UNIVERSIDADE ESTADUAL PAULISTA
“JÚLIO DE MESQUITA FILHO”
INSTITUTO DE BIOCIÊNCIAS

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS
(BIOLOGIA VEGETAL)

LUÍS FELIPE DAIBES DE ANDRADE

O PAPEL DO FOGO NA GERMINAÇÃO DAS SEMENTES DE LEGUMINOSAS DO CERRADO

Rio Claro – SP
2017

LUÍS FELIPE DAIBES DE ANDRADE

O PAPEL DO FOGO NA GERMINAÇÃO DAS SEMENTES DE LEGUMINOSAS DO CERRADO

Orientadora: Profa. Dra. Alessandra Fidelis

Coorientador: Prof. Dr. Fernando A.O. Silveira

Tese apresentada ao Instituto de Biociências da Universidade Estadual Paulista "Júlio de Mesquita Filho", *campus* de Rio Claro, como requisito para obtenção do grau de Doutor em Ciências Biológicas (Biologia Vegetal)

Rio Claro – SP
2017

582.0467 Daibes, Luís Felipe
D132p O papel do fogo na germinação das sementes de leguminosas do cerrado / Luís Felipe Daibes de Andrade. - Rio Claro, 2017
140 f. : il., figs., gráfs., tabs., fots., mapas

Tese (doutorado) - Universidade Estadual Paulista, Instituto de Biociências de Rio Claro
Orientador: Alessandra Fidelis
Coorientador: Fernando A.O. Silveira

1. Semestes. 2. Ecologia do fogo. 3. Choque térmico. 4. Dormência física. 5. Fabaceae. 6. Flutuação térmica. I. Título.

Ficha Catalográfica elaborada pela STATI - Biblioteca da UNESP
Campus de Rio Claro/SP - Márcia Correa Bueno Degasperi / CRB 8/5694

unesp

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Rio Claro

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: O papel do fogo na germinação das sementes de leguminosas do cerrado

AUTOR: LUÍS FELIPE DAIBES DE ANDRADE
ORIENTADORA: ALESSANDRA TOMASELLI FIDELIS
COORIENTADOR: FERNANDO AUGUSTO DE OLIVEIRA E SILVEIRA

Aprovado como parte das exigências para obtenção do Título de Doutor em CIÊNCIAS BIOLÓGICAS (BIOLOGIA VEGETAL), pela Comissão Examinadora:

Profa. Dra. ALESSANDRA TOMASELLI FIDELIS
Departamento de Botânica / Instituto de Biociências de Rio Claro - SP

Profa. Dra. VANIA REGINA PIVELLO
Departamento de Ecologia / Universidade de São Paulo

Prof. Dr. MARCELO FRAGOMENI SIMON
Recursos Genéticos e Biotecnologia / Embrapa - Brasília/DF

Davi Rodrigo Rossatto
Prof. Dr. DAVI RODRIGO ROSSATTO
Departamento de Biologia Aplicada a Agropecuária / Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal/ UNESP

Prof. Dr. FABIAN BORGHETTI
Departamento de Botânica / Instituto de Ciências Biológicas/Universidade de Brasília

Rio Claro, 01 de dezembro de 2017

Instituto de Biociências - Câmpus de Rio Claro -
Av. 24-A no. 1515, 13506900, Rio Claro - São Paulo
http://ib.rc.unesp.br/#!/pos-graduacao/secao-tecnica-de-pos/programas/biologia-vegetal/apresentacao/CNPJ: 48.031.918/0018-72.

Dedico este trabalho à minha família

AGRADECIMENTOS

Agradeço a todas as agências de fomento que tornaram este trabalho possível: à FAPESP (2015/06743-0), ao CNPq (455183/2014-7), e especialmente à CAPES pela bolsa de doutorado concedida por meio do programa de pós-graduação, bem como pela bolsa de doutorado sanduíche CAPES/PDSE (88881.131702/2016-01). Muito obrigado também à Fundação Grupo Boticário (0153_2011_PR) por possibilitar o trabalho envolvendo a coleta de sementes e queimas experimentais na Reserva Natural Serra do Tombador.

Agradeço ao Programa de Pós-graduação em Ciências Biológicas (Biologia Vegetal), e também ao Departamento de Botânica da Unesp Rio Claro, principalmente aos professores e demais funcionários. Obrigado por toda infraestrutura, apoio logístico e intelectual no desenvolvimento dos estudos, desde o Mestrado até hoje. Rio Claro foi minha casa durante sete anos, e sempre fui muito bem acolhido pelo carinho Unespiano de todos. Agradeço à Profa. Dra. Alessandra Ike Coan, que coordenou o PPG durante a maior parte do desenvolvimento desta Tese, muito obrigado pelas conversas e pelo apoio absoluto. Obrigado também ao Dr. Douglas Domingues, que coordena atualmente o PPG, e às secretárias Celinha Hebling e Ivana Brandt, sempre solícitos.

Em especial, agradeço à minha orientadora, Alessandra Fidelis, pela dedicação, empenho e atenção com o trabalho. Por me ensinar o valor da perseverança e de nunca desistir de um sonho. Um sonho pelo qual sonhamos juntos. Obrigado por todas as leituras, correções e discussões. Especialmente aquelas nas quais não concordávamos e que, de alguma maneira, nos levaram adiante. Obrigado pelas idas a campo, coletas de sementes, ajuda no laboratório. Obrigado também pela força e incentivo na minha saída para o estágio na Espanha e na participação em congressos internacionais para divulgação do nosso trabalho.

Ao meu co-orientador, Fernando Silveira (Grande Lelê!), agradeço pela amizade, por colocar meu trabalho como prioridade entre os seus afazeres, sempre de prontidão para dar novas ideias, lendo e corrigindo o texto rapidamente. Valeu demais! Seguiremos juntos nas próximas etapas.

Ao Dr. Juli Pausas, que supervisionou meu estágio sanduíche na Espanha (CIDE/CSIC, Valência), obrigado pela parceria, pelo exemplo e pela inspiração, e por todos os ensinamentos que tornaram possível o quarto capítulo desta Tese. Agradezco también a todos los amigos del CIDE, muchísimas gracias por el ánimo y fuerza durante la estancia en Valencia.

Agradeço imensamente aos meus ex-orientadores, Profa. Dra. Renita Frigeri e Prof. Dr. Victor Cardoso, por todo conhecimento compartilhado, sem o qual eu não poderia estar concluindo este doutorado. Esta vitória é de vocês também! Obrigado por terem acreditado em mim. Obrigado ainda a cada um dos professores com os quais cursei (inúmeras) disciplinas e que participaram diretamente da minha formação como pesquisador. Em especial, agradeço aos professores Gustavo Habermann, Massanori Takaki e Patrícia Morellato por também facilitarem o meu acesso aos equipamentos e manterem as portas de seus laboratórios abertas para mim. Obrigado às professoras Vera Scatena e Aline Martins pelos ensinamentos envolvendo a anatomia ecológica das plantas. Aos professores Julio Lombardi, Marco Assis e Pedro Moraes, eu agradeço o uso do laboratório de sistemática e o acesso ao herbário. Estendo este agradecimento também aos professores do curso de Ecologia e Biodiversidade, onde cursei algumas disciplinas e sempre fui muito bem recebido.

Às Doutoras Elizabeth Gorgone-Barbosa, Mariana Rissi e Talita Zupo: faltam palavras pra agradecer a amizade, as conversas e a terapia de grupo. Obrigado por todo amparo, todos os campos, todos os almoços juntos, todos os churrascos. Obrigado por não medirem esforços em ler (e reler) o texto, em discutir incansavelmente o assunto. Agradeço pela força, por me fazerem acreditar que era possível, por me escutar sempre (até os áudios de cinco minutos!).

Aos bravos funcionários da Serra do Tombador, Anderson Terra, Gilson Berberino, Rogério de Almeida e Silvanio Borges (Parceirinho): muito obrigado por todos os ensinamentos sobre o fogo e o Cerrado. Tem um pouquinho de cada um de vocês neste trabalho. Agradeço todo apoio na Reserva (também ao Didi) e, especialmente, durante as queimas nas parcelas. Agradeço ao Maurício Cunha pelo suporte nas expedições de campo e por nos guiar nas belas paisagens do Brasil Central.

Agradeço a todos que auxiliaram na coleta e identificação das sementes utilizadas neste estudo, especialmente aos futuros Doutores Diego Escobar, pelas coletas em Itirapina, e Gabriel Sabino (Mió), por me apresentar ao pessoal do Parque Estadual de Porto Ferreira. Agradeço a toda equipe do PEPF pela gentileza e boa recepção, especialmente ao Ernesto Pedro e ao André Luís. Obrigado também ao Instituto Florestal de São Paulo pela autorização de coleta (Cotec 260108 – 008.322/2015). Aos Drs. Marcelo Simon e Leonardo Borges (Caqui): obrigado pela parceria e especialmente pela identificação das espécies de *Mimosa*.

Às alunas e alunos de graduação que tive o prazer de coorientar durante o decorrer desta Tese: Jessika Nunes, Nathalia Bonani, Rafael Novaes (Pajé) e Henrique Pinho (Gaga). Muito obrigado pela dedicação e parceria no laboratório. Agradeço ainda aos demais alunos do Leveg, que também sempre participaram ativamente dos experimentos de germinação, em especial à Heloiza Zirondi.

Agradeço à minha família, a quem dedico esta Tese, especialmente à minha mãe, Ivani Daibes, minha irmã, Ana Lídia, meus sobrinhos Ana Beatriz e João Pedro. Agradeço minha avó Lídia e minhas tias Marivone e Naime Daibes pelo amor infinito. Dedico este trabalho também à memória de meu pai, Bolivar Andrade.

Ao Akauan Arrais, obrigado pelo apoio incondicional sempre, por me escutar e aguentar o meu mau-humor nos momentos mais difíceis. Igualmente, agradeço à Elaine Nunes e Paula Maldonado, pela amizade sincera, pelos puxões de orelha ocasionais, pelos bons momentos compartilhados. Vocês são como família pra mim, e esta Tese também é dedicada a vocês.

I wish to thank very specially to Marcella Oleman for being the best roomie ever and making my Spanish days so incredible. Hope to see you soon, in Europe or in Brazil. Miss you so much! Wish we could find a *gezellig* place to have a *clarita con limón* and celebrate together. A Lorena Cea y Manoli Alarcón, agradezco muchísimo por toda hospitalidad y amistad en Valencia. Echo de menos a vosotras, a nuestra casita y a mi habitación. Han sido algunos de los mejores días de mi vida, lo sabéis.

Agradeço também aos amigos da Botânica, por compartilharem as alegrias e angústias da vida acadêmica (e da vida em si), e que acompanharam minha trajetória até aqui. Em especial aos Doutores Camila Kissmann, Blanca Dugarte e Thales Henrique, e aos futuros Doutores Kaire Nardi, Mari Maciel e Kleber Rezende. À Dra. Natalinha Costa (Azia), que também me acompanha desde sempre, obrigado especialmente pela força e apoio nessa reta final da(s) Tese(s). Ao Dr. Paulo Souza Filho pela ajuda com as análises do terceiro capítulo. Ao futuro Dr. Vitor Kamimura (Lilo) obrigado pela companhia durante o IVAS na Itália, pela força e pelas ideias trocadas. Sucesso e *tamo junto*!

Às amigas, Cíntia Portela, Frances Tatiane e Pri Funada, que estão comigo desde a graduação, na Universidade Federal de Rondônia (e desde antes, nos tempos de colégio) e as quais tenho certeza que estão orgulhosas de mim. Muito obrigado pela amizade e parceria de longa data. Ao Alexandre Casagrande (Xandi), também: obrigado pela amizade, pela torcida, pelos papos (engraçadíssimos). Às futuras Doutoras Semirian Amoêdo e Gabi Cerqueira, a quem desejo todo êxito do mundo: muito obrigado pela força.

Agradeço ao Hélio Hoshina por me acompanhar durante tantos anos, por tantos *papos de maluco*, tantos *insights*, tanta paciência e compreensão. Você conhece todos os detalhes desta história e vale muito a pena agradecê-lo aqui. Muito obrigado também aos companheiros do Instituo AME-SE pelas aulas de yoga, que ajudam a manter um pouco da minha saúde física e mental.

Enfim, esta tese é fruto de muitas parcerias, amizades e familiares. Estou muito orgulhoso do resultado final, e eu não teria conseguido sem a ajuda de cada um de vocês. Muito obrigado.

“Mi fracaso personal no me ha impedido de agradecer los favores que el fuego nos hizo.

Nos defendió del frio y de las bestias enemigas, nos cocinó la comida,

nos alumbró la noche y nos invitó a sentarnos, juntos, a su lado”

Eduardo Galeano

RESUMO

O fogo é o principal distúrbio em diversas vegetações ao redor do mundo, denominadas ecossistemas inflamáveis. Neste contexto, muitas espécies possuem estratégias de regeneração e colonização do ambiente pós-fogo, o que tipicamente envolve a sobrevivência (tolerância) das sementes e/ou quebra da dormência. Em especial, muitas sementes de leguminosas possuem o tegumento impermeável (dormência física), que pode ser rompido por meio de choques térmicos relacionados ao fogo. Outro fator que pode auxiliar no processo de quebra da dormência física é a flutuação térmica no solo, cuja amplitude é aumentada nas clareiras formadas pela remoção da vegetação durante a queima. Ambos os fatores, fogo e flutuação térmica, são relativamente bem estudados nos ecossistemas inflamáveis da Austrália e em vegetações Mediterrâneas. Por outro lado, os padrões relacionados à quebra da dormência e germinação das sementes ainda são controversos e menos conhecidos nas savanas tropicais da África e da América do Sul. Assim, esta Tese de doutorado teve como objetivo avaliar o papel do fogo na germinação e sobrevivência das sementes de leguminosas do Cerrado. Para tanto, realizamos tratamentos em campo, incluindo queimas experimentais, e também aplicamos tratamentos no laboratório, simulando a flutuação térmica nas clareiras e a passagem do fogo (choques térmicos). Após os tratamentos, observamos a germinação em condições ótimas, fazendo contagens três vezes por semana, e realizamos testes de viabilidade ao final de 30 dias dos experimentos. As análises estatísticas consistiram basicamente em GLMMs com distribuição binomial, considerando as réplicas como efeitos aleatórios. Os resultados apontam que as sementes morrem quando diretamente expostas ao fogo na superfície do solo. Por outro lado, há uma maior probabilidade de sobrevivência quando as sementes estão localizadas em clareiras (*gaps*) da vegetação. Nos gaps, a maior porcentagem de solo nu proporciona temperaturas do fogo mais amenas, queimando por menos tempo. Quando enterradas 1-cm sob o solo, as sementes sempre sobrevivem e pode haver quebra de dormência. A flutuação térmica também pode quebrar uma proporção significativa da dormência em condições de campo, especialmente em *Mimosa leiocephala*. Em laboratório, ao contrário do esperado, não há quebra da dormência, indicando que a flutuação térmica em si não consiste um mecanismo para quebra da dormência física em espécies do Cerrado. Nos choques térmicos, poucas espécies (seis de 46) apresentaram quebra da dormência. Observamos também que a mortalidade das sementes está relacionada a um *trade-off* entre forma de crescimento, tamanho das sementes e presença de dormência. Isso se deve ao tamanho pequeno das sementes de arbustos, que a despeito de tipicamente ocorrerem em savana aberta, podem morrer sob condições severas dos choques térmicos, mais do que as espécies arbóreas. Dentre as espécies arbóreas, a filogenia influencia no tamanho e, consequentemente, na mortalidade das sementes frente ao fogo. Concluímos que a germinação das sementes não está diretamente ligada à presença do fogo no mosaico Cerrado-floresta, contrastando com os padrões reconhecidos para outros ecossistemas inflamáveis.

