incapacidade de colonizar novos habitats não está relacionada à filogenia das espécies, o que sugere conservantismo de nicho.

Palavras-chave: conservantismo de nicho, dormência secundária, ciclos de dormência, campos rupestres, heterogeneidade de habitat.

Introdução

Em escala local, fatores ecológicos e evolutivos determinam a distribuição de plantas em comunidades ricas em espécies, especialmente naquelas com alta heterogeneidade espacial (Brown & Lomonilo 1998). Em habitats complexos, a estruturação das comunidades vegetais pode ser influenciada por limitações na chegada de sementes (*seed arrival*), limitação de recrutamento, conservantismo de nicho, estrutura filogenética e filtragem ecológica (Webb et al. 2002, Losos 2008, Myers & Harms 2009, Donohue et al. 2010, Wiens et al. 2010).

A presença de uma planta em uma determinada comunidade, não é determinada somente pela germinação de suas sementes, mas também pelos processos que permitem sua sobrevivência e a reprodução neste local. A época e o local de germinação podem determinar as condições que o indivíduo vai experimentar ao longo de sua vida (Turnbull et al. 2000, Donohue 2005). Portanto, em ambientes heterogêneos, espera-se que a seleção natural tenha favorecido a evolução de mecanismos para detectar características do habitat que forneçam às plantas condições ideais de estabelecimento (Walters 1998, Donohue et al. 2010).

Os bancos de sementes, o conjunto de sementes na superfície do solo ou enterrado nele (Leck et al. 1989), são fundamentais para a reprodução e manutenção de populações após distúrbio (Leck et al. 1989, Butler & Chazdon 1998, Wills & Read 2007) e manutenção da variabilidade genética (Simpson et al. 1989). O entendimento da dinâmica dos bancos de sementes torna-se, portanto, chave para a compreensão de processos como regeneração natural e restauração ecológica (Vazquez-Yanes & Orozco-Segovia 1993, Cap. 1). A importância funcional dos bancos varia de acordo com o tipo de vegetação (Vazquez-Yanes & Orozco-Segovia 1993, Medina & Fernandes 2007, Salazar et al. 2011). Estudos de bancos de sementes são muito comuns em florestas tropicais chuvosas (Vazquez-Yanes & Orozco-Segovia 1993) e permitiram avanços significativos sobre o conhecimento do recrutamento nestes ambientes. Atualmente se conhece bem a composição e estrutura espacial dos bancos (Butler & Chazdon 1998, Baider et al. 2001), o papel de microhabitats (Pearson et al. 2003, Daws et al. 2008) e sazonalidade (Butler & Chazdon 1998) no recrutamento e mecanismos de detecção de condições ótimas para o estabelecimento de plântulas (Vazquez-Yanes & Orozco-Segovia 1993, Pearson et al. 2002, 2003).

Em contraste com os solos permanentemente úmidos de florestas chuvosas, as camadas superficiais dos solos de savanas tropicais demonstram marcante redução do potencial hídrico durante a estação seca (Salazar et al. 2011). Este fato sugere que espécies de savanas, sob a força de diferentes pressões seletivas, evoluíram mecanismos diferentes para detectar flutuações ambientais que sinalizam condições adequadas para o estabelecimento. Em ambientes sazonais, a alta mortalidade de plântulas resultante da dessecação deve ter sido uma importante pressão seletiva dirigindo estratégias reprodutivas de plantas. Portanto, nestes ambientes o período de estabelecimento a partir da chuva de sementes e do banco de sementes está restrito aos meses correspondentes à estação chuvosa (Cap. 1, 4).

A evolução da regeneração por bancos de sementes é muito pouco conhecida em ambientes espacialmente heterogêneos (Matias & Kisdi 2001). Ambientes heterogêneos apresentam-se como ambientes complexos, nos quais os diferentes microhabitats sustentam floras com composição e traços funcionais distintos (Clarke 2002). Uma característica marcante de formações rupestres é o desenvolvimento de vegetação arbustiva distribuída de forma semelhante a arquipélagos em meio a uma matriz de vegetação graminóide (Clarke 2002, Alves & Kolbek 2010). Os campos rupestres compreendem uma diversidade considerável de pedoambientes, e o mosaico de vegetações é determinado por aspectos microambientais (Benites et al. 2007). Os substratos sobre os quais se estabelecem estas distintas vegetações são de diferente origem e provocam isolamento edáfico na comunidade vegetal (Alves & Kolbek 2010). Solos associados aos afloramentos rochosos contêm altos níveis de material fibroso e acumulam matéria orgânica. Por outro lado, os solos associados aos campos graminosos são compostos em sua maioria por areia grossa, que tem baixa capacidade de retenção de água (Benites et al. 2007). A elevada heterogeneidade ambiental dos campos rupestres cria microhabitats que sustentam comunidades vegetais diferentes na sua composição florística e em caracteres funcionais (Clarke 2002, Conceição & Pirani 2005, Alves & Kolbek 2010).