Palavras-chave: choque térmico, dormência física, ecologia do fogo, Fabaceae, flutuação térmica, regeneração, savana

ABSTRACT

Fire is the principal disturbance in several vegetation types around the world, being called flammable ecosystems. In this context, many species have strategies to regenerate and colonize the post-fire environment, which typically involve seed survival (tolerance) and/or breaking of dormancy. In special, legume seeds usually have an impermeable seed coat (physical dormancy), which may be disrupted by fire-related heat shocks. Another factor that can help in the process of dormancy-breaking is the temperature fluctuation in the soil, which amplitude is increased in gaps formed by the removal of vegetation as a result of the passage of fire. Both factors, fire and temperature fluctuation, are relatively well studied in flammable ecosystems from Australia and Mediterranean vegetation. On the other hand, the patterns related to dormancy-breaking and seed germination are still controversial and less known in tropical savannas from Africa and South America. Therefore, this doctoral Thesis has aimed to evaluate the role of fire on germination and survival of legume seeds from Cerrado. Therefore, we conducted experiments in the field, including experimental burning, and also applied treatments in the laboratory, simulating temperature fluctuation in the gaps and fire passage (heat shocks). After the treatments, we observed germination under optimal conditions, making counting three times a week, and accomplishing viability tests by the end of 30 days of the experiment. The statistical analyses basically consisted on GLMMS with binomial distribution, considering replicates as random effects. Results pointed that seeds die when directly exposed to fire in the soil surface. On the other hand, there is a higher probability of survival when seeds are placed in vegetation gaps. In the gaps, the higher percentage of bare soil provides milder fire temperatures, lasting for less time. When buried at 1-cm belowground, seeds always survive and dormancy-breaking may occur. Temperature fluctuation can also break a significant proportion of dormancy under field conditions, especially for *Mimosa leiocephala*. In the laboratory, there is no dormancy-breaking, showing that temperature fluctuation itself might not be a mechanism for physical dormancy-breaking in Cerrado species. When heat shocks were applied, six out of 46 species showed dormancy-breaking. We also observed that seed mortality is related to a trade-off between growth-form, seed size and the presence of dormancy. This is due to the small size of seeds from shrubs, that although typically occurring in the open savannas, they might die under severe conditions of temperatures. Among the tree species, phylogeny influences on size and, consequently, mortality of seeds in face to fire. We concluded that seed germination is not directly linked to the presence of fire in the Cerrado-forest mosaic, contrasting to the patterns recognized for other flammable ecosystems.

Key words: heat shock, physical dormancy, fire ecology, Fabaceae, temperature fluctuation, regeneration, savanna

SUMÁRIO

INTRODUÇÃO GERAL

O fogo está presente em diversos tipos de vegetação, como nas regiões de clima mediterrâneo e nas savanas mundo afora (Whelan, 1995; Bond & Keeley, 2005; Keeley et al., 2012), incluindo o Cerrado (Coutinho, 1982; Furley, 1999; Simon et al., 2009; Pivello, 2011). Estes ambientes são chamados de "ecossistemas inflamáveis", ou *fire-prone ecosystems*, por terem em comum as pressões seletivas da queima sobre o metabolismo, a morfologia e ecologia das plantas (Pausas & Keeley, 2009; Keeley et al., 2011).

Espera-se, *a priori*, que as espécies nestes ambientes compartilhem estratégias semelhantes de regeneração (Keeley & Bond, 1997), incluindo a capacidade de rebrote e/ou a resistência dos propágulos (sementes e outras unidades de dispersão) às altas temperaturas, (Pausas et al., 2004; Pausas & Keeley, 2014). Assim, a sobrevivência das sementes ao fogo é um importante atributo para garantir suprimento de propágulos após o distúrbio no ambiente, permitindo sua recolonização (Paula & Pausas, 2008; Jaureguiberry & Díaz, 2015; Fichino et al., 2016). Outros atributos da semente podem estar ligados a sua capacidade de sobreviver, como seu tamanho e forma (Ribeiro et al., 2015; Gómez-González et al., 2016), ou mesmo a presença de dormência (Rolston, 1978; Ramos et al., 2016).

Neste contexto, as leguminosas tipicamente apresentam dormência física, ou seja, tegumento da semente impermeável à água (Baskin et al., 2000; Rubio de Casas et al., 2017). O tegumento destas sementes é composto por uma ou mais camadas paliçádicas de células impermeáveis, que proporcionam proteção mecânica ao embrião, podendo auxiliar na sua sobrevivência a fatores externos, como o fogo (Rolston, 1978; Baskin & Baskin, 2014). Além da proteção das sementes, a quebra deste tipo de dormência está frequentemente associada ao fogo em um número de espécies da vegetação Mediterrânea (por exemplo, Herranz et al., 1998; Moreira et al., 2010) e da Austrália (por exemplo, Auld & O'Connell, 1991; Williams et al., 2003; Scott et al., 2010). Nestes ecossistemas, as altas temperaturas do fogo podem romper o tegumento, permitindo a germinação (Morrison et al., 1998; Ooi et al., 2014).

Por outro lado, o fogo também possui efeitos indiretos, pois remove a vegetação de maneira eficiente, aumentando os espaços abertos e modificando o microclima, aumentando a oscilação de temperatura dia/noite (Fidelis et al., 2012). Portanto, a queima pode agir na quebra da dormência física tanto direta como indiretamente, por meio da flutuação térmica (Auld & Bradstock, 1996; Santana et al., 2010; 2013). Isso permite a classificação das espécies em *obrigatórias* ou *facultativas* em relação ao fogo, de acordo com o mecanismo necessário para quebra da sua dormência física (Ooi et al., 2014)

Além da quebra da dormência, há outros mecanismos por meio dos quais o fogo influencia na germinação. A fumaça, por exemplo, pode agir como estímulo em sementes com tegumento permeável, permitindo uma rápida colonização do ambiente pós-queima (van Staden et al., 2000; Keeley & Pausas, *in press*). O regime de fogo, entretanto, muda conforme o tipo de vegetação e clima ao redor do mundo (Archibald et al., 2013), e o *feedback* da vegetação em resposta a estes fatores também deve mudar nos diferentes continentes (Lehmann et al., 2014).

Nos ambientes sujeitos à queima relativamente pouco frequente (décadas de intervalo), como as vegetações mediterrâneas, o fogo é bastante severo, consumindo material lenhoso das árvores e arbustos como combustível (Keeley et al., 2012). Nestes ambientes, muitos indivíduos adultos podem ser destruídos pelo fogo, e devem possuir adaptações para rebrotamento e/ou germinação pós-fogo (Pausas et al., 2004; Paula & Pausas, 2008). As espécies *germinadoras* possuem um banco de sementes permanente e resistente ao fogo, usualmente estimulado após o distúrbio (Auld & Denham, 2006; Pausas & Keeley, 2014).

Em savanas com alta produtividade de herbáceas e gramíneas, apresentando alta frequência de queima, a sobrevivência das sementes ao fogo seria mais importante do que apresentar estímulo da germinação (Le Stradic et al., 2015; Fichino et al., 2016). Tal estímulo não seria viável porque os indivíduos recém-recrutados estariam sujeitos novamente ao fogo em um espaço de tempo relativamente curto. A queima frequente, porém menos intensa, poderia favorecer a persistência de mais indivíduos por meio do rebrotamento (Bond & Midgley, 2003; Keeley & Pausas, 2009; Clarke et al., 2013).

De toda maneira, os mecanismos envolvendo o recrutamento das sementes em relação tanto ao fogo como às flutuações térmicas ainda parecem muito variáveis e não há padrões claros sobre quebra de dormência e germinação de sementes nas espécies das savanas da África (ver Gashaw & Michelsen, 2002; Dayamba et al., 2008) e do Cerrado (Ribeiro et al., 2013; Fichino et al., 2016). Nesse contexto, o Cerrado é um *hotspot* de biodiversidade (Myers et al., 2000), formando um mosaico de vegetação, que compreende desde as fisionomias florestadas (cerradão), que praticamente não são influenciadas pelo fogo, até as formações savânicas e as campestres, nas quais o fogo ocorre em menor ou maior frequência (Coutinho, 1978; Oliveira-Filho & Ratter, 2002).

A frequência do fogo é muito alta nas savanas abertas do Cerrado (campo sujo), podendo recorrer a cada três-quatro anos de intervalo (Coutinho 1982; Miranda et al., 2009). O fogo limita o estabelecimento de espécies de floresta nas savanas do Cerrado (Hoffmann, 2000), mas o banco de sementes no campo sujo não parece ser estimulado pelo fogo (Andrade & Miranda, 2014). Portanto, há pouca evidência de que altas temperaturas possam quebrar a dormência física em espécies do Cerrado (Ribeiro et al., 2013), embora suas sementes sejam reconhecidas como resistentes ao fogo (Rizzini, 1976; Fichino et al., 2016). Espécies de floresta, por outro lado, parecem ser menos resistentes aos choques térmicos e à dessecação (Ribeiro & Borghetti, 2014; Ribeiro et al., 2015). A flutuação térmica também foi estudada em algumas espécies do Cerrado (ver Musso et al., 2015; Zupo et al., 2016), mas não está claro se tal fator seria limitante pra espécies florestais germinarem no ambiente aberto do Cerrado.

Estudos de campo e laboratório, que avaliem o papel do fogo *versus* flutuações térmicas na quebra da dormência e na germinação das sementes do Cerrado, podem levar a uma melhor compreensão do quanto estas sementes estão relacionas (ou toleram) tais fatores. Mesmo para os demais ecossistemas inflamáveis, há controvérsias se o fogo agiu como fator de pressão evolutiva em moldar atributos (em especial da dormência e da germinação) das sementes (Bradshaw et al., 2011; Keeley et al. 2011; Jaganathan, 2015; Lamont & He, 2017). Assim, é importante a compilação de dados e sua comparação com a filogenia e com o ambiente de ocorrência das espécies, a fim de esclarecer a importância do fogo na manutenção da biodiversidade em ecossistemas inflamáveis.

Esta tese de doutorado tem como objetivo avaliar o papel do fogo na germinação de sementes de leguminosas do Cerrado. Para tanto, realizamos queimas experimentais em campo (Fig. 1) e observamos a germinação e viabilidade das sementes em condições de laboratório (Fig. 2). A tese está dividida em quatro capítulos, nos quais pretendemos responder às seguintes perguntas:

(I) Qual o papel do fogo e das variações térmicas, em condições de campo, na germinação das sementes e quebra da dormência física em espécies de leguminosas do Cerrado? *Hipótese:* Quando expostas na superfície, as sementes devem em sua maioria morrer em face das altas temperaturas do fogo e da exposição direta às chamas. Por outro lado, pode haver quebra de dormência quando enterradas a 1-cm no solo, assim como as flutuações térmicas também devem influenciar positivamente na quebra da dormência/germinação.

(II) Quais são as temperaturas (e tempo de residência) do fogo em pequena escala em área de campo sujo, e como as clareiras (% de solo nu) na vegetação influenciam na sobrevivência das sementes expostas durante a queima? *Hipótese:* Quanto maior o tamanho da clareira, menores devem ser as temperaturas e tempo de residência do fogo, aumentando a probabilidade de sobrevivência das sementes.

(III) Como a flutuação térmica, simulada em laboratório, influencia na germinação das sementes de Cerrado e floresta? *Hipótese:* A flutuação térmica pode quebrar a dormência física em sementes do Cerrado, diminuindo ou atrasando a germinação em espécies de floresta.

(IV) Como o fogo afeta a germinação de sementes de Cerrado *vs.* floresta, e qual a importância dos atributos das sementes (tamanho, forma, permeabilidade) e da proximidade filogenética em determinar a mortalidade das sementes de leguminosas? *Hipóteses:* Algumas sementes do Cerrado podem ter uma parte da sua dormência física quebrada pelo fogo, enquanto a maioria deve ser tolerante aos choques térmicos em comparação às espécies de floresta, que devem apresentar maior mortalidade. Alternativamente, a mortalidade das sementes pode ser influenciada pelos atributos, que por sua vez são mais semelhantes conforme sua proximidade filogenética.

Referências bibliográficas

ANDRADE, L. A. Z.; MIRANDA, H. S. The dynamics of the soil seed bank after a fire event in a woody savanna in central Brazil. Plant Ecology, 215, 1199-1209. 2014.

ARCHIBALD, S.; LEHMANN, C. E. R.; GÓMEZ-DANS, J. L.; BRADSTOCK, R.A. Defining pyromes and global syndromes of fire regimes. Proceedings of the National Academy of Sciences, 110, 6442-6447. 2013.

AULD, T. D.; BRADSTOCK, R. A. Soil temperatures after the passage of a fire: Do they influence the germination of buried seeds? Australian Journal of Ecology, 21, 106-109. 1996.

AULD, T. D.; DENHAM, A. J. How much seed remains in the soil after a fire? Plant Ecology, 187, 15-24. 2006.

AULD, T. D.; O’CONNELL, M. A. Predicting patterns of post-fire germination in 35 eastern Australian Fabaceae. Australian Journal of Ecology, 16, 53-70. 1991.

BASKIN, J. M.; BASKIN, C. C.; LI, X. Taxonomy, anatomy and evolution of physical dormancy in seeds. Plant Species Biology, 15, 139-152. 2000.

BOND, W. J.; KEELEY, J. E. Fire as a global ‘herbivore’: the ecology and evolution of flammable ecosystems. Trends in Ecology and Evolution, 20, 387-394. 2005.

BOND, W. J.; MIDGLEY, J. J. The evolutionary ecology of sprouting in woody plants. International Journal of Plant Sciences, 164, 103-114. 2003.

BRADSHAW, S. D.; DIXON, K. W.; HOPPER, S. D.; LAMBERS, H.; TURNER, S. R. Little evidence for fire-adapted plant traits in Mediterranean climate regions. Trends in Plant Science, 16, 69-76. 2011.

CLARKE, P. J.; LAWES, M. J.; MIDGLEY, J. J.; LAMONT, B. B.; OJEDA, F.; BURROWS, G. E.; ENRIGHT, N. J.; KNOX, K. J. E. Resprouting as a key functional trait: how buds, protection and resources drive persistence after fire. New Phytologist, 197, 19-35. 2013.

COUTINHO, L. M. O conceito de cerrado. Revista Brasileira de Botânica, 1, 17-23. 1978.

COUTINHO, L. M. Ecological effects of fire in Brazilian Cerrado. In: Huntley BJ, Walker BH (Ed.). Ecology of tropical savannas, Berlin: Springer-Verlag. 273-291 pp. 1982.

DAYAMBA, S. D.; TIGABU, M.; SAWADOGO, L.; ODEN, P. C. Seed germination of herbaceous and woody species of the Sudanian savanna-woodland in response to heat shock and smoke. Forest Ecology and Management, 256, 462-470. 2008.

FICHINO, B.; DOMBROVSKI, J. R. G.; PIVELLO, V. R.; FIDELIS, A. Does fire trigger seed germination in the Neotropical savannas? Experimental tests with six Cerrado species. Biotropica, 48, 181-187. 2016.

FIDELIS, A.; BLANCO, C. C.; MÜLLER, S. C.; PILLAR, V. D.; PFADENHAUER, J.; OTTO, W. Short-term changes caused by fire and mowing in Brazilian *campos* grasslands with different long-term fire histories. Journal of Vegetation Science, 23, 552-562. 2012.

FURLEY, P. A. The nature and diversity of neotropical savanna vegetation with particular reference to the Brazilian cerrados. Global Ecology & Biogeography, 8, 223-241. 1999.

GASHAW, M.; MICHELSEN, A. Influence of heat shock on seed germination of plants from regularly burnt savanna woodlands and grasslands in Ethiopia. Plant Ecology, 159, 83-93. 2002.

GÓMEZ-GONZÁLEZ, S.; OJEDA, F.; TORRES-MORALES, P.; PALMA, J. E. Seed pubescence and shape modulate adaptive responses to fire cues. PLoS ONE, 11, e0159655. 2016.

HERRANZ, J. M.; FERNANDIS, P.; MARTÍNEZ-SANCHEZ, J. J. Influence of heat on seed germination of seven Mediterranean *Leguminosae* species. Plant Ecology, 136, 95-103. 1998.

HOFFMANN, W. A. Post-establishment seedling success in the Brazilian Cerrado: a comparison of savanna and forest species. Biotropica, 32: 62-69. 2000.

JAGANATHAN, G. K. Are wildfires an adapted ecological cue breaking physical dormancy in the Mediterranean basin? Seed Science Research, 25, 120-126. 2015.

JAUREGUIBERRY, P.; DÍAZ, S. Post-burning regeneration of the Chaco seasonally dry forest: germination response of dominant species to experimental heat shock. Oecologia, 177, 689-699. 2015.

KEELEY, J. E.; BOND. Convergent seed germination in South African fynbos and Californian chaparral. Plant Ecology, 133, 153-167. 1997.

KEELEY, J. E.; BOND, W. J.; BRADSTOCK, R. A.; PAUSAS, J. G.; RUNDEL, P. W. Fire in Mediterranean Ecosystems, Cambridge University Press. 2012.

KEELEY, J. E.; PAUSAS, J. G. Evolution of ‘smoke’ induced seed germination in pyroendemic plants. South African Journal of Botany, *in press*. doi: 10.1016/j.sajb.2016.07.012.

KEELEY, J. E.; PAUSAS, J. G.; RUNDEL, P. W.; BOND, W. J.; BRADSTOCK, R. A. Fire as an evolutionary pressure shaping plant traits. Trends in Plant Science, 16, 406-411. 2011.

LAMONT, B. B.; HE, T. Fire-proneness as a prerequisite for the evolution of fire-adapted traits. Trends in Plant Science, 22, 278-288. 2017.

LEHMANN, C. E. R.; ANDERSON, T. M.; SANKARAN, M.; HIGGINS, S. I.; ARCHIBALD, S.; HOFFMANN, W. A.; HANAN, N. P.; WILLIAMS, R. J.; FENSHAM, R. J.; FELFILI, J.; HUTLEY, L. B.; RATNAM, J.; SAN JOSE, J.; MONTES, R.; FRANKLIN, D.; RUSSELL-SMITH, J.; RYAN, C. M.; DURIGAN, G.; HIERNAUX, P.; HAIDAR, R.; BOWMAN, D. M. J. S.; BOND, W. J. Savanna vegetation-fire-climate relationships differ among continents. Science, 343, 548-552. 2014.

LE STRADIC, S.; SILVEIRA, F. A. O.; BUISSON, E.; CAZELLES, K.; CARVALHO, V.; FERNANDES, G. W. Diversity of germination strategies and seed dormancy in herbaceous species of *campo rupestre* grasslands. Austral Ecology, 40, 537-546. 2015.

MOREIRA, B.; PAUSAS, J. G. Tanned or burned: The role of fire in shaping physical seed dormancy. Plos One, 7, 1-8. 2012.

MOREIRA, B.; TORMO, J.; ESTRELLES, E.; PAUSAS, J. G. Disentangling the role of heat and smoke as germination cues in Mediterranean Basin flora. Annals of Botany, 105, 627-635. 2010.

MIRANDA, H.S., SATO, M.N., NETO, W.N. AND AIRES, F.S. Fires in the cerrado, the Brazilian savanna. In: Cochrane MA (Ed.). Tropical Fire Ecology: Climate change, land use and ecosystem dynamics, Berlin: Springer-Praxis, 427-450 pp. 2009.

MUSSO, C.; MIRANDA, H. S.; AIRES, S. S.; BASTOS, A. C.; SOARES, A. M. V. M.; LOUREIRO, S. Simulated post-fire temperature affects germination of native and invasive grasses in cerrado (Brazilian savanna). Plant Ecology and Diversity, 8, 219-227. 2015.

MYERS, N.; MITTERMEIER, R. A.; MITTERMEIER, C. G.; FONSECA, G. A. B.; KENT, J. Biodiversity hotspots for conservation priorities. Nature, 403, 853-858. 2000.

OLIVEIRA-FILHO, A. T.; RATTER, J. A. Vegetation Physiognomies and Woody Flora of the Cerrado Biome. In: Oliveira PS, Marquis RJ (Ed.). The cerrados of Brazil: ecology and natural history of a neotropical savanna, Columbia University Press. 91-120 pp. 2002.

OOI, M. K. J.; DENHAM, A. J.; SANTANA, V. M.; AULD, T. D. Temperature thresholds of physically dormant seeds and plant functional response to fire: variation among species and relative impact of climate change. Ecology and Evolution, 4, 656-671. 2014.

PAULA, S.; PAUSAS, J. G. Burning seeds: germinative response to heat treatments in relation to resprouting ability. Journal of Ecology, 96, 543-552. 2008.

PAUSAS, J. G.; BRADSTOCK, R. A.; KEITH, D. A.; KEELEY, J. E.; GCTE FIRE NETWORK. Plant functional traits in relation to fire in crown-fire ecosystems. Ecology. 85, 1085-1100. 2004.

PAUSAS, J. G.; KEELEY, J. E. A burning story: the role of fire in the history of life. BioScience, 59, 593-601. 2009.

PAUSAS, J. G.; KEELEY, J. E. Evolutionary ecology of resprouting and seeding in fire-prone ecosystems. New Phytologist, 204, 55-65. 2014.

PIVELLO, V. R. The use of fire in the Cerrado and Amazonian Rainforests of Brazil: Past and present. Fire Ecology. 7, 24-39. 2011.

RAMOS, D. M.; LIAFFA, A. B. S.; DINIZ, P.; MUNHOZ, C. B. R.; OOI, M. K. J.; BORGHETTI, F.; VALLS, J. F. M. Seed tolerance to heating is better predicted by seed dormancy than by habitat type in Neotropical savanna grasses. International Journal of Wildland Fire, 25, 1273-1280. 2016.

RIBEIRO, L. C.; BARBOSA, E. R. M.; VAN LANGEVELDE, F.; BORGHETTI, F. The importance of seed mass for the tolerance to heat shocks of savanna and forest tree species. Journal of Vegetation Science, 26, 1102-1111. 2015.

RIBEIRO, L. C.; BORGHETTI, F. Comparative effects of desiccation, heat shock and high temperatures on seed germination of savanna and forest tree species. Austral Ecology, 39, 267-278. 2013.

RIBEIRO, L. C.; PEDROSA, M.; BORGHETTI, F. Heat shock effects on seed germination of five Brazilian savanna species. Plant Biology, 15, 152-157. 2013.

RIZZINI, C. T. Influência da temperatura sobre a germinação de diásporos do cerrado. Rodriguésia, 28, 341-381. 1976.

ROLSTON, M. P. Water impermeable seed dormancy. The Botanical Review, 44, 365-396. 1978.

RUBIO DE CASAS, R.; WILLIS, C. G.; PEARSE, W. D.; BASKIN, C. C.; BASKIN, J. M.; CAVENDER-BARES, J. Global biogeography of seed dormancy is determined by seasonality and seed size: a case study in the legumes. New Phytologist, 214, 1527-1536. 2017.

SANTANA, V. M.; BAEZA, M. J.; BLANES, M. C. Clarifying the role of fire heat and daily temperature fluctuations as germination cues for Mediterranean Basin obligate seeders. Annals of Botany, 111, 127-134. 2013.

SANTANA, V. M.; BRADSTOCK, R. A.; OOI, M. K. J.; DENHAM, A. J.; AULD, T. D.; BAEZA, M. J. Effects of soil temperature regimes after fire on seed dormancy and germination in six Australian Fabaceae species. Australian Journal of Botany, 58, 539-545. 2010.

SCOTT, K.; SETTERFIELD, S.; DOUGLAS, M.; ANDERSEN, A. Soil seed banks confer resilience to savanna grass-layer plants during seasonal disturbance. Acta Oecologica, 36, 202-210. 2010.

SIMON, M. F.; GRETHER, R.; QUEIROZ, L. P.; SKEMA, C.; PENNINGTON, R. T.; HUGHES, C. E. Recent assembly of the Cerrado, a neotropical plant diversity hotspot, by in situ evolution of adaptations to fire. Proceedings of the National Academy of Sciences, 106, 20359-20364. 2009.

VAN STADEN, J.; BROWN, N. A. C.; JÄGGER, A. K.; JOHNSON, T. A. Smoke as a germination cue. Plant Species Biology, 15, 167-178. 2000.

WHELAN, R. J. The Ecology of Fire. Cambridge: Cambridge University Press. 346 p. 1995.

WILLIAMS, P. R.; CONGDON, R. A.; GRICE, A. C.; CLARKE, P. J. Fire-related cues break seed dormancy of six legumes of tropical eucalypt savannas in north-eastern Australia. Austral Ecology, 28, 507-514. 2003.

ZUPO, T.; BAEZA, M. J.; FIDELIS, A. The effect of simulated heat-shock and daily temperature fluctuations on seed germination of four species from fire-prone ecosystems. Acta Botanica Brasilica, 30, 514-519. 2016.

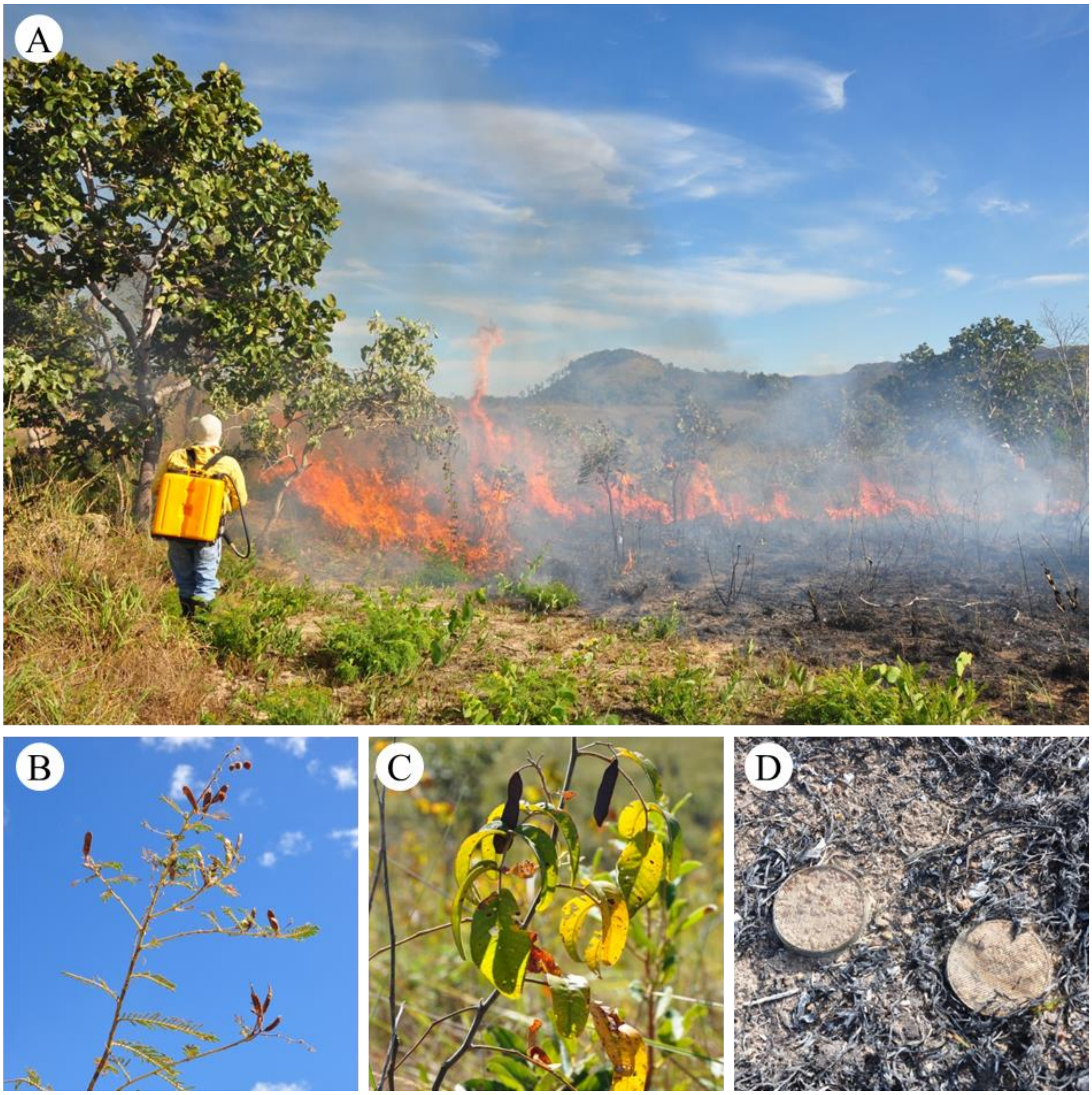


Figura 1. (A) Queima experimental na Reserva Natural Serra do Tombador (RNST; Norte de Goiás). (B e C) Espécies arbustivas utilizadas nos experimentos de campo dos Capítulos 1 e 2: *Mimosa leiocephala* e *Harpalyce brasiliana*. (D) Placas de alumínio preenchidas com solo e contendo sementes durante os experimentos de fogo na RNST.

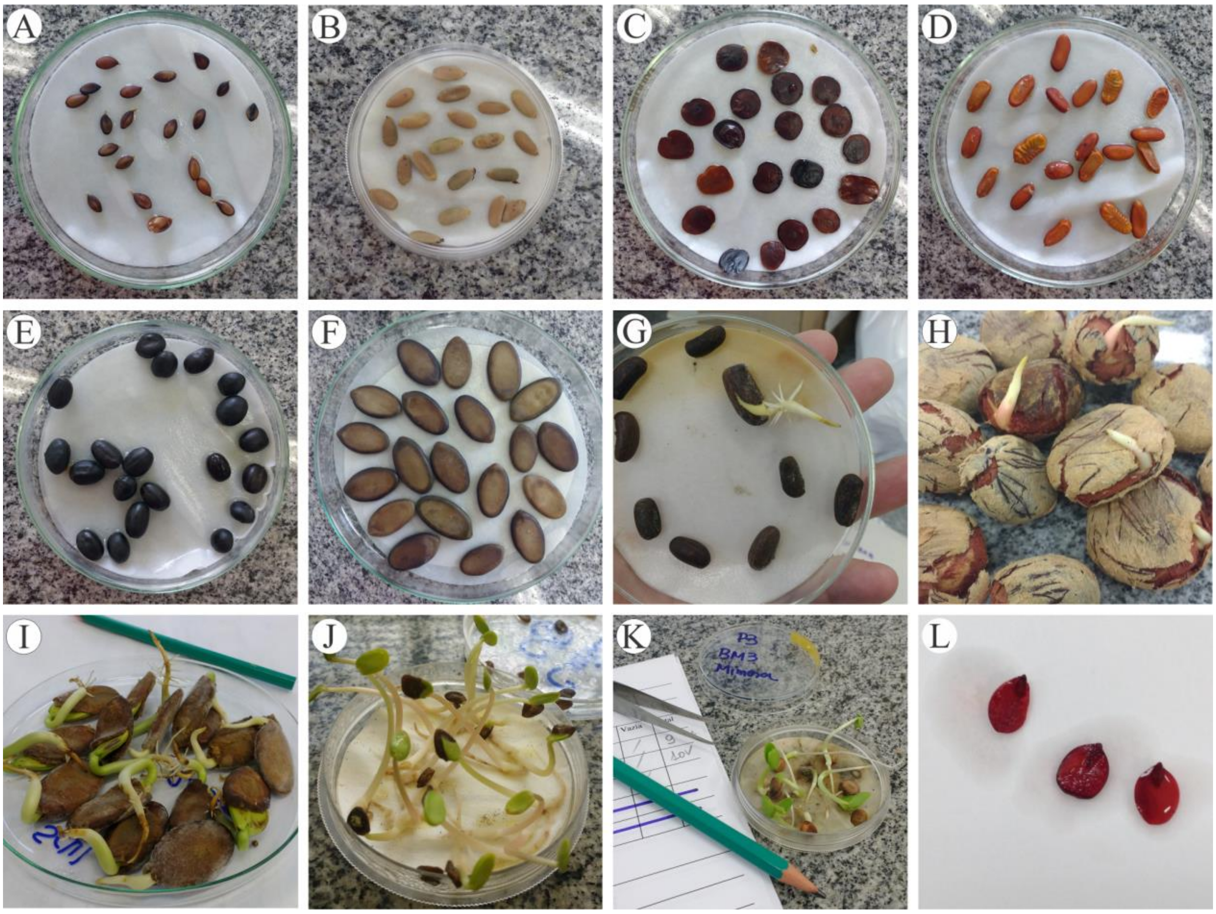


Figura 2. Sementes de algumas espécies utilizadas no estudo (Capítulos 3 e 4): (A) *Stryphnodendron obovatum*; (B) *Peltophorum dubium*; (C) *Anadenathera falcata*; (D) *Dimorphandra mollis*; (E) *Copaifera langsdorffii*; (F) *Schizolobium parahyba*; (G) *Erythrina speciosa*; (H) *Hymenaea stigonocarpa*. (I; J; K) Sementes deixadas para germinar uma semana após escarificação (teste de viabilidade). (L) Sementes remanescentes não-germinadas após escarificação, submetidas a teste de tetrazólio.

CAPÍTULO 1

A field perspective on effects of fire and temperature fluctuation on Cerrado legume seeds

Ensine-a a questionar a linguagem.
A linguagem é o repositório de nossos preconceitos,
de nossas crenças, de nossos pressupostos
Chimamanda Ngozi Adichie

A field perspective on effects of fire and temperature fluctuation on Cerrado legume seeds[1]

L. Felipe Daibes, Talita Zupo, Fernando A. O. Silveira, Alessandra Fidelis

Abstract

Information from a field perspective on temperature thresholds related to physical dormancy (PY) alleviation and seed resistance to high temperatures of fire is crucial to disentangle fire and non-fire related germination cues. We investigated seed germination and survival of four leguminous species from a frequently burned open Neotropical savanna in Central Brazil. Three field experiments were conducted according to seed location in/on the soil: (1) fire effects on exposed seeds; (2) fire effects on buried seeds; and (3) effects of temperature fluctuations on exposed seeds in gaps and shaded microsites in vegetation. After field treatments, seeds were tested for germination in the laboratory, together with the control (nontreated seeds). Fire effects on exposed seeds decreased viability in all species. However, germination of buried *Mimosa leiocephala* seeds was enhanced by fire in an increased fuel load treatment, in which we doubled the amount of aboveground biomass. Germination of two species (*M. leiocephala* and *Harpalyce brasiliana*) was enhanced with temperature fluctuation in gaps, but this condition also decreased seed viability. Our main conclusions are: (1) most seeds died when exposed directly to fire; (2) PY could be alleviated during hotter fires when seeds were buried in the soil; and (3) daily temperature fluctuations in gaps also broke PY of seeds on the soil surface, so many seeds could be recruited or die before being incorporated into the soil seed banks. Thus, seed dormancy-break and germination of legumes from Cerrado open savannas seem to be driven both by fire and temperature fluctuations.