Neste estudo, avaliamos a dinâmica do banco de sementes de 18 espécies de Melastomataceae em um ambiente espacialmente heterogêneo. Sementes foram enterradas e exumadas periodicamente a fim de se avaliar a germinação em dois tipos de substratos. Esta técnica permite avaliar com precisão a sobrevivência das sementes no banco e não apenas a sua persistência (Saatkamp et al. 2009). Especificamente testamos as seguintes hipóteses: 1) as sementes de Melastomataceae formam bancos de sementes persistentes; 2) a flutuação dos sinais ambientais regula a germinação e dormência de sementes no banco; e 3) filtros ecológicos limitam a longevidade de sementes em solos de microhabitats distintos dos adultos.

Material e métodos

Área de estudo e espécies estudadas

Este estudo foi realizado na Serra do Cipó (19º17' S, 43º35' O), localizado na porção Sul da Cadeia do Espinhaço, sudeste do Brasil. O material vegetal foi coletado em uma vegetação denominada campo rupestre, que ocorre em áreas acima de 900m acima do nível do mar em solos quartzíticos. A vegetação de campos rupestres abriga um elevado número de espécies e apresenta altos índices de endemismos (Alves & Kolbek 2010, Echternacht et al. 2011). As comunidades vegetais dos campos rupestres são de maneira geral, esclerófilas e se estabelecem em solos ácidos, superficiais e pobres em nutrientes (Giulietti *et al.* 1997, Benites et al. 2007, Jacobi *et al.* 2007) e algumas de suas espécies apresentam baixa plasticidade fenotípica (Negreiros et al. 2009). O clima é sazonal com uma estação fria e seca com duração aproximada de cinco meses e uma estação quente e úmida com duração aproximada de sete meses (Giulietti *et al.* 1997, Madeira & Fernandes 1999). Durante a estação seca, a umidade do solo cai vertiginosamente (Coelho et al. 2006), restringindo a estação de estabelecimento e crescimento aos sete meses do ano correspondentes à estação chuvosa (Cap. 4).

Neste estudo, foram selecionadas 18 espécies de Melastomataceae distribuídas em 10 gêneros e três tribos (Tabela 1). Dez espécies estudadas ocorrem nos afloramentos rochosos e oito nos solos arenosos. Estas espécies apresentam diferentes modos de dispersão, formas de vida e história de vida (Tabela 1).

Coleta de sementes e experimentos

Frutos e sementes foram coletados no pico de frutificação. Após triagem e processamento, as sementes foram colocadas em sacos de nylon de malha fina e enterradas nos locais onde as espécies naturalmente ocorrem a 5cm de profundidade devido à superficialidade dos bancos nesta vegetação (Garcia & Oliveira 2007, Medina & Fernandes 2007).

Para cada espécie, quatro sacos contendo 100-200 sementes cada foram enterrados em estação favorável ao estabelecimento e em estação desfavorável ao estabelecimento. Sacos foram enterrados em dois tipos de substrato, solos arenosos e em solos de afloramentos rochosos (exceto *M. ibaguensis*). Estes solos diferem em suas propriedades físicas e químicas (Benites et al. 2007), mas não há diferenças na flutuação diária de temperatura (Cap. 3). Um baixo número de sementes por saco foi utilizado a fim de se diminuir as probabilidades de contaminação por patógenos de solo e para se evitar pseudo-réplicas (van Mourik et al 2005). Os sacos contendo sementes foram exumados em intervalos regulares de seis meses até um período de 12 (sete espécies) a 18 meses (11 espécies), período que é suficiente para confirmar a incorporação de sementes em bancos persistentes (veja discussão em Walck et al. 2005). Para que os períodos de exumação das sementes coincidissem com as estações favoráveis e desfavoráveis para espécies com diferentes fenologias de dispersão, o enterramento dos sacos ocorreu em épocas distintas. *Miconia stenostachya, Leandra aurea* e *Miconia ibaguensis* foram agrupadas como espécies dispersoras na estação chuvosa (Tabela 1). Suas sementes foram enterradas em Janeiro de 2009 e exumadas em Julho de 2009 (estação desfavorável) e Janeiro de 2010 (estação favorável). As demais espécies foram agrupadas como espécies dispersoras de seca (Tabela 1). Suas sementes foram enterradas em Outubro de 2008 e exumadas em Abril de 2009 e 2010 (estação desfavorável) e Outubro de 2009 (estação favorável).

O conteúdo relativo de água de sementes recém-coletadas foi determinado através da seguinte formula: CRA= PF-PS/PS*100, onde PF= peso fresco da semente e PS= peso seco da semente após secagem a 70^{o}C por cinco dias. Outro lote de sementes recém-coletadas foi pesado e colocado para germinar sob variadas condições de luz e temperatura e testes com sementes enterradas foram realizados sob condições ótimas (Cap. 3). As sementes exumadas foram levadas para o laboratório, onde os testes de germinação foram imediatamente realizados. Para cada espécie, quatro repetições de 25-50 sementes foram montadas por tratamento. As sementes foram colocadas em placas de Petri forradas com camada dupla de papel filtro umedecida com solução de Nistatina (2%). As placas de Petri foram incubadas em câmaras BOD para realização dos testes de germinação em condições ótimas (Cap. 3). As sementes foram monitoradas em intervalos diários por 30 dias consecutivos e foram consideradas germinadas após a emissão da radícula.

As espécies foram agrupadas de acordo com sua estratégia funcional. Para definir os grupos funcionais, utilizamos três critérios: classe de longevidade, ocorrência de ciclos sazonais e capacidade de detecção de microhabitat (Tabela 2).

Tabela 1. História de vida e habitat de espécies de 18 Melastomataceae de campos rupestres.

Tribo	**Espécies**	**Forma de vida**	**Habitat**	**Dispersão**	**Fenologia**[1]
Melastomeae	*Acisanthera alsinaefolia*	Arbusto	Afloramento	Abiótica	Seca
Miconieae	*Clidemia urceolata*	Arbusto	Arenoso	Biótica	Seca
Melastomeae	*Comolia sertularia*	Arbusto	Afloramento	Abiótica	Seca
Microlicieae	*Lavoisiera campos-portoana*†	Arbusto	Afloramento	Abiótica	Seca
Microlicieae	*Lavoisiera confertiflora*	Sub-arbusto	Arenoso	Abiótica	Seca
Microlicieae	*Lavoisiera cordata*†	Arbusto	Afloramento	Abiótica	Seca
Microlicieae	*Lavoisiera imbricata*	Arbusto	Arenoso	Abiótica	Seca
Microlicieae	*Lavoisiera subulata*†	Sub-arbusto	Afloramento	Abiótica	Seca
Miconieae	*Leandra aurea*	Arbusto	Afloramento	Biótica	Chuva
Melastomeae	*Marcetia taxifolia*	Arbusto	Arenoso	Abiótica	Seca
Miconieae	*Miconia alborufescens*	Arbusto	Afloramento	Biótica	Seca
Miconieae	*Miconia ferruginata*	Árvore	Afloramento	Biótica	Seca
Miconieae	*Miconia ibaguensis*	Arbusto	Arenoso	Biótica	Chuva
Miconieae	*Miconia stenostachya*	Arbusto	Arenoso	Biótica	Chuva
Microlicieae	*Microlicia* sp.	Sub-arbusto	Arenoso	Abiótica	Seca
Melastomeae	*Tibouchina martiusiana*	Arbusto	Afloramento	Abiótica	Seca
Microlicieae	*Trembleya laniflora*	Arbusto	Afloramento	Abiótica	Seca

Microlicieae	*Trembleya parviflora*	Arbusto	Arenoso	Abiótica	Seca

† Espécies incluídas na lista de plantas raras do Brasil ou ameaçadas de extinção por listas vermelhas.

1 Dados a partir de observações pessoais.

Tabela 2. Critérios utilizados para determinar grupos funcionais em bancos de sementes de Melastomataceae de campo rupestre.

Característica funcional	Estado	Evidência
Longevidade	Alta	A germinabilidade final é estatisticamente igual à germinabilidade inicial
	Baixa	A germinabilidade final é estatisticamente inferior à germinabilidade inicial
Ciclos sazonais	Presente	Diminuição significativa da germinabilidade durante períodos desfavoráveis seguida de aumento significativo em período favorável.
	Ausente	Ausência de variação significativa ou aumento da germinabilidade durante períodos desfavoráveis.
Detecção de microhabitat	Presente	Diferenças significativas na germinabilidade de sementes enterradas nos dois microhabitats
	Ausente	Sem diferenças na germinabilidade de sementes enterradas nos dois microhabitats

Análises estatísticas

Os dados de germinabilidade foram transformados em seu valor angular para atingir os pressupostos das análises paramétricas. Cada saco de nylon foi considerado uma amostra independente. Diferenças nas médias foram determinadas através de ANOVA de duas vias seguida do teste de Tukey para comparações múltiplas. O valor de 0,05% para α foi estabelecido para todas as análises.

Resultados

As sementes das espécies estudadas são diminutas, com variação de massa entre 0,00048g e 0,027g (valor correspondente a massa média de 25 sementes). O conteúdo relativo da água de sementes maduras variou entre 12,5% e 51,4%. Não foi observada mortalidade causada por insetos ou fungos e tão pouco foi germinação de sementes enterradas. O tempo de armazenamento *in situ* e o tipo de substrato afetaram a germinabilidade final. Para nove espécies, o armazenamento *in situ* das sementes não alterou a viabilidade, já que não houve diminuição significativa na germinabilidade de sementes recém-coletadas e após o período final de armazenamento (Figuras 1 e 2). Para as demais espécies, houve uma diminuição significativa na germinabilidade ao final do experimento, mas em nenhuma das espécies encontrou-se evidência de fitopatógenos nas sementes.