Keywords: *campo sujo*, experimental fire, Fabaceae, fire-prone, Neotropical savannas, physical dormancy

---

[1] Publicado no periódico *Seed Science Research*

Introduction

Recruitment from seeds following fire is a key factor for the maintenance of populations of many species with physically dormant seeds throughout the world (e.g. Auld and O'Connell, 1991; Moreira and Pausas, 2012; Ooi *et al.*, 2014). Physical dormancy (PY), or seed coat impermeability to water (Rolston, 1978; Baskin and Baskin, 2004; Finch-Savage and Leubner-Metzger, 2006), is common in many ecosystems and in many plant families, but it is especially relevant in tropical Fabaceae (Baskin *et al.*, 2000; Jayasuriya *et al.*, 2013; Baskin and Baskin, 2014). Although PY may have emerged as a response to distinct selective pressures rather than fire, such as drought and seasonality (Bradshaw *et al.*, 2011; Santana *et al.*, 2013; Willis *et al.*, 2014; Jaganathan, 2015; Jayasuriya *et al.*, 2015), its alleviation under natural conditions has been suggested to be fire-dependent in many fire-prone habitats (Auld and O'Connell, 1991; Auld and Denham, 2006; Keeley *et al.*, 2011; Moreira and Pausas, 2012; Ooi *et al.*, 2014).

Nevertheless, daily temperature fluctuations also may play a role in PY alleviation in fire-prone ecosystems (Auld and Bradstock, 1996; Santana *et al.*, 2010; 2013). Relatively high temperature fluctuations occur in post-fire vegetation due to increased solar irradiance (Auld and Bradstock, 1996; Fidelis and Blanco, 2014), but temperature fluctuations also occur in undisturbed vegetation gaps (Santana *et al.*, 2013). On the other hand, there are many cases in which the temperature required to break PY is higher than those reached in vegetation gaps, and thus, fire passage would be necessary to break dormancy (Moreira and Pausas, 2012). PY has been reported to be broken at different temperature thresholds, and these data have been used to classify species into functional groups according to their fire-dependency: fire-obligate or fire-facultative, depending on the temperatures required to break PY (Ooi *et al.*, 2014).

Determination of the temperature thresholds related to PY alleviation is crucial to disentangling the role of fire and temperature fluctuations as germination cues in fire-prone environments (Moreira and Pausas, 2012; Ooi *et al.*, 2014), and it is also important to clarify seed tolerance to fire-related temperatures (Jaureguiberry and Díaz, 2015; Ribeiro *et al.*, 2015). Moreover, it is necessary to determine such temperature thresholds from a field perspective (e.g. Santana *et al.*, 2013). Therefore, if PY break is fire-dependent one should expect dormancy to be alleviated only at temperatures higher than those found in daily temperature fluctuations at the soil surface (Moreira and Pausas, 2012; Ooi *et al.*, 2014).

The question regarding fire-mediated PY break has been mainly addressed in Australia (e.g. Auld and O’Connell, 1991; Knox and Clarke, 2006; Scott *et al.*, 2010; Ooi *et al.*, 2014) and Mediterranean vegetation (e.g. Herranz *et al.*, 1998; Reyes and Trabaud, 2009; Moreira *et al.*, 2010; Santana *et al.*, 2013). In the Cerrado, few data are derived from field experiments (but see Hoffmann, 2000; Dodonov *et al.*, 2014) and most data obtained from laboratory studies suggests that fire-related heat shock does not break PY (Ribeiro *et al.*, 2015; Fichino *et al.*, 2016; Zupo *et al.*, 2016). Therefore, we still lack a comprehensive idea of the overall effects of fire on dormancy alleviation and seed germination in grassy biomes, which have been typically neglected (Parr *et al.*, 2014). Such a field-related perspective should help us understand germination responses to fire in the Neotropical savannas, such as the open Cerrado formations (*campo sujo*, Coutinho, 1982). The Cerrado is a Neotropical complex of open and closed savannas (Furley, 1999; Oliveira-Filho and Ratter, 2002), where fire has been present for at least four million years (Simon *et al.*, 2009).

Our purpose was to evaluate different scenarios for seed germination and survival from a field perspective, aiming to disentangle the role of fire-related and non-fire related germination cues in a Neotropical savanna. Thus, we carried out three field experiments with legume shrub seeds from Cerrado open savannas, according to where seeds could be located in/on the soil. Specifically, we tested the effects of (1) fire on exposed seeds (in the upper layer of the soil), (2) fire on buried seeds and (3) temperature fluctuations under non-fire related conditions on dormancy break and maintenance of seed viability.

Additionally, we addressed the following hypotheses. (1) Because recurrent Cerrado fires consume up to 90% of the grassy fuel load (Coutinho, 1982; Kauffman *et al.*, 1994; Miranda *et al.*, 2009), we expected fire to kill most of the seeds located on the soil surface, as reported for other fire-prone ecosystems (Carrington, 2010; Dayamba *et al.*, 2010). (2) Belowground soil heating during fires could be increased by a higher amount of fuel load (Choczynska and Johnson, 2009; Gagnon *et al.*, 2012; 2015), which in this case is the amount of live and dead grassy biomass, so we expected fires to have a positive effect on seed germination of buried seeds at increased fuel load conditions.(3) Natural temperature fluctuations, especially in vegetation gaps, could alleviate PY in the Cerrado, as reported for Australian legumes (Santana *et al.*, 2010) and Mediterranean Basin obligate seeders (Santana *et al.*, 2013).

Materials and Methods

Study site and seed collection

Three separate field experiments (Table 1) were carried out at the *Reserva Natural Serra do Tombador*, in Central Brazil (47°45’-51’W and 13°35’-38’S, 8900 ha, 560-1118 m a.s.l.). The area has been subjected to natural and anthropogenic fires, with a mean return interval of three years (Daldegan *et al.*, 2014). The climate has a marked dry season from May to September (total precipitation during this period <60 mm) and a wet season from October to April, when most of the annual precipitation (1300-1500 mm/year) occurs. Annually, mean temperatures range from 22 to 25°C and September is usually the hottest month. Minimal temperatures may be <15°C and maximal up to 36°C (Fundação Grupo Boticário, 2011). Vegetation is predominantly *campo sujo*, which is an open savanna dominated by a species-rich herbaceous layer with scattered shrubs and small trees (Coutinho, 1982).

We collected seeds from four leguminous shrub species commonly found in the area: *Mimosa leiocephala* Benth., *Harpalyce brasiliana* Benth., *Senna corifolia* (Benth.) H.S. Irwin and Barneby, and *Bauhinia dumosa* Benth. (Table 1). Seeds from all species were collected from several individuals (n >10) and stored at 5°C for up to one year until the beginning of the experiments. All species had physically dormant seeds (Table 1), except *B. dumosa*, which has permeable seed coats (Zupo *et al.*, 2016).

Fire effects on exposed seeds

We evaluated fire effects on survival of exposed seeds by carrying out experimental fires with seeds placed in the upper soil layer. The experiment was performed in July 2014 (mid-dry season), a period in which the study species are shedding seeds (personal observation) and when most anthropogenic fires occur in the Cerrado (Ramos-Neto and Pivello, 2000; Miranda *et al.*, 2009). We avoided patches invaded by *Urochloa brizantha*, an invasive African grass species, because it can influence fire parameters (Gorgone-Barbosa *et al.*, 2015).

Seeds were placed in experimental plots subjected to fires (Fig. 1). We independently burned four distinct plots (15 x 20 m, two years excluded from fire), which were placed at least 20 m from each other. Seeds were placed in aluminium dishes (7 cm diameter x 0.5 cm deep) filled with local soil and positioned on the soil surface. Seeds were carefully sat within the aluminium dishes, so they would have at least part of their seed coat exposed directly to fire.

Three aluminium dishes were placed under the native vegetation (grass tussocks) in each plot (3 dishes x 4 plots = 12 samples). We conducted head fires (wind direction), and fires were set in the morning for safety reasons (Whelan, 1995); all plots were burned in the same week. Fire temperatures were recorded every two seconds with thermocouple sensors type K attached to data loggers (Novus ® log box). We calculated fire residence times at temperatures ≥60°C, which is a standardized temperature threshold lethal for plant tissue (Daniell *et al.*, 1969; Dayamba *et al.*, 2010). Additionally, we calculated residence time of fire temperatures ≥100°C, a threshold easily reached by fire under exposed conditions.

The number of seeds per dish varied between species due to constraints in seed availability (see Table 1). Control seeds were transported to the field and kept stored in thermal bags at room temperature, thus not heated. After fires, both control and treatment seeds were tested for germination in the laboratory (see germination procedures below).

Fire effects on buried seeds

To test the effects of fire on the survival and germination of buried seeds, we used seeds of two species with different traits in relation to seed coat permeability: one with impermeable-coated seeds (PY, *M. leiocephala*) and the other one with permeable seeds (non-dormant, *B. dumosa*, see Table 1). Since the amount of fuel (live and dead grassy species) can influence both fire intensity and temperatures reached during fires (Gagnon *et al.*, 2015; M.N. Rissi *et al.*, *unpubl. data*), we manipulated the fuel load to assess the effects of doubling the amount of aboveground biomass on soil heating and thus on seed germination and survival of buried seeds. Therefore, the following treatments were carried out in the field: (1) no manipulation of fuel load (~700 g.m^{-2}) and (2) increased fuel load by adding 700 g.m^{-2} of biomass collected from neighbouring plots to double the fuel load.

For this treatment, four 30 x 30 m plots were individually burned, and we established three subplots (2 x 2 m) for each biomass manipulation treatment within each plot (3 subplots x 4 plots = 12 subplots/treatment). In each subplot, we placed an aluminium dish with 10 seeds/species buried 1 cm belowground (Fig. 1; Table 1). Fires were set in October 2015 (late-dry season), using experimental procedures similar to those described above, and fire temperatures were recorded (thermocouple type K attached to data loggers) every two seconds at 1 cm belowground in each subplot/treatment. Nevertheless, measurements in most sensors at the increased fuel load treatment failed and therefore they are not presented in detail. Control seeds were transported to the field, but kept stored at room temperatures, and after fires both control and treatment seeds were tested for germination in the laboratory (see below).

Temperature fluctuation effects

We used seeds of the four species at two different field treatments to test the effects of temperature fluctuations on seed germination: (1) shaded = seeds placed under native grass tussocks; and (2) gap = seeds placed in bare soil gaps. The soil-filled aluminium dishes (0.5 mm deep) with seed samples were placed at soil level in shade or in a gap in the field for *ca.* 75 days from July to October (dry season). Soil temperatures were recorded every 30 minutes by sensors coupled to data loggers. The sensors were placed at the soil surface in each treatment, near the aluminium dishes: in the shaded treatment, sensors were placed under tussocks of native grasses and in the gap treatment they were exposed to sunlight. The treatments were applied in four unburned plots and followed the same experimental design as described above (3 dishes x 4 plots = 12 samples/treatment; Fig. 1; Table 1). After 75 days, treatment and control (stored at room temperatures) seeds were tested for germination, as described below.

Germination tests after field treatments

Upon the completion of the field treatments, seeds were packed in thermal bags and immediately transported to the laboratory. Treatment and control seeds were then placed in Petri dishes (60 mm diam.) with a double layer of filter paper saturated with distilled water and set to germinate in germination chambers at 27°C (12 h of light), the average temperature recorded at soil level in the study site and in accordance to other studies performed with Cerrado seeds (Fichino *et al.*, 2016). We used a constant temperature to avoid possible additional effects of alternating temperature in seed germination. Seeds were considered germinated when exhibiting radicle protrusion (Bewley *et al.*, 2013) and germination was recorded three times a week during four consecutive weeks.

At the end of germination tests, nongerminated seeds were scarified, allowed to imbibe and then cut and tested for the viability using tetrazolium solution (1%, buffer pH 7). Seeds with embryos that stained red were considered to be viable, and those with nonstained embryo or were visibly burned or showed flaccid tissues were considered to be dead (Lakon, 1949).

Data analyses

All our experiments followed a similar experimental design (Fig. 1), with the seed samples nested within the experimental plots (3 dishes x 4 plots = 12 samples/species/treatment). Therefore, we performed generalized linear mixed models (GLMMs) with a binomial distribution (see Santana *et al.*, 2013) to separately evaluate: seed germination (binary data considering that a seed could germinate or not) and seed viability (a seed could be viable or not at the end of the trials). Since the dishes were nested within the plots, they were considered as the random effects in the analyses. The field treatments (fire treatments or shaded/gap temperature fluctuations, as well as their respective controls) were treated as fixed effects. The controls were considered as the baseline for the intercept in the models. Models were fitted for each species separately in each experiment. Post-hoc Tukey tests were performed to evaluate multiple comparisons among treatments. All analyses were performed using the packages lme4 (Bates *et al.*, 2015) and multcomp (Hothorn *et al.*, 2008) in R software 3.2.5 (R Core Team, 2016) and significance was determined with $\alpha = 0.05$.

Table 1. Experiment setup, species, date of collection, date of experiment, presence of dormancy (PY = physical dormancy and ND = non-dormant), and number of seeds per dish used in the tests at field conditions in an open savanna in Central Brazil.

Experiment	Species	Seed collection date	Date of experiment	Dormancy	Seeds per dish (n)
Fire effects on exposed seeds	*Mimosa leiocephala*	October 2013	July 2014 (mid-dry season)	PY	10
	Harpalyce brasiliana	July 2013		PY	10
	Senna corifolia	October 2013		PY	9
	Bauhinia dumosa	October 2013		ND	8
Fire effects on buried seeds	*Mimosa leiocephala*	October 2014	October 2015 (late-dry season)	PY	10
	Bauhinia dumosa	October 2015		ND	10
Temperature fluctuation effects	*Mimosa leiocephala*	October 2013	July-October 2014 (dry season)	PY	10
	Harpalyce brasiliana	July 2013		PY	10
	Senna corifolia	October 2013		PY	9
	Bauhinia dumosa	October 2013		ND	8

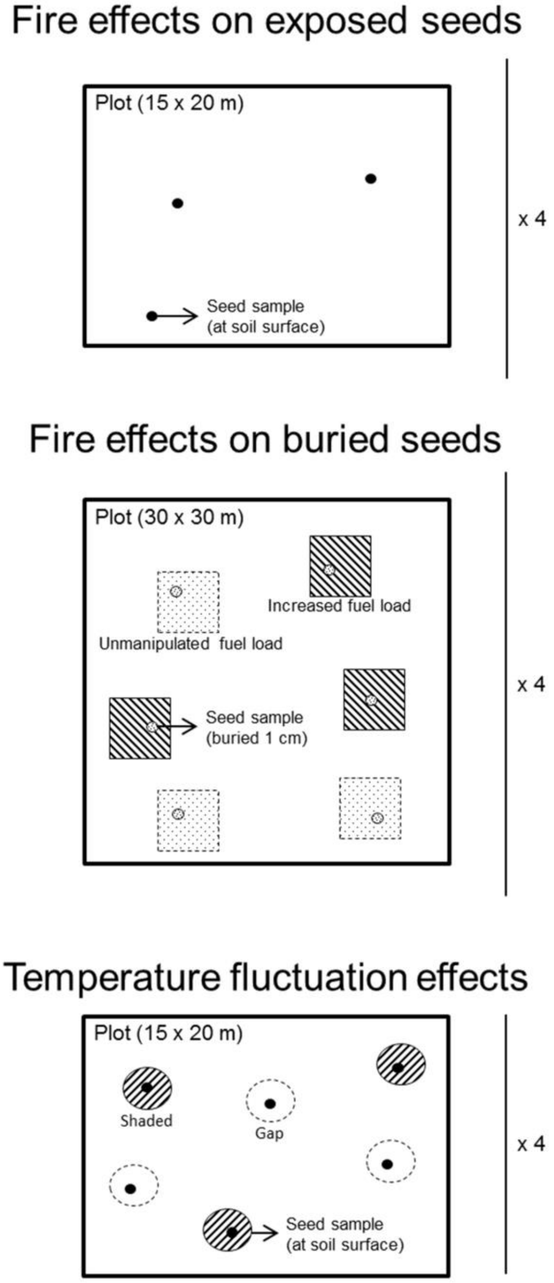


Figure 1. Experimental design of the different experiments tested under field conditions in an open savanna in Central Brazil: (1) fire effects on exposed seeds; (2) fire effects on buried seeds at different fuel load treatments: unmanipulated and increased amount of aboveground biomass; and (3) temperature fluctuations in shaded and gap conditions. Black circles show aluminium dishes containing seed samples at soil surface and grey dotted circles show aluminium dishes containing seed samples at 1 cm belowground. Number of seeds per species/sample is shown in Table 1.

Results

Fire effects on exposed seeds

Maximum fire temperatures were 225±152°C, with residence time of temperatures ≥60°C equal to 80±26 seconds and ≥100°C always less than one minute. For *M. leiocephala*, germination decreased from 26±9% (control) to 9±7% (fire treatment, *P* = 0.002; Table 2a; Fig. 2a). Germination of *H. brasiliana* and *S. corifolia* was nearly null in both the control and the fire exposure treatments (Table 2a; Fig. 2a). However, for *B. dumosa*, a species with permeable seeds, fire exposure reduced germination from 88±4% to 11±22% (*P* <0.001; Table 2a; Fig. 2a). Initial seed viability ranged from 63% (*H. brasiliana*) to 90% (*B. dumosa*), and seed viability of all four species decreased after fire exposure to less than 30% (all *P* <0.001; Table 2a; Fig. 2b).

Fire effects on buried seeds

Belowground temperatures reached mean maxima of 54±14°C, and thus they did not exceed the threshold of 60°C for residence time calculations in the unmanipulated treatment. Sensors failed to measure belowground temperatures in the increased fuel load condition, however, recordings of fire temperatures in the same area point out that mean maxima temperatures reach around 80°C at 1 cm buried in sites under high biomass accumulation (see Discussion).

Regarding seed germination, there was no difference in germination percentage of *M. leiocephala* seeds between the control (5±4%) and the unmanipulated treatment (3±5%, Table 2b; Fig. 3a). Moreover, the increased fuel load treatment had a positive effect on seed germination of *M. leiocephala*, resulting in a six-fold increase in germination (*P* = 0.011; Table 2b; Fig. 3a). There was no difference in seed viability among treatments for this species (Table 2b), which was over 70% for all treatments (Fig. 3b). For *B. dumosa* seeds, both seed germination and viability percentages were not affected by the treatments (Table 2b), remaining ≥88% (Fig. 3a, b).

Temperature fluctuation effects

Soil temperature under the shaded condition ranged from 20 to 38°C (Fig. 4) and had little or no effect on seed germination for any species (Table 2c; Fig. 5a). On the other hand, soil temperature in gaps ranged from 18 to 53°C and remained ≥50°C for at least two hours each day (Fig. 4).

Temperature fluctuations in the gap treatment had a positive effect in alleviating PY in *M. leiocephala* (*P* <0.001) and *H. brasiliana* (*P* = 0.001; Table 2c). For *M. leiocephala*, the gap condition enhanced germination percentage (58±17%) in relation to both shaded treatments (16±4%) and the control (26±9%; Fig. 5a). For *H. brasiliana*, the gap condition increased germination to 20±5% compared to the control (5±3%, Fig. 5a). However, none of the treatments alleviated dormancy in *S. corifolia* seeds (Table 2c; Fig. 5a). Seeds of *B. dumosa* were negatively affected by temperature fluctuation in the gaps (*P* <0.001; Table 2c; Fig. 5a).

Seed viability of all species in the shaded treatment did not differ from their respective controls (Table 2c; Fig 5b). Nonetheless, the gap treatment had a negative effect on seed viability from 20 to 30% across species (Table 2c; Fig. 5b).

Table 2. Parameters estimated by the GLMM models for seed germination and viability of four legume shrub species exposed to: fire effects on exposed seeds (a); fire effects on buried seeds (b); and temperature fluctuations (c), in an open savanna in Central Brazil. Significant *P*-values (≤0.05) highlighted in bold.

Experiment	Species	Treatment	*Seed germination*			*Seed viability*		
			Estimate	s.e.	*P*-value	Estimate	s.e.	*P*-value
Fire effects on exposed seeds (a)	*Mimosa leiocephala*	Intercept	-1.093	0.264	**<0.001**	2.034	0.736	**0.006**
		Fire	-1.362	0.448	**0.002**	-3.862	1.051	**<0.001**
	Harpalyce brasiliana	Intercept	-3.136	0.457	**<0.001**	0.632	0.696	0.364
		Fire	-1.644	1.103	0.136	-4.051	1.202	**<0.001**
	Senna corifoilia	Intercept	-2.632	0.522	**<0.001**	1.344	0.436	**0.002**
		Fire	-2.020	1.133	0.075	-2.357	0.621	**<0.001**
	Bauhinia dumosa	Intercept	2.331	0.873	**0.008**	2.530	0.874	**0.004**
		Fire	-6.2931	1.615	**<0.001**	-6.468	1.604	**<0.001**
Fire effects on buried seeds (b)	*Mimosa leiocephala*	Intercept	-3.700	0.729	**<0.001**	1.418	0.339	**<0.001**
		Unmanip.	-0.565	1.151	0.578	-0.031	0.476	0.950
		Increased	2.225	0.869	**0.011**	-0.339	0.469	0.470
	Bauhinia dumosa	Intercept	6.058	1.496	**<0.001**	6.058	1.496	**<0.001**
		Unmanip.	-2.250	1.511	0.137	-2.250	1.511	0.137
		Increased	-2.434	1.510	0.107	-2.434	1.510	0.107
Temperature fluctuation effects (c)	*Mimosa leiocephala*	Intercept	-1.098	0.272	**<0.001**	1.874	0.398	**<0.001**
		Shaded	-0.641	0.406	0.114	-0.381	0.549	0.488
		Gap	1.435	0.376	**<0.001**	-1.231	0.535	**0.021**
	Harpalyce brasiliana	Intercept	-3.216	0.489	**<0.001**	0.551	0.293	0.060
		Shaded	0.947	0.579	0.102	-0.121	0.413	0.769
		Gap	1.754	0.546	**0.001**	-0.900	0.414	**0.030**
	Senna corifolia	Intercept	-2.543	0.393	**<0.001**	1.192	0.306	**<0.001**
		Shaded	-1.318	0.815	0.106	0.065	0.423	0.879
		Gap	0.156	0.539	0.772	-1.402	0.415	**<0.001**
	Bauhinia dumosa	Intercept	1.989	0.296	**<0.001**	2.190	0.334	**<0.001**
		Shaded	-0.682	0.378	0.071	-0.425	0.440	0.335
		Gap	-1.788	0.358	**<0.001**	-1.780	0.403	**<0.001**

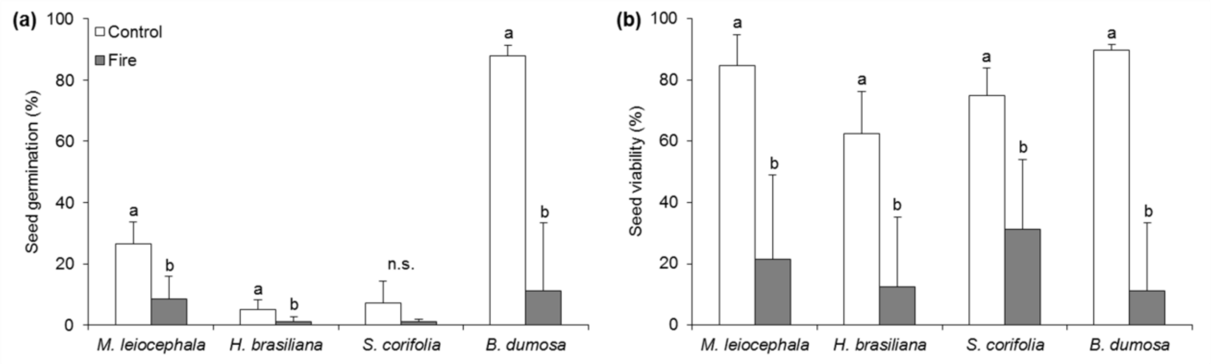


Figure 2. Fire effects on exposed seeds: (a) seed germination and (b) seed viability (%, mean ± SD) of four leguminous shrub species from an open savanna subjected to experimental fires in Central Brazil. Control refers to untreated seeds, set to germinate at the laboratory. Different letters mean significant differences (*P*≤0.05) among treatments; statistical comparisons were performed separately for each species.

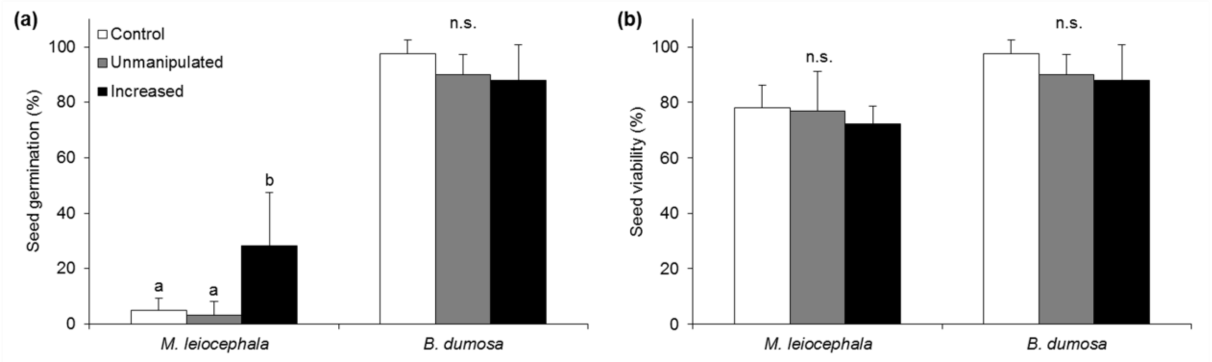


Figure 3. Fire effects on buried seeds: (a) seed germination and (b) seed viability (%, mean ± SD) of buried seeds (1 cm belowground) of two legume shrub species from an open savanna, subjected to experimental fires in Central Brazil. Control = untreated seeds, set to germinate at the laboratory; Unmanipulated = treatment with no manipulation of fuel load; and Increased = aboveground biomass was manipulated to double its average amount. Different letters mean significant differences (*P*≤0.05) among treatments; statistical comparisons were performed separately for each species.

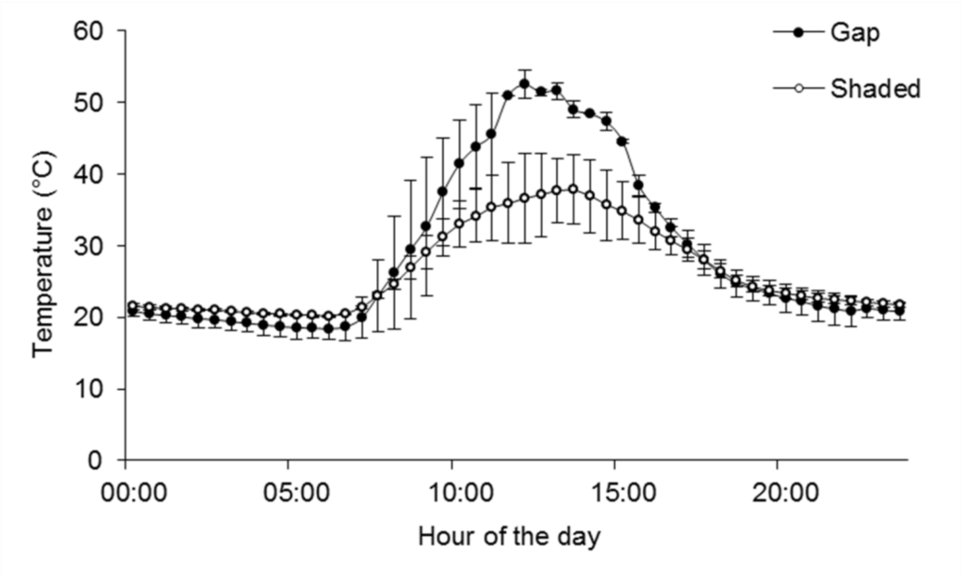


Figure 4. Daily temperature fluctuations (mean±SD) at the soil surface under vegetation gaps (filled circles) and under shaded conditions (open circles) in an open savanna in Central Brazil for 75 days in the dry season (July-October 2014).

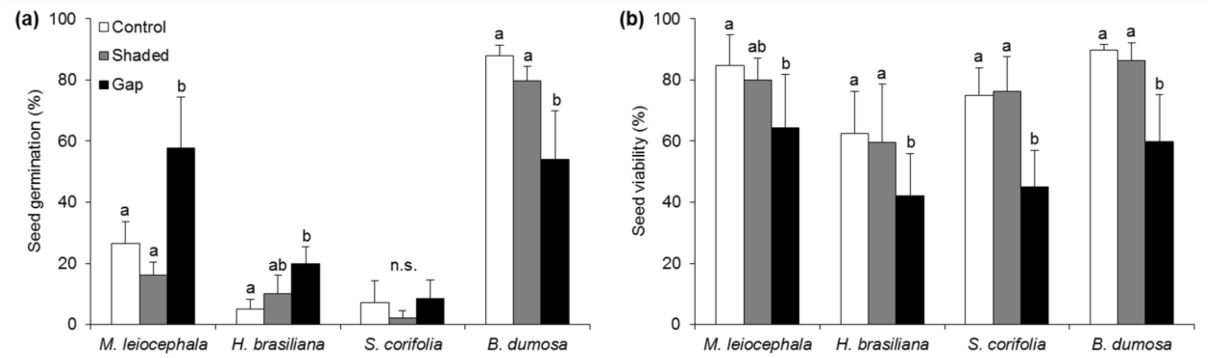


Figure 5. Temperature fluctuation effects (a) on seed germination and (b) on seed viability (%, mean ± SD) of four legume shrub species from an open savanna, subjected to daily temperature fluctuation in Central Brazil for 75 days. Control = untreated seeds, set to germinate at the laboratory; Shaded = temperature fluctuations under the shade of grasses; and Gap = temperature fluctuations in the gaps of bare soil. Different letters mean significant differences ($P \leq 0.05$) among treatments; statistical comparisons were performed separately for each species.

Discussion

There is an ongoing debate on the relative importance of fire-related temperatures *vs.* daily temperature fluctuations in alleviating PY in fire-prone ecosystems (Moreira *et al.*, 2010; Santana *et al.*, 2010; 2013; Ooi *et al.*, 2014), and our study helps to disentangle these germination cues from a field perspective in a Neotropical savanna. Theoretically, PY should be broken at different temperature thresholds, so dormancy alleviation could be classified as being fire-related or not (Moreira and Pausas, 2012; Ooi *et al.*, 2014). However, we argue that Cerrado leguminous seeds have their PY (or at least part of it) alleviated by two different mechanisms: (1) during hotter fires, if seeds are stored in the soil, or (2) when exposed to daily temperature fluctuations.

Although not tested under the buried conditions in our field experiments, some seeds of *H. brasiliana* have part of their PY alleviated after heat shocks applied in the laboratory (H.L. Zirondi *et al.*, *unpubl. data*). Therefore, seeds of both *M. leiocephala* and *H. brasiliana* should be classified as facultative regarding their functional responses to fire (see Ooi *et al.*, 2014). PY of *S. corifolia* seeds, on the other hand, was neither alleviated from laboratory heat shocks (data not shown) nor from the enhanced field temperature fluctuations, which could suggest that these legume seeds have other habitat-specific dormancy-breaking mechanisms under natural conditions, perhaps related to wetter conditions (van Klinken and Goulier, 2013).

Fire effects on exposed seeds caused a high mortality, as reported for most seeds throughout the world when placed in the upper layer of the soil (Auld and O’Connell, 1991; Bradstock and Auld, 1995; Dayamba *et al.*, 2010; Carrington, 2010). However, seeds buried in the soil would be insulated and could have PY alleviated, as reported for Australian leguminous seeds (Auld and O’Connell, 1991; Bradstock and Auld, 1995). Conversely, belowground temperatures required for PY break seem to be only reached under more intense fires (Knox and Clarke, 2006). Despite that, Cerrado fires are mostly fast and belowground soil heating usually negligible (~50°C, Miranda *et al.*, 1993), with minor consequences to the seed bank (Andrade and Miranda, 2014).

Nevertheless, hotter fires may occur in the Cerrado when the return intervals take four years or more, due to a substantial accumulation of fuel load (Coutinho, 1982), which would also enhance belowground fire temperatures, as reported for other high-diversity flammable communities (e.g. Gagnon *et al.*, 2015). In our study area, previous measurements of fire temperatures 1cm belowground in plots with four years of fuel load accumulation, detected temperatures reaching ≥60°C for about three minutes, occasionally recording temperatures ≥100°C (mean maxima = 80°C; maximum observed = 154°C; T. Zupo *et al.*, *unpubl. data*). On the other hand, anthropogenic fires have been suggested to change fire regimes in Cerrado open savannas, by shifting season of occurrence from wet to the dry season and shortening the frequency of return (Ramos-Neto and Pivello, 2000; Miranda *et al.*, 2009).

Fire-stimulated germination traits should not have been favoured in fire-prone systems that undergo surface fires (Bond and Midgley, 2003; Bradshaw *et al.*, 2011), such as the open savannas where fire episodes are frequent and less severe (Bond and Keeley, 2005). Although there has been no evidence of fire-stimulated germination from laboratory experiments (Ribeiro *et al.*, 2015; Le Stradic *et al.*, 2015; Fichino *et al.*, 2016; Zupo *et al.*, 2016), we argue that fire-related conditions can potentially break PY, as was observed in at least 30% of *M. leiocephala* buried seeds under increased fuel load conditions. Therefore, fire could play a role in seed recruitment for a proportion of seeds from the Cerrado soil seed bank.