Diferenças significativas na germinabilidade de sementes estocadas entre os dois tipos de solos foram verificadas para 12 espécies (Figuras 1 e 2). Para outras 12 espécies, houve interação significativa entre tempo e tipo de solo, indicando que para estas, o efeito do tipo de solo modulou a resposta ao tempo de armazenamento.

Seis espécies apresentaram ciclos sazonais de germinabilidade. Estes ciclos se caracterizaram por diminuição significativa da germinabilidade em períodos desfavoráveis para o estabelecimento, seguida por um aumento significativo de germinabilidade em períodos ótimos para o estabelecimento (Figura 2).

Doze entre 15 espécies (80%) apresentaram diferenças significativas na germinabilidade entre os tipos de solo em pelo menos um dos intervalos. Porém, contrariando nossa expectativa, as diferenças entre os tipos de solo foram consistentes com o habitat dos adultos em todos os intervalos de tempo apenas em *T. parviflora*, *M. taxifolia* e *L. cordata* (Figura 2). A hipótese de consistência entre habitat das sementes e adultos foi parcialmente aceita para *A. alsinaefolia*.

As duas espécies com dormência primária (*C. sertularia* e *Microlicia* sp.) apresentaram germinabilidade inferior a 10% sob todas as condições e ambas não foram incluídas nos cálculos de porcentagem acima nem nas análises estatísticas.

As espécies de Melastomataceae de campo rupestre apresentam uma grande variedade de estratégias funcionais no banco de sementes. De acordo com as características funcionais das sementes no banco, as espécies foram agrupadas em seis grupos. *Miconia ibaguensis* não foi enquadrada em nenhum grupo, pois dados sobre detecção de microhabitats não estão disponíveis.

Grupo 1- espécies com alta longevidade, dormência secundária e com detecção de microhabitat. *L. aurea, T. parviflora* e *T. martiusiana*.

Grupo 2- espécies com alta longevidade, sem dormência secundária e com detecção de microhabitat. *M. stenostachya, M. taxifolia, L. imbricata, C. urceolata* e *A. alsinaefolia*.

Grupo 3- espécies com baixa longevidade, dormência secundária e com detecção de microhabitat. *L. cordata, L. confertiflora* e *L. campos-portoana*.

Grupo 4- espécies com baixa longevidade, sem dormência secundária e com detecção de microhabitat. *L. subulata*.

Grupo 5- espécies com baixa longevidade, sem dormência secundária e sem detecção de microhabitat. *M. alborufescens, T. laniflora* e *M. ferruginata*.

Grupo 6- espécies que apresentam dormência primária. *C. sertularia* e *Microlicia* sp.

Discussão

Nossos dados demonstram que, apesar de reduções na longevidade para algumas espécies, de modo geral as sementes de Melastomataceae de campo rupestre são capazes de formar bancos de sementes. A maioria destas sementes apresenta CRA dentro da faixa estabelecida para sementes ortodoxas (Pammenter & Berjak 2008), e portanto são capazes de tolerância à dessecação. Sementes de Melastomataceae são quase universais nas camadas superficiais do solo (Pereira-Diniz & Ranal, 2006) de florestas e savanas neotropicais (Cap. 1), e podem ser os componentes dominantes do banco em determinadas vegetações (Baider et al. 2001, Medina & Fernandes 2007). A interação entre variáveis ambientais e características das sementes determina a entrada destas no banco e para as espécies estudadas, o pequeno tamanho de suas sementes (Cap. 3), mesmo na ausência de dormência primária (Thompson et al. 2003), parece ser o principal fator que permite a entrada e persistência no banco. Sementes pequenas são geralmente fotoblásticas e têm maior probabilidade de enterramento (Milberg et al.

2000), e na ausência de condições apropriadas para germinação após a dispersão, podem incorporar-se nos bancos (Cap. 6).

O tamanho das sementes é um caractere que co-evoluiu com a resposta fotoblástica (Milberg et al. 2000) e com a persistência no solo (Thompson et al. 1998). Portanto, para Melastomataceae, a persistência e sobrevivência no solo não devem ser interpretadas como uma adaptação, devido ao sinal filogenético para o tamanho das sementes (Cap. 5). A persistência de sementes não dormentes de Melastomataceae em bancos (Cap. 1) deve ser um traço ancestral e representa um exemplo de convergência funcional com outras linhagens de sementes pequenas de campo rupestre (Velten & Garcia 2005, Garcia & Oliveira 2007).

Embora tenhamos verificado grande potencial para a formação de bancos de sementes, algumas espécies apresentaram significativa redução de longevidade (espécies pertencentes aos grupos 3, 4 e 5). O envelhecimento das sementes acarreta em perda de funções enzimáticas e deterioração de estruturas sub-celulares, que aumentam o stress e eventualmente levam à morte da semente (Walters 1998). Inúmeros fatores internos e externos controlam o envelhecimento e sobrevivência de sementes no solo (Walters 1998, Dalling et al. 2010). Neste estudo, não foram encontradas evidências de mortalidade causadas por fungos (fatores externos) nas espécies estudadas. A ausência de evidências de mortalidade induzida por patógenos nas espécies estudadas pode estar relacionada com a presença de compostos fenólicos (Werker 1997, Dalling et al. 2010) no tegumento destas sementes (Cap. 4, Ribeiro et al., dados não publicados).