Fire also has been recognized as having an indirect effect on PY alleviation by removing vegetation and consequently increasing daily temperatures at upper soil layers (~0.5 cm), therefore reaching temperature thresholds required for gap-dependent seeds to break PY (Ooi *et al.*, 2014), which do not seem to occur in vegetated sites (Auld and Bradstock, 1996; Santana *et al.*, 2010). However, gaps in unburned sites seem to have the same effect in seedling recruitment, suggesting that biomass removal, which increases the daily temperature fluctuations, plays a role in regeneration from seeds irrespective of fire (Santana *et al.*, 2013).

Previous laboratory simulations of temperature fluctuations had already recognized a decrease in germination in the permeable-coated *B. dumosa* seeds, but such experiments had no effect on PY alleviation of *M. leiocephala* (Zupo *et al.*, 2016). Instead, our results show that temperature fluctuation in gaps enhanced germination for *M. leiocephala* and *H. brasiliana* seeds, even though PY was not fully alleviated. Thus, from a field perspective, vegetation gaps provide an important micro-environment with higher temperature fluctuations that could lead to the alleviation of PY in legume seeds, enabling their germination in both post-fire environments (where temperature fluctuations will be higher due to the removal of the aboveground vegetation) as well as in fire-free intervals if seeds reach such gaps.

PY release has not yet been fully explained for Cerrado open savannas. Our four study species are dominant in their plant community, and distinct traits related to dormancy relief and germination strategies could explain a diversity of regeneration niches and species coexistence (see Grubb, 1977). Therefore, other species-specific processes may be related to releasing PY in these species. Further studies are warranted to understand if water availability could play any role coupled with daily heating temperatures (Moreno-Casasola *et al.*, 1994; van Klinken *et al.*, 2006), and if seeds could become sensitive to dormancy-breaking conditions as they age in soil seed banks (van Assche *et al.*, 2003; Jayasuriya *et al.*, 2009; Gama-Arachchige *et al.*, 2012; Liyanage and Ooi, *in press*).

This was the first attempt to test fire and non-fire related cues of seed germination within an experimental field approach for the Cerrado open savannas. We addressed three main conclusions from our experiments: (1) most seeds die when exposed directly to fire; (2) PY could be alleviated during hotter fires when seeds were buried, and thus stored in the soil seed bank; and (3) daily temperature fluctuations in gaps also break PY in seeds of some species on the soil surface, and thus many seeds may germinate or die instead of being incorporated into the soil seed banks.

Acknowledgments

We thank the fire team from the *Reserva Natural Serra do Tombador*: A.F. Terra, G.A. Berberino, R.B. de Almeida and S.A. Borges. We also thank E. Gorgone-Barbosa, M.N. Rissi and M.B. Cunha for the collaboration in field work. The first author has a scholarship from *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES). This study was funded by *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP 2015/06743-0), *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq 455183/2014-7) and Fundação Grupo Boticário (0153_2011_PR; 0106_2011_PR). F.A.O. Silveira (482720/2014) and A. Fidelis (306170/2015-9) receive research grants from CNPq.

References

Andrade, L.A.Z. and Miranda, H.S. (2014) The dynamics of the soil seed bank after a fire event in a woody savanna in central Brazil. *Plant Ecology* **215,** 1199-1209.

Auld, T.D. and Bradstock, R.A. (1996) Soil temperatures after the passage of a fire: Do they influence the germination of buried seeds? *Australian Journal of Ecology* **21,** 106-109.

Auld, T.D. and Denham, A.J. (2006) How much seed remains in the soil after a fire? *Plant Ecology* **187,** 15-24.

Auld, T.D. and O'Connell, M.A. (1991) Predicting patterns of post-fire germination in 35 eastern Australian Fabaceae. *Australian Journal of Ecology* **16,** 53-70.

Baskin, C.C. and Baskin, J.M. (2014) *Seeds: Ecology, biogeography, and evolution of dormancy and germination.* 2nd edition, San Diego: Academic Press.

Baskin, J.M. and Baskin, C.C. (2004) A classification system for seed dormancy. *Seed Science Research* **14,** 1-16.

Baskin, J.M., Baskin, C.C. and Li, X. (2000) Taxonomy, anatomy and evolution of physical dormancy in seeds. *Plant Species Biology* **15,** 139-152.

Bates, D., Maechler, M., Bolker, B. and Walker, S. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67,** 1-48.

Bewley, J.D., Bradford, K., Hilhorst, H.W.M. and Nonogaki, H. (2013) *Seeds: Physiology of development, germination and dormancy.* 3rd edition, New York: Springer Science.

Bond, W.J. and Keeley, J.E. (2005) Fire as a global 'herbivore': the ecology and evolution of flammable ecosystems. *Trends in Ecology and Evolution* **20,** 387-394.

Bond, W.J. and Midgley, J.J. (2003) The evolutionary ecology of sprouting in woody plants. *International Journal of Plant Sciences* **164,** 103-114.

Bradshaw, S.D., Dixon, K.W., Hopper, S.D., Lambers, H. and Turner, S.R. (2011) Little evidence for fire-adapted plant traits in Mediterranean climate regions. *Trends in Plant Science* **16,** 69-76.

Bradstock, R.A. and Auld, T.D. (1995) Soil temperatures during experimental bushfires in relation to fire intensity: consequences for legume germination and fire management in South-Eastern Australia. *Journal of Applied Ecology* **32,** 76-84.

Carrington, M.E. (2010) Effects of soil temperature during fire on seed survival in Florida sand pine scrub. *International Journal Forestry Research* doi: 10.1155/2010/402346

Choczynska, J. and Johnson, E.A. (2009) A soil heat and water transfer model to predict belowground grass rhizome bud death in a grass fire *Journal of Vegetation Science* **20,** 277-287.

Coutinho, L.M. (1982) Ecological effects of fire in Brazilian Cerrado. In: Huntley BJ, Walker BH, eds. *Ecology of tropical savannas.* Berlin: Springer-Verlag, pp. 273-291.

Daldegan, G.A., Carvalho Júnior, O.A., Guimarães, R.F., Gomes, R.A.T., Ribeiro, F.F. and McManus, C. (2014) Spatial patterns of fire recurrence using remote sensing and GIS in the Brazilian savanna: Serra do Tombador Nature Reserve, Brazil. *Remote Sensing* **6,** 9873-9894.

Daniell, J.W., Chappell, W.E. and Couch, H.B. (1969) Effect of sublethal and lethal temperatures on plant cells. *Plant Physiology* **44,** 1684-1689.

Dayamba, S.D., Savadogo, P., Zida, D., Sawadogo, L., Tiveau, D. and Oden PC. (2010) Fire temperature and residence time during dry season burning in a Sudanian savanna-woodland of West Africa with implication for seed germination. *Journal of Forestry Research* **21,** 445-450.

Dodonov, P., Xavier, R.O., Tiberio, F.C.S., Lucena, I.C., Zanelli, C.B. and Matos, D.M.S. (2014) Driving factors of small-scale variability in a savanna plant population after a fire. *Acta Oecologica* **56,** 47-55.

Fichino, B., Dombrovski, J.R.G., Pivello, V.R. and Fidelis, A. (2016) Does fire trigger seed germination in the Neotropical savannas? Experimental tests with six Cerrado species. *Biotropica* **48,** 181-187.

Fidelis, A. and Blanco, C. (2014) Does fire induce flowering in Brazilian subtropical grasslands? *Applied Vegetation Science* **17,** 690-699.

Finch-Savage, W.E. and Leubner-Metzger, G. (2006) Seed dormancy and the control of germination. *New Phytologist* **171,** 501-523.

Fundação Grupo Boticário (2011) *Plano de Manejo da Reserva Natural Serra do Tombador.* Supervisor: G.A. Gatti. Curitiba, Brazil http://www.fundacaogrupoboticario.org.br

Furley, P.A. (1999) The nature and diversity of neotropical savanna vegetation with particular reference to the Brazilian cerrados. *Global Ecology and Biogeography* **8,** 223-241.

Gagnon, P.R., Harms, K.E., Platt, W.J., Passmore, H.A. and Myers, J.A. (2012) Small-scale variation in fuel loads differentially affects two co-dominant bunchgrasses in a species-rich pine savanna. *PLoS ONE* **7,** e29674.

Gagnon, P.R., Passmore, H.A., Slocum, M., Myers, J.A., Harms, K.E., Platt, W.J. and Paine, C.E.T.(2015) Fuels and fires influence vegetation via above- and belowground pathways in a high-diversity plant community. *Journal of Ecology* **103,** 1009-1019.

Gama-Arachchige, N.S., Baskin, J.M., Geneve, R.L. and Baskin, C.C. (2012) The autumn effect: timing of physical dormancy break in seeds of two winter annual species of Geraniaceae by a stepwise process. *Annals of Botany* **110,** 637-651.

Gorgone-Barbosa, E., Pivello, V.R., Bautista, S., Zupo, T., Rissi, M.N. and Fidelis, A. (2015) How can an invasive grass affect fire behavior in a tropical savanna? A community and individual plant level approach. *Biological Invasions* **17,** 423-431.

Grubb, P. J. (1977) The maintenance of species-richness in plant communities: the importance of the regeneration niche. *Biological Reviews* **52,** 107-145.

Herranz, J.M., Ferrandis, P. and Martínez-Sánchez, J.J. (1998) Influence of heat on seed germination of seven Mediterranean *Leguminosae* species. *Plant Ecology* **136,** 95-103.

Hoffmann, W.A. (2000) Post-establishment seedling success in the Brazilian Cerrado: a comparison of savanna and forest species. *Biotropica* **32,** 62-69.

Hothorn, T., Bretz, F. and Westfall, P. (2008) Simultaneous inference in general parametric models. *Biometrical Journal* **50,** 346-363.

Jaganathan, G.K. (2015) Are wildfires an adapted ecological cue breaking physical dormancy in the Mediterranean basin? *Seed Science Research* **25,** 120-126.

Jaureguiberry, P. and Díaz, S. (2015) Post-burning regeneration of the Chaco seasonally dry forest: germination response of dominant species to experimental heat shock. *Oecologia* **177,** 689-699.

Jayasuriya, K.M.G.G., Athugala, Y.S., Wijayasinghe, M.M., Baskin, J.M., Baskin, C.C. and Mahadevan, N. (2015) The crypsis hypothesis: a stenopic view of the selective factors in the evolution of physical dormancy in seeds. *Seed Science Research* **25,** 127-137.

Jayasuriya, K.M.G.G., Baskin, J.M. and Baskin, C.C. (2009) Sensitivity cycling and its ecological role in seeds with physical dormancy. *Seed Science Research* **19,** 3-13.

Jayasuriya K.M.G.G., Wijetunga, A.S.T.B., Baskin, J.M. and Baskin, C.C. (2013) Seed dormancy and storage behaviour in tropical Fabaceae: a study of 100 species from Sri Lanka. *Seed Science Research* **23,** 257-269.

Kauffman, J.B., Cummings, D.L. and Ward, D.E. (1994) Relationships of fire, biomass and nutrient dynamics along a vegetation gradient in the Brazilian Cerrado. *Journal of Ecology* **82,** 519-531.

Keeley, J.E., Pausas, J.G., Rundel, P.W., Bond, W.J. and Bradstock, R.A. (2011) Fire as an evolutionary pressure shaping plant traits. *Trends in Plant Science* **16,** 406-411.

Knox, K.J.E. and Clarke, P.J. (2006) Fire season and intensity affect shrub recruitment in temperate sclerophyllous woodlands. *Oecologia* **149,** 730-739.

Lakon, G. (1949) The topographical tetrazolium method for determining the germination capacity of seeds. *Plant Physiology* **24,** 389-394.

Le Stradic, S., Silveira, F.A.O., Buisson, E., Cazelles, K., Carvalho, V. and Fernandes, G.W. (2015) Diversity of germination strategies and seed dormancy in herbaceous species of *campo rupestre* grasslands. *Austral Ecology* **40,** 537-546.

Liyanage, G.S. and Ooi, M.K.J. (*in press*) Do dormancy-breaking temperature thresholds change as seeds age in the soil seed bank? *Seed Science Research* doi: 10.1017/S0960258516000271.

Miranda, A.C., Miranda, H.S., Dias, I.F.O and Dias, B.F.S. (1993) Soil and air temperatures during prescribed cerrado fires in Central Brazil. *Journal of Tropical Ecology* **9,** 313-320. 1993.

Miranda, H.S., Sato, M.N., Neto, W.N. and Aires, F.S. (2009) Fires in the cerrado, the Brazilian savanna. In: Cochrane MA, ed. *Tropical Fire Ecology: Climate change, land use and ecosystem dynamics.* Berlin: Springer-Praxis, pp. 427-450.

Moreira, B. and Pausas, J.G. (2012) Tanned or burned: the role of fire in shaping physical seed dormancy. *PLoS ONE* **7,** e51523.

Moreira, B., Tormo, J., Estrelles, E. and Pausas, J.G. (2010) Disentangling the role of heat and smoke as germination cues in Mediterranean Basin flora. *Annals of Botany* **105,** 627-635.

Moreno-Casasola, P., Grime, J.P. and Martínez, M.L. (1994) A comparative study of the effects of fluctuations in temperature and moisture supply on hard coat dormancy in seeds of coastal tropical legumes in Mexico. *Journal of Tropical Ecology* **10,** 67-86.

Oliveira-Filho, A.T. and Ratter, J.A. (2002) Vegetation physiognomies and woody flora of the Cerrado biome. In: Oliveira PS, Marquis RJ, eds. *The cerrados of Brazil: ecology and natural history of a neotropical savanna.* New York, USA: Columbia University Press, 91-120.

Ooi, M.K.J., Denham, A.J., Santana, V.M. and Auld, T.D. (2014) Temperature thresholds of physically dormant seeds and plant functional response to fire: variation among species and relative impact of climate change. *Ecology and Evolution* **4,** 656-671.

Parr, C.L., Lehmann, C.E.R., Bond, W.J., Hoffmann, W.A., Andersen, A.N. (2014) Tropical grassy biomes: misunderstood, neglected, and under threat. *Trends in Ecology and Evolution* **29:** 205-213.

R Core Team. (2016) *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria https://www.R-project.org/

Ramos-Neto, M.B. and Pivello, V.R. (2000) Lightning fires in a Brazilian savanna national park: Rethinking management strategies. *Environmental Management* **26,** 675-684.

Reyes, O. and Trabaud, L. (2009) Germination behaviour of 14 Mediterranean species in relation to fire factors: smoke and heat. *Plant Ecology* **202,** 113-121.

Ribeiro, L.C., Barbosa, E.R.M., van Langevelde, F. and Borghetti, F. (2015) The importance of seed mass for the tolerance to heat shocks of savanna and forest tree species. *Journal of Vegetation Science* **26,** 1102-1111.

Rolston, M.P. (1978) Water impermeable seed dormancy. *The Botanical Review* **44,** 365-396.

Santana, V.M., Baeza, M.J. and Blanes, M.C. (2013) Clarifying the role of fire heat and daily temperature fluctuations as germination cues for Mediterranean Basin obligate seeders. *Annals of Botany* **111,** 127-134.

Santana, V.M., Bradstock, R.A., Ooi, M.K.J., Denham, A.J., Auld, T.D. and Baeza, M.J. (2010) Effects of soil temperature regimes after fire on seed dormancy and germination in six Australian Fabaceae species. *Australian Journal of Botany* **58:** 539-545.

Scott, K., Setterfield, S., Douglas, M. and Andersen, A. (2010) Soil seed banks confer resilience to savanna grass-layer plants during seasonal disturbance. *Acta Oecologica* **36,** 202-210.

Simon, M.F., Grether, R., Queiroz, L.P., Skema, C., Pennington, R.T. and Hughes, C. (2009) Recent assembly of the Cerrado, a neotropical plant diversity hotspot, by in situ evolution of adaptations to fire. *Proceedings of the National Academy of Sciences* **106,** 20359-20364.

van Assche, J.A., Debucquoy, K.L.A. and Rommens, W.A.F. (2003) Seasonal cycles in the germination capacity of buried seeds of some Leguminosae (*Fabaceae*). *New Phytologist* **158,** 315-323.

van Klinken, R.D., Flack, L.K. and Pettit, W. (2006) Wet-season dormancy release in seed banks of a tropical leguminous shrub is determined by wet heat. *Annals of Botany* **98,** 875-883.

van Klinken, R.D. and Goulier, J.B. (2013) Habitat-specific seed dormancy-release mechanisms in four legume species. *Seed Science Research* **23,** 181-188.

Whelan, R.J. (1995) *The Ecology of Fire.* Cambridge, UK: Cambridge University Press.

Willis, C.G., Baskin, C.C., Baskin, J.M., Auld, J.R., Venable, D.L., Cavender-Bares, J., Donohue, K., Casa, R.R. and The NESCent Germination Working Group. (2014) The evolution of seed dormancy environmental cues, evolutionary hubs, and diversification of the seed plants. *New Phytologist* **203,** 300-309.

Zupo, T., Baeza, M.J. and Fidelis, A. (2016) The effect of simulated heat-shock and daily temperature fluctuations on seed germination of four species from fire-prone ecosystems. *Acta Botanica Brasilica* **30,** 514-519.

CAPÍTULO 2

Gaps are safe sites for the survival of exposed seeds during Cerrado fires

Sim, eu sei de onde venho!
Insatisfeito como o fogo
Ardo para me consumir
Aquilo em que toco torna-se flama,
Em carvão aquilo que abandono:
Sou fogo, com certeza.
Friedrich Nietzsche

Gaps are safe sites for the survival of exposed seeds during Cerrado fires[2]

L. Felipe Daibes, Elizabeth Gorgone-Barbosa, Fernando A. O. Silveira, Alessandra Fidelis

Abstract

Fine-scale effects of fire and consequences for seed survival are poorly understood, especially in the Cerrado (Brazilian savannas). Thus, we aimed to investigate whether vegetation gaps (bare soil patches) influence the survival of exposed seeds during fire events in the Cerrado by serving as safe sites. We performed field fire experiments in Central Brazil to examine how gap size (% of bare soil) influences on fire heat (fire temperatures and residence time) and seed survival (Experiment 1), and to determine how seed survival is affected by fixed conditions: gaps *versus* grass tussocks during fires (Experiment 2). We used seeds of two common Cerrado legumes, *Mimosa leiocephala* Benth. and *Harpalyce brasiliana* Benth. Seed survival was analyzed using GLMMs with a binomial distribution. In Experiment 1, seeds survived (38% and 35% for *M. leiocephala* and *H. brasiliana*, respectively) only when gaps had >40% of bare soil. In Experiment 2, all seeds under grass tussocks died when exposed to fire, while up to 40% of seeds survived in vegetation gaps, relative to their respective controls. Because gaps influence on fire heat, they are important as safe sites for seed survival in Cerrado, allowing a significant proportion of seeds to survive when exposed in the soil surface.

Keywords: Fabaceae; Fire ecology; Leguminosae; Regeneration; Savanna ecology; Seed ecology

Introduction

Local variation in the soil vegetation cover plays a major role in seed recruitment after disturbances (Odion and Davis 2000; Ojeda et al. 2010). Vegetation gaps (here defined as patches with varying levels of bare soil) may influence seed germination and survival in many fire-prone ecosystems (Auld 1986; Santana et al. 2013; Keeley et al. 2016). Fire-related seed mortality is

---

[2] Submetido ao periódico *Australian Journal of Botany*

mostly caused by direct exposure to heat, which is particularly hazardous to living tissues placed at the ground level (Bradstock and Auld 1995; Carrington 2010; Dayamba et al. 2010). Fire temperatures and residence time (fire temperature duration at one point) would thus decrease with increasing gap size, thereby enhancing the probability of seed survival (Wiggers et al. 2013; Dodonov et al. 2014; Keeley et al. 2016).

Nevertheless, fine-scale variation in fire behavior is poorly understood, except for some woody ecosystems (Hiers et al. 2009; Loudermilk et al. 2012; O’Brien et al. 2016a). Small changes in soil cover, during fire events, drive seed survival in legumes from pine forests, for example (Wiggers et al. 2013; O’Brien et al. 2016b). Gap size is also recognized as important for seedling recruitment after fire in the Californian chaparral (Keeley et al. 2016). However, fire regimes and vegetation-fire-climate feedbacks differ among continents (Archibald et al. 2013; Lehmann et al. 2014), with grassy ecosystems being generally the most neglected ecosystems in this regard (Parr et al. 2014). Fires may be much different in grasslands and open savannas than in forests, because of their different vegetation structure, fuel supply and climatic conditions (see Daubenmire 1968; Cheney et al. 1993).

Within this context, the Cerrado is a biodiversity hotspot (Myers et al. 2000), which harbors more than 12,000 plant species (BFG 2015), and is comprised of a mosaic of fire-prone vegetation, from grasslands to open and closed savannas and forest (Oliveira-Filho and Ratter 2002). The open savannas of the Cerrado, referred to as *campo sujo*, consist of a continuous herbaceous layer with scattered shrubs and trees, frequently subjected to surface fires [at each 3-4 year of interval (Coutinho 1982; Miranda et al. 2009)]. Although fire temperatures can reach from <100 to >600°C near the soil surface (Coutinho 1978; Miranda et al. 1993; Schmidt et al. 2017; Daibes et al. 2017), there is limited understanding of its fine-scale variation across the soil and its direct consequences to seed survival in the Cerrado.

Therefore, we aimed to disentangle the importance of gaps on influencing fire temperatures and residence time, as well as the survival of exposed seeds (at ground surface) in the *campo sujo*. We hypothesized that the absence of fuel load in gaps could result in locally milder fires that would lead to greater seed survival. Specifically, we focused on seed viability of two legume shrub species that are common in Central Brazil, *Mimosa leiocephala* Benth. and *Harpalyce brasiliana* Benth. We performed two experimental-fire field experiments, which tested: (1) how the gap size (% of bare soil) influences fire heating and seed survival on a fine-scale; and (2) how seed survival is affected by two different fixed conditions during fire: gap *versus* grass tussock.

Materials and Methods

Study area and seed collection

Field experiments were carried out at the *Reserva Natural da Serra do Tombador* (RNST), located in Central Brazil (47°45'W, 13°35'S; 8900 ha; 560-1118 m a.s.l. – Fig. 1a). The climate at RNST has a marked dry season from May to September (total precipitation <60 mm) and a rainy season from October to April, in which most of the annual precipitation is concentrated (~1500 mm/year). Annual mean temperatures of RNST are between 15 and 36°C, and the soils are mostly nutrient-poor, red-yellow latosols (Fundação Grupo Boticário 2011).

The vegetation of RNST comprises various Cerrado formations, but predominantly *campo sujo*, an open savanna dominated by a rich layer of herbaceous grassy species, with scattered shrubs and dwarf trees (Coutinho 1982; Furley 1999). The region has a history of anthropogenic fires related to cattle ranching, and a mean fire return interval of three years, which also includes natural (lightning) fires (Daldegan et al. 2014). Most anthropogenic fires in Cerrado occur in the dry season, while natural fires may occur during the wet or seasonally transitional months (Ramos-Neto and Pivello 2000). Therefore, we performed the experiments in the interface between dry and wet seasons [i.e. the late-dry (early-wet) period (October)], when a higher amount of dead fuel load is found (Rissi et al. 2017).

We used seeds of two common legume shrubs at RNST for testing our hypotheses: *Mimosa leiocephala* Benth. (Mimosoideae) and *Harpalyce brasiliana* Benth. (Papilionoideae). Both species are small-seeded (<0.1 g, H.L. Zirondi et al. *unpubl. data*), have water-impermeable seed coats (physical dormancy) and are dispersed throughout the dry season. Therefore, most seeds would have been shed and could be exposed during fires.

Seeds were collected from at least 10 individuals per species in 2013 (late-dry season) for *M. leiocephala* and 2014 (mid-dry season) for *H. brasiliana.* After collection, seeds were screened in the laboratory for removing the predated ones and then stored in plastic tubes and kept refrigerated (5°C) until use in experiments (October 2014). Seed viability prior beginning of the experiments was determined by setting to germinate 100 scarified seeds of each species under room temperature, showing 85% of viability for *M. leiocephala* and 62% for *H. brasiliana*.

Experiment 1: gap size, fire heating and seed survival

Four 12-m long transects were established within four 15 x 20 m experimental plots (one transect per plot – Fig. 1b) for characterization of fine-scale bare soil patchiness and evaluation of fire heating under field conditions. The plots were randomly placed and belonged to fire experiments performed in a *campo sujo* area at RNST (Gorgone-Barbosa et al. 2015), which includes different fire frequencies (annual and biennial). Subplots (0.5 x 0.5 m) were set at each meter of each transect (12 subplots per transect x 4 plots = 48 total subplots). The percentage of bare soil (gaps with no vegetation or litter) was visually estimated in each subplot by using a modification of the Braun-Blanquet cover-abundance scale (see Wikum and Shanholtzer 1978), with a finer subdivision of cover classes (5% intervals; i.e., 0, 5, 10, 15, 20, 30%, and so on). Cover estimates were always made by the same person to eliminate observer bias. This method has been recognized as a robust sampling procedure, and is comparable to other sampling methods (Damgaard 2014).

Seeds from both species were mixed in the same soil-filled aluminum dishes (7 cm diameter x 0.5 cm deep, 10 seeds/species/dish), which were placed on the soil surface, each in the center of a subplot. The dishes were filled with local soil, and covered with a malleable wire mesh to avoid seed loss. Seeds were placed carefully in the soil-filled dishes (0.5 cm deep), being thus partially buried, yet had at least a part of their seed coat exposed directly to fire. After fire, seeds were put in paper bags and plastic packs and kept in a thermal bag to avoid large temperature fluctuations during transportation to the laboratory, where post-fire procedures were performed to assess seed survival (see below).

In order to record fire temperature and residence times we used thermocouple (type K) sensors coupled to data loggers (Novus ® LogBox). The sensors were placed next to, but not in contact with the aluminum dishes in each transect subplot (1 sensor per subplot, total of 12 sensors/transect) and made measurements every two seconds at the soil surface. We calculated fire residence time (seconds) ≥60ºC, typically assessed as the lethal threshold temperature for plant tissues (Daniell et al. 1969). In addition, we calculated residence times of fire temperatures ≥100ºC, which is a temperature threshold tolerated by most legume seeds in other fire-prone ecosystems (Auld and O’Connell 1991; Herranz et al. 1998). Experimental plots were burned separately during the same week in October 2014. Fires were set in the morning and followed the direction of the wind (head fires, Whelan 1995).

Experiment 2: fire effects on seed survival in gap versus tussock conditions

To evaluate whether gaps would act as ‘safe sites’ for exposed seeds during fire passage, we applied two fire treatments (see Fig. 1b) with seeds placed in soil-filled aluminum dishes (0.5 cm deep) at ground level: (1) Gap = fire in a gap (100% bare soil) with ≥40 cm diameter; and (2)

Tussock = fire under the tussocks of native grasses, which are the main fuel load for fires in the Cerrado *campo sujo* (Kauffman et al., 1994). We used seeds of the same species, which were burned under field conditions and taken to the laboratory for post-fire viability assessment (see below).

Fires were applied in the same season as Experiment 1 (October 2014, late-dry season), in four 15 x 20 m plots, following two years of fire exclusion. We set three aluminum dishes per treatment (gap or tussock) in each plot (10 seeds x three dishes x four plots = 120 seeds/treatment). Plots were burned independently with head fires. Environmental parameters were monitored and fire temperatures recorded as for Experiment 1.

Post-fire evaluation of seed survival

In the laboratory, seeds of both species were carefully removed from the aluminum dishes, separated from one another and cleaned from the soil sand particles. They were then set in 60 mm diameter Petri dishes layered with a double sheet of moistened filter paper, and maintained for four weeks in germination chambers (27°C and 12h light, see Fichino et al. 2016). Observations were made three times per week and seeds that germinated (as indicated by radicle protrusion (Bewley et al. 2013) were scored as viable. By the end of the fourth week of the germination test, non-germinating seeds that were visibly damaged by fire were scored as dead. Intact, but non-germinating, seeds were individually scarified with sandpaper to overcome physical dormancy and then incubated in the same conditions for an additional week. Germinated seeds were then scored as viable, while the remaining seeds were tested for viability using a tetrazolium test (Lakon 1949). Seeds were cut and set in a tetrazolium buffer solution (1%, pH 7). Seeds with embryos stained red were scored as viable, whereas those with flaccid tissues or did not stain were scored as dead. Final viability was calculated as the percentage of germinated + stained seeds.

Data analyses

To evaluate fire heating (temperature and residence times) in relation to bare soil patches, we first fitted linear mixed effects models, considering the subplots nested within the plots as random factors in the analysis. Since these random factors had no effect, we performed simple linear models, aiming to relate bare soil cover (%) with maximum fire temperature (°C), residence time ≥60°C, and residence time ≥100°C.

We evaluated seed viability in each subplot as a function of the gap size (% of bare soil in each subplot; see Experiment 1). We then performed generalized linear mixed models (GLMMs) with a binomial distribution, considering binary data where a seed could be viable or not at the end of the trials (see Santana et al. 2013). The aluminum dishes containing the seed samples at each subplot, nested within their respective plots, were considered as random effects in the models. A *predict* function was used to estimate the probability curve of seeds being viable across the different gap sizes.

In the Experiment 2, we also fitted GLMMs with a binomial distribution (Santana et al. 2013) to evaluate seed survival. Because the aluminum dishes were nested within the plots (three dishes x four plots = 12 samples/species/treatment), they were considered the random effects in the analysis. The treatments (control, gap and tussock) were treated as fixed effects and the control was the baseline for the intercept in the models. Models were always fitted separately for *M. leiocephala* and *H. brasiliana* seeds. All analyses were performed using the packages lme4 (Bates et al. 2015) and stats in R environment 3.2.5 (R Core Team 2016), with significance level of $\alpha \leq 0.05$.

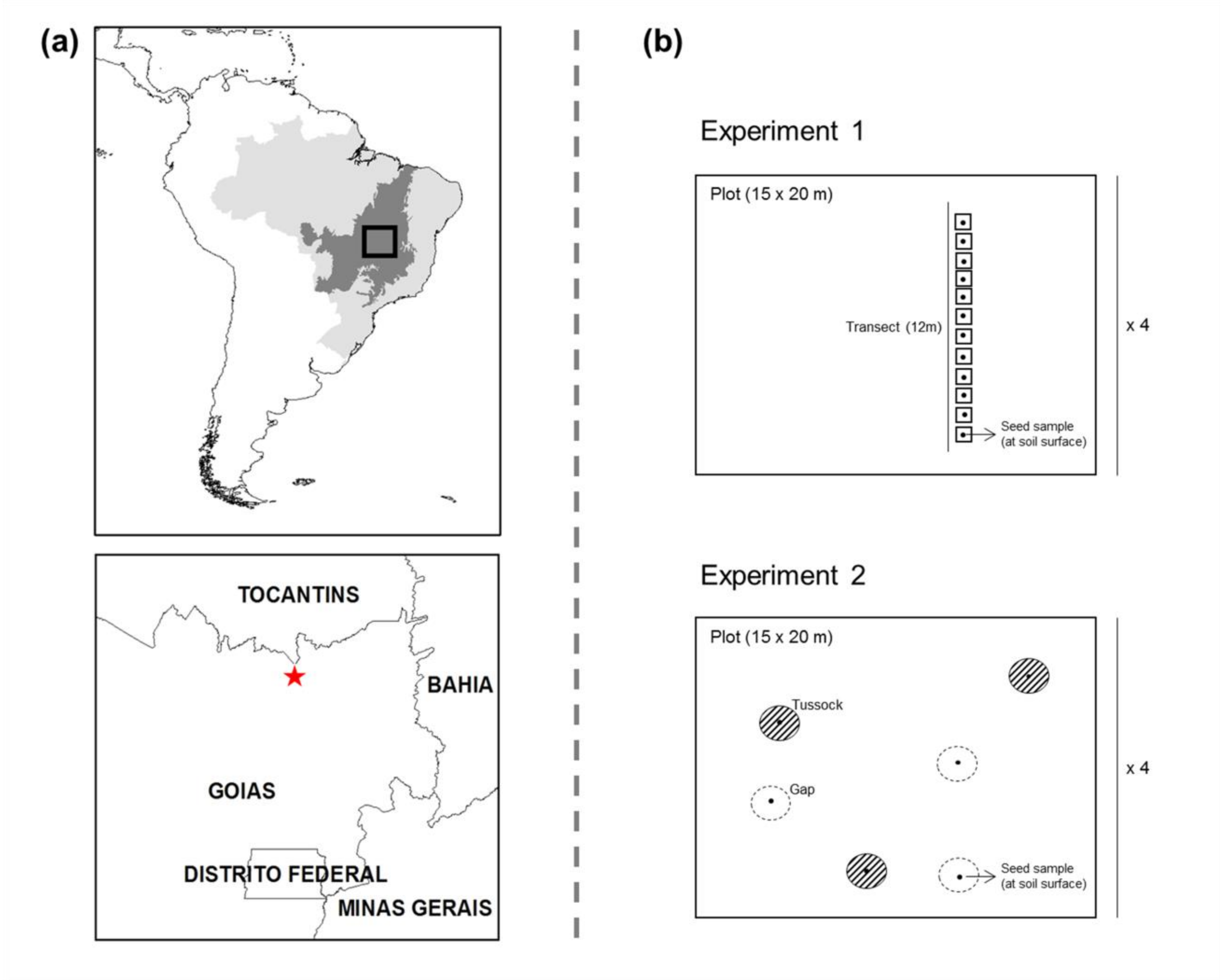


Fig. 1. (a) Location of the *Reserva Natural Serra do Tombador* in Central Brazil, Northern to the Goiás State. **(b)** Experimental design of the fire *vs* seed survival experiments conducted in a Cerrado open savanna in Central Brazil.

Results

Experiment 1: gap size, fire heating and seed survival

Fire temperature curves varied widely within the experimental plots (Fig. S1). Maximum temperatures reached by fire varied between 75 and 576°C. Residence time of fire was also had an eight-fold variation, lasting from 116 to 894 s ≥60°C, and from 70 to 282 s ≥100°C. Despite variations in fire temperatures and residence time, the peak of maximum temperature mostly occurred at around 50 s in the curves (see Fig. S1).

Fire heat was highly dependent on the percentage of bare soil cover. Patches with higher percentages of bare soil had both lower maximum temperatures (P <0.001; Fig. 2a) and lower residence times at temperatures ≥60°C (P = 0.03; Fig. 2b) and ≥100°C (P <0.001; Fig. 2c). Seed viability was positively influenced by gap size (Fig. 3; Table 1). All seeds died in gaps with <40% of bare soil during the fire passage. Seed survival only occurred in gaps with >40% of bare soil, where the probability of seeds being viable was significantly higher for both *M. leiocephala* (P = 0.002) and *H. brasiliana* (P = 0.01; see Table 1).

Experiment 2: fire effects on seed survival in gap versus tussock conditions

Maximum fire temperatures under tussocks (425±92°C) were almost two-times higher than in gaps (219±58°C; Table 2). Residence time at temperatures ≥60°C lasted around 250 seconds for both gap and tussock treatments, while at temperatures ≥100°C residence time lasted less than 180 s for both conditions (Table 2).

Seed viability in the control treatment was 85±10% for *M. leiocephala* and 63±14% for *H. brasiliana.* Fire killed all seeds placed in tussocks (Fig. 4), while a proportion of seeds survived fires in gaps, but with reduced viability in relation to the controls (P = 0.002 for *M. leiocephala* and P = 0.03 for *H. brasiliana*). Seed survival in gaps varied widely for both species: 36±30% for *M.*

leiocephala and 24±36% for *H. brasiliana* (Fig. 4). If adjusted in relation to their initial viability (control), these values represent 42 and 37%, respectively, of survival in the gap treatment.

Table 1. Parameters estimated by the GLMM models for the survival of exposed seeds of two legume shrub species, in relation to gap size (% of bare soil) during experimental fires (Experiment 1) in a Cerrado open savanna in Central Brazil. *P*-values ≤0.05 highlighted in bold.

Species	Fixed effects	Estimate	s.e.	*P*-value
Mimosa leiocephala	Intercept	-12.572	3.365	
	Gap size	0.204	0.066	**0.002**
Harpalyce brasiliana	Intercept	-10.066	2.736	
	Gap size	0.127	0.051	**0.013**

Table 2. Maximum temperature and residence times of experimental fires at soil level in different treatments: gap (bare soil ≥40 cm diameter) and tussock (under tussocks of native grasses) during experimental fires (Experiment 2) in a Cerrado open savanna in Central Brazil.

Fire treatment	Maximum temperature (°C, mean ± SD)	Residence time (s, mean ± SD)	
		≥60°C	≥100°C
Gap	219 ± 58	254 ± 134	162 ± 179
Tussock	425 ± 92	249 ± 94	139 ± 33

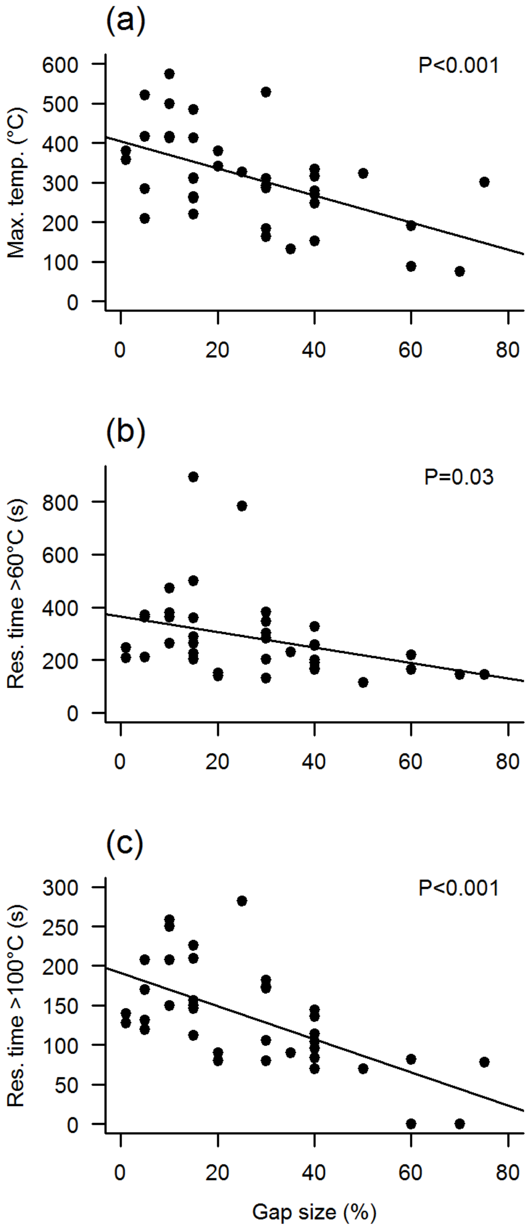


Fig. 2. Relationship of **(a)** maximum temperature (°C), **(b)** residence time ≥60°C (seconds), and **(c)** residence time ≥100°C (s) in relation to bare soil cover (%) in a Cerrado open savanna during experimental fires (Experiment 1) in Central Brazil.

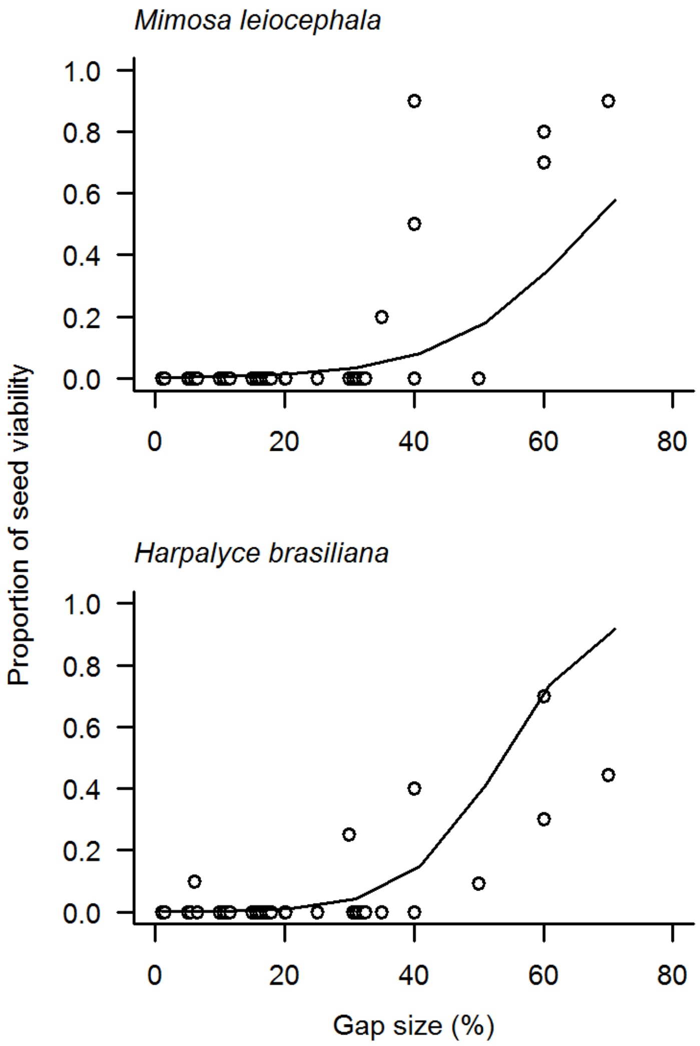


Fig. 3. Proportion of seed viability in the seed samples in relation to the gap size (% of bare soil) of two legume shrub species from a Cerrado open savanna in Central Brazil subjected to experimental fires (Experiment 1). Lines show the probability curve of seeds being viable (seed survival) based in a binomial distribution. For statistical parameters, see Table 1.

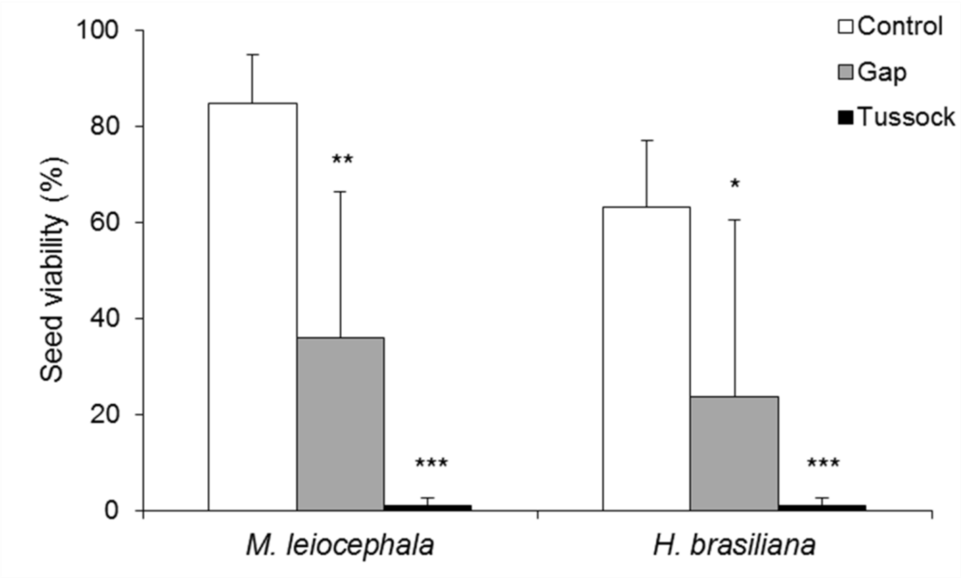


Fig. 4. Seed viability percentage (mean ± SD) of two legume shrub species from a Cerrado open savanna in Central Brazil subjected to experimental fires (Experiment 2). Control = untreated seeds; Gap = fire at gaps of bare soil (≥40 cm diam.); and Tussock = fire under tussocks of native grasses. Asterisks mean significant differences in relation to Control: * = *P* ≤0.05 and ≥0.01, ** = *P* ≤0.01 and ≥0.001, *** = *P* ≤0.001.

Discussion

Our study shows how gaps influence both fire temperatures and residence time, untangling the linkage between fire and the survival of exposed seeds in a grassy ecosystem. This finding allowed us to better understand the direct consequences of fire for seed regeneration. Unburned patches were previously considered to drive seed survival of *Miconia albicans* (Melastomataceae) during Cerrado fires (Dodonov et al. 2014), but such study has not recorded fire temperatures and residence time. Therefore, here we provide informative, former unconsidered data on fine-scale measurements of fire temperatures and residence time for tropical savannas. These field measurements of fire parameters might help us to better understand Cerrado fires and facilitate laboratory studies to simulate fire effects on seeds.

Gap size was negatively related to both fire temperatures and residence time, supporting our hypothesis that larger gap sizes would result in milder fire temperatures. Therefore, gaps are an important source of variation driving fire parameters and seed survival. We recorded an eight-fold variation in the fire parameters of temperature and residence time in the same area and season. These findings are similar to values previously recorded for the Cerrado (Miranda et al. 1993; Schmidt et al. 2017; Daibes et al. 2017), but never recorded at the fine-scale. Hence, variation of fuel bed might create discontinuities in the soil cover (Hobbs and Atkins 1988), thereby driving seed survival during fire passage (Harper et al. 1965; Wiggers et al. 2013).

Most seeds in the upper layer of the soil certainly die during fires (Auld 1986; Carrington 2010; Dayamba et al. 2010; Daibes et al. 2017), but nearly 40% of the initially viable (control) seeds survived for both *M. leiocephala* and *H. brasiliana* under gap temperatures (mean T max. = 219 ± 58°C). Therefore, we argue that if seeds are able to survive temperatures ≥200°C, they could survive fire under exposed conditions in the Cerrado. However, residence time never exceeded five minutes at temperatures ≥100°C, and the peak of temperature was usually reached at less than one minute. Thus, some laboratory studies simulating fire temperatures for more than these thresholds of time might be overestimating the duration of Cerrado fires (Ribeiro et al. 2013). Moreover, most studies at the plot level might generalize results to whole species (the species has tolerant seeds or not) or to a whole condition (fire in the soil surface kills all seeds). In a more individualistic level, we argue the fate (survival or death) of a given seed once dispersed in the soil surface to be determined by its chance to find a gap or be incorporated (buried) to the soil seed bank, or be dispersed in a year without fire.

Cerrado legume seeds survive fires under buried conditions (reaching from 60 to 100°C 1 cm underground), although showing little to no dormancy-breaking (Daibes et al. 2017). Hence, fire does not deplete seed bank recruitment in the *campo sujo* (Andrade and Miranda 2014). However, many seeds might be dispersing seeds during the transitional months between dry and wet season (Salazar et al. 2011), which coincides with the natural fire season in the Cerrado (Ramos-Neto and Pivello 2000). Therefore, those seeds could be found exposed on the soil surface during fires, and their capacity to survive temperatures between 100 and 200°C is thus crucial to drive their persistence.

Seeds of some Cerrado plants have been considered tolerant to high temperatures due to some specific traits, such as seed mass and the presence of dormancy (Ribeiro et al. 2015; Ramos et al. 2016; L.F. Daibes et al. *unpubl. data*). On the other hand, most legume species from Australia and the Mediterranean Basin can be recruited following physical dormancy-breaking at temperatures between 80 and 100°C (Moreira and Pausas 2012; Ooi et al. 2014), but die under 120-150°C (Auld and O’Connell 1991; Herranz et al. 1998; Williams et al. 2003). Therefore, seed survival (Le Stradic et al. 2015; Fichino et al. 2016) modulated by vegetation gaps, rather than germination promotion is the main response of Cerrado species to fire.

Conversely, referring to open sites (gaps) as ‘safe’ might be quite paradoxical given they could also expose seeds to critical conditions of temperature and water loss. Traditionally, the term ‘safe site’ has been described as moister microsites (litter patches, for instance) favorable for seed germination (Fowler 1988; Lamont et al. 1993). However, litter patches might be advantageous for seed germination only if they remain as unburned patches, otherwise they will burn and kill exposed seeds (Carrington 2010; Wiggers et al. 2013). Thus, our results suggest that for seed survival during the passage of fire, the ‘safe site’ could be a bare soil microsite (gap) that breaks the fuel bed and decreases fire temperature. Additionally, open sites would be better for seedling establishment, avoiding competition, even though they would have to show some strategies to avoid water loss (Lamont et al. 1993).

We argue that fire-free gaps are an important dimension of seed regeneration niches in fire-prone ecosystems (Keeley et al. 2016), just as light gaps are important for forest regeneration (Grubb 1977; Whitmore 1989). Both our study species are small-seeded and have physically dormant seeds, which are dispersed during the dry season, thus possessing broadly overlapping seed regeneration niches (see Grubb 1977), including the probability to survive in the face of fires. Our results indicate that seeds would die in face of fires when placed under vegetated sites, and gaps are here recognized as safe sites for seed survival, allowing persistence of at least a proportion of their population in frequently burned open savannas. Further studies are warranted to understand these patterns from a community perspective (e.g. Paula and Pausas 2008; Jaureguiberry and Díaz 2015). Exploring these patterns will be important for disentangling how small differences in seed survival could favor regeneration of a given species over another in the Cerrado.

Concluding Remarks

Gap size was crucial for structuring fine-scale variability in fire temperatures and residence time, both of which are fundamental parameters for the creation of safe sites where exposed seeds experienced a higher probability of surviving fire passage. The effects of fire on regeneration and community assembly still need to be better addressed in grassy biomes, given that global fire regimes are changing due to warmer and/or dryer seasons (see Westerling et al. 2006; Krawchuk et al. 2009; Flannigan et al. 2009; Liu et al. 2010). Further studies should examine whether this fine-scale variation accounts for seedling establishment and post-fire changes at the community-level, which would help to increase our capacity for predicting the assemblage of natural communities in tropical savannas.

Acknowledgements

We thank the RNST fire team, A. F. Terra, G. A. Berberino, R. B. de Almeida, and S. A. Borges, for assistance. We also thank M. B. Cunha for support during field excursions