Por outro lado, compostos fenólicos também podem estar relacionados com propriedades antioxidantes (Pukacka & Ratajczak 2007). Espécies reativas de oxigênio estão envolvidas com o envelhecimento e dano aos tecidos de sementes (Pukacka & Ratajczak 2007), e mecanismos enzimáticos e a ação de compostos antioxidantes desempenham importante papel na sobrevivência da semente e manutenção da viabilidade no solo (Hendry 1993, Bailly 2004, Pukacka & Ratajczak 2007, Munné-Bosch et al. 2011).

Compostos antioxidantes podem estar envolvidos em atividades metabólicas associadas com a regulação de ciclos sazonais de dormência (Munné-Bosch et al. 2011). Encontramos seis espécies com evidência de dormência secundária. A dormência secundária é uma adaptação predominantemente associada com o comportamento de sementes nos bancos. A temperatura e o potencial hídrico do solo são os fatores que determinam os ciclos de dormência secundária (Hilhorst 1998), especialmente em ambientes sazonais (Ren & Tao 2003, Batlla & Benech-Arnold 2006). A regulação da dormência por condições ambientais evoluiu como uma estratégia que garante a germinação em períodos favoráveis para o estabelecimento (Mollard et al. 2007). Os solos de ambientes sazonais, como os dos campos rupestres, apresentam significativo decréscimo do potencial hídrico durante os meses da estação seca (Coelho et al. 2006, Salazar et al. 2011) e a evolução da dormência faz parte do repertório de estratégias para prevenir a mortalidade de plântulas induzida pela seca (Cap. 4). Contudo, seqüências de ciclos de hidratação-desidratação causam stress nas sementes enterradas (van Assche & Vanderlook 2010), e espera-se o envolvimento de rigorosos mecanismos bioquímicos que controlam a produção e degradação de espécies reativas de oxigênio quando as sementes estiverem enterradas em solo encharcado.

Este estudo documenta de forma inédita a dormência secundária na família. Embora a classe de dormência não tenha sido determinada, provavelmente se trata de dormência fisiológica (veja Cap. 4). A evolução da dormência primária e secundária em Melastomataceae demonstra a diversidade de estratégias ecofisiológicas na família. Todas as espécies de baixa longevidade e com dormência secundária pertencem ao gênero *Lavoisiera*, sinalizando um possível papel da filogenia na evolução da dormência secundária.

Sabe-se que variações na temperatura e umidade do solo controlam a dormência secundária e emergência de plântulas (Battla & Benech-Arnold 2006). Assim, espera-se que as sementes dos campos rupestres tenham evoluído a capacidade de detectar sinais ambientais que indiquem momentos apropriados para sincronizar a germinação (Donohue 2005). No entanto, a maioria das espécies investigadas aqui não foi capaz de detectar condições impróprias para o estabelecimento e foram plenamente capazes de germinar em altas porcentagens em época desfavorável (transição chuva-seca, Cap. 3). Aparentemente a temperatura e umidade do solo não difere entre o início e final da estação chuvosa (Madeira & Fernandes 1999, Coelho et al. 2006). Se sementes no banco utilizam a variação nestes parâmetros como gatilhos para iniciar a germinação, seria impossível para elas detectarem por quanto tempo as condições se manterão ótimas o suficiente para o sucesso de estabelecimento. Aparentemente, o tempo de germinação está sob forte pressão seletiva, de forma que a seleção natural favorece rápida germinação sob condições ótimas (Cap. 5). Dados empíricos suportam esta hipótese (Cap. 4, 5).

Múltiplos fatores ecológicos e evolutivos limitam a distribuição de plantas. A estrutura das comunidades vegetais em vegetações rupestres é controlada principalmente por fatores abióticos e o isolamento edáfico é uma das características mais marcantes desta vegetação (Clarke 2002, Simon & Hay 2003, Alves & Kolbek 2010). Embora dados sobre chuva de sementes estejam indisponíveis, a baixa similaridade na composição florística de bancos de afloramentos rochosos e solos arenosos (Medina & Fernandes 2007) sugere que a limitação de dispersão seja fator estruturador de comunidades em campos rupestres (Turnbull et al. 2000, Myers & Harms 2009). Nossas observações de campo demonstram baixa distância de dispersão para alguns sub-arbustos típicos de solos arenosos (*Microlicia*, *Chaetostoma* e *Lavoisiera*), sugerindo limitação de sementes (Myers & Harms 2009). A limitação de dispersão deve ser especialmente forte para Microlicieae porque a distância de dispersão é menor em plantas pequenas (Thomson et al. 2011).

Nossos dados sugerem ainda, que além da limitação de dispersão, a diversidade local é controlada por filtros ecológicos. Embora, a maior parte das espécies seja capaz de manter longevidade nos dois tipos de solos, adultos são encontrados apenas em um dos dois tipos de habitats. Nós hipotetizamos que os filtros ecológicos atuam durante a etapa de estabelecimento de plântulas (Turnbull et al. 2000, Donohue et al. 2010). Portanto, filtros bióticos e/ou abióticos e limitação de estabelecimento são sugeridos como fatores estruturadores das assembléias para as espécies estudadas (Myers & Harms 2009, Donohue et al. 2010).

O conservantismo de nicho, definido como a tendência de espécies reterem seus nichos e características ecológicas através do tempo (Wiens et al. 2010), foi amplamente distribuído na estrutura filogenética das espécies investigadas. Nossos resultados sugerem que a maioria das espécies estudadas apresenta restrições na colonização de outros habitats além daquele no qual os adultos normalmente ocorrem, o que denota conservantismo de nicho. No entanto, espécies filogeneticamente relacionadas não apresentam nichos mais similares do que espécies mais distantes (veja Tabela 1) e exemplos de divergências de nicho são comuns entre espécies de um mesmo gênero (Cap. 5). Deste modo, não parece haver conservantismo de nicho filogenético, definido como a retenção de nicho em espécies filogeneticamente relacionadas (Losos 2008).

Não observamos liberação de dormência com o armazenamento no solo para nenhuma das duas espécies com dormência primária (*C. sertularia* e *Microlicia* sp., Cap. 4). Muitas espécies requerem mais de um ano para saírem da dormência (Walck et al. 2005) e somente estudos de longo prazo podem determinar ciclos sazonais de emergência em espécies dormentes. As possíveis explicações para a baixa ou nula germinação destas espécies incluem: 1) necessidade de múltiplos ciclos de temperatura e umidade (Batlla & Benech-Arnold 2006, van Assche & Vanderlook 2010); 2) necessidade de mecanismos específicos de quebra de dormência; e 3) alteração de requerimentos germinativos após a quebra da dormência.

Nossos dados demonstram que as espécies de Melastomataceae apresentam múltiplos caracteres que co-evoluíram e atualmente favorecem a sobrevivência e persistência em bancos de sementes (*sensu* Saatkamp et al. 2009). A dominância de sementes desta família nos campos rupestres (Medina & Fernandes 2007) aponta para o importante papel que estas plantas têm na regeneração natural após distúrbio. Sementes e plântulas de Melastomataceae são extremamente sensíveis ao fogo (Hoffmann 2000, Lahoureau et al. 2006), e seu enterramento pode fornecer um escape a este distúrbio. Assim, a emergência pós-fogo pode ser uma estratégia que permita a emergência de plântulas em uma situação de relaxada competição, devido à diminuição de biomassa do estrato herbáceo.

Nossos dados sugerem que a limitação de dispersão, filtros ambientais e conservantismo de nicho são processos ecológicos importantes que estruturam a vegetação dos campos rupestres. De toda forma, existe a necessidade de se avaliar aspectos regenerativos desta vegetação como a chuva de sementes para entendimento processos-base que controlam a vegetação de campo rupestre. Particularmente, no que diz respeito aos bancos de sementes, é relevante avaliar as respostas do banco frente a distúrbios para definir os mecanismos que controlam a dinâmica de recrutamento em ambientes espacialmente heterogêneos.

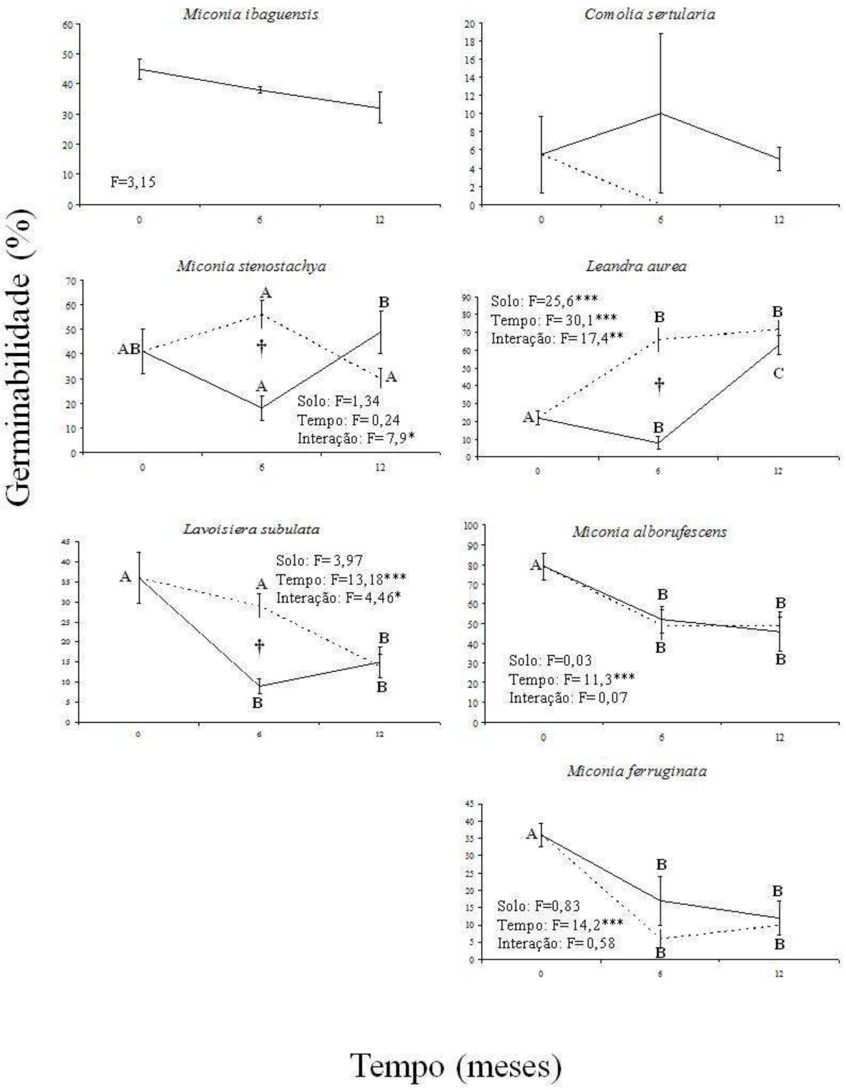

Figura 1. Média (± EP) da germinabilidade de sementes recém-coletadas e armazenadas em solos de afloramentos rochosos de quartzito (linha contínua) e solo arenoso (linha pontilhada) por até 12 meses. Os dados são de sete espécies de Melastomataceae de campos rupestres da Serra do Cipó. A coluna da esquerda representa espécies cujos adultos ocorrem em solo arenoso e a coluna da direita representa espécies cujos adultos ocorrem em solo de afloramento rochoso. Letras diferentes indicam médias significativamente diferentes pelo teste de Tukey (α= 0.05).† indica diferença significativa entre tipos de solo dentro de um mesmo tempo.

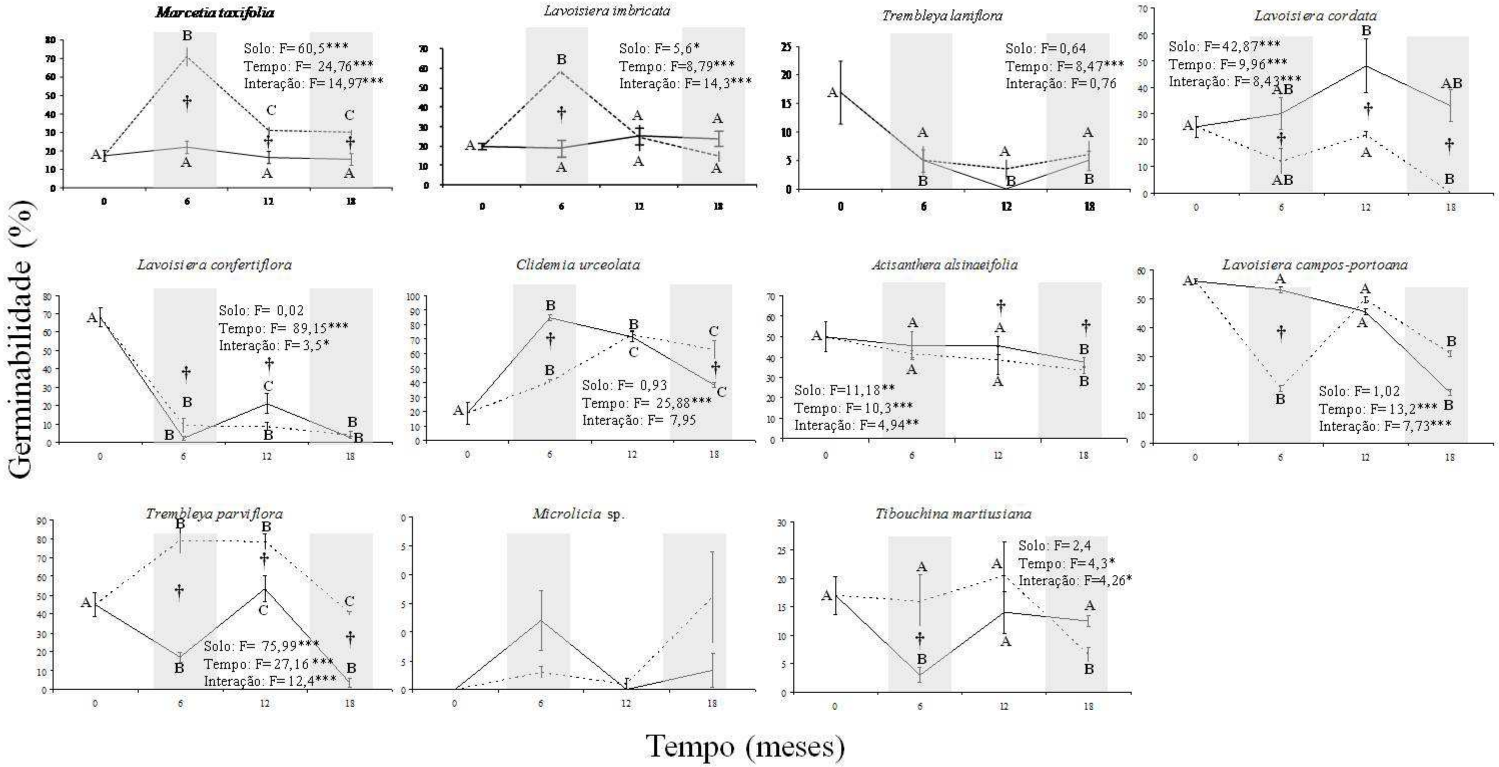

Figura 2. Média (± EP) da germinabilidade de sementes recém-coletadas e armazenadas em solos de afloramentos rochosos de quartzito (linha contínua) e solo arenoso (linha pontilhada) por até 18 meses. Os dados são de 11 espécies de Melastomataceae de campos rupestres da Serra do Cipó. As duas colunas da esquerda representam espécies cujos adultos ocorrem em solo arenoso e as duas colunas da direita representam espécies cujos adultos ocorrem em solo de afloramento rochoso. Áreas em cinza indicam períodos desfavoráveis para o estabelecimento de plântulas. Letras diferentes indicam médias estatisticamente diferentes pelo teste de Tukey (α= 0.05) entre os tempos de armazenamento. † indica diferença significativa entre tipos de solo dentro de um mesmo tempo.

CONSIDERAÇÕES FINAIS

Nesta tese os padrões ecofisiológicos da germinação de sementes de Melastomataceae de campo rupestre foram examinados utilizando métodos filogenéticos comparativos. Tentei incluir informações e realizar experimentos desde a formação das sementes, até a formação das plântulas, passando pelos complexos processos de maturação, dispersão, germinação, dormência e formação de bancos.

Espécies de Melastomataceae representam um excelente modelo para testar correlações ecológicas e evolutivas com traços funcionais. A enorme variação filogenética, ecológica e biogeográfica proporciona uma oportunidade ímpar para entender a evolução de caracteres ecofisiológicos em ambientes tropicais. O tamanho das sementes também é bastante variável nas espécies estudadas, entretanto, todas as espécies que estudei aqui produzem sementes consideradas diminutas quando se considera toda a variação existente nas espermatófitas. Este forte sinal filogenético no tamanho da semente é uma característica que determinou grande parte dos padrões que encontramos aqui:

- Aparentemente a seleção natural favoreceu a evolução de estratégias generalistas em Melastomataceae. O sistema de dispersão é tipicamente generalista e o *trade-off* entre número e tamanho das sementes é tendenciado para a produção de muitas sementes pequenas ao invés de poucas sementes grandes.
- Os frutos de Melastomataceae são consumidos por ampla gama de frugívoros que desempenham um papel determinante no recrutamento: a limpeza das sementes.
- Melastomataceae produzem sementes fotoblásticas positivas, com longevidade variada, mas alta sensibilidade ao fogo.
- Estas sementes têm maiores oportunidades de serem enterradas e o fotoblastismo é responsável pela sua manutenção nos bancos.
- A dormência fisiológica evoluiu múltiplas vezes em espécies/populações de Cerrado que dispersam sementes em ambientes secos durante a transição chuva-seca.
- A variação na germinação em resposta a estímulos abióticos não está relacionada à distribuição em escala global, mas sim à distribuição em escala local (microhabitats).
- A dormência secundária faz parte de um espectro de estratégias adaptativas que evoluiu em Melastomataceae em resposta à mortalidade induzida pela seca.
- A evolução dos traços germinativos esteve associada a mudanças no modo de dispersão e na forma de vida.
- Altas temperaturas, diminuições na pluviosidade e aumento da freqüência de incêndios devem impactar severalmente o recrutamento de Melastomataceae no futuro.
- Espécies de Melastomataceae apresentam uma série de caracteres que favorecem sua utilização em programas de restauração ecológica.

A utilização de métodos comparativos filogenéticos é um paradigma dominante para futuros estudos sobre a ecologi e evolução de atributos da flora do Cerrado e do Brasil. Embora, tais estudos venham sendo conduzidos há mais de 35 anos no mundo, somente nos últimos anos estudos desta natureza vêm sendo realizados para nossa flora. Espero que esta tese, mesmo com todas as suas deficiências, possa fomentar a abertura de linhas de pesquisa que incorporem a informação filogenética em estudos comparativos nos campos rupestres.

Esta tese deixa mais perguntas em aberto do que perguntas respondidas. Sugiro que estudos futuros incluam outros taxa de forma a ampliar a variação em caracteres filogeneticamente conservados. A ampliação do banco de dados de sementes de plantas de campo rupestre vai permitir a reconstrução da história evolutiva desta flora que vem fascinando cientistas ao longo de séculos. Tais estudos constituirão a base teórica para programas mais eficientes de conservação e manejo deste frágil e ameaçado ecossistema.

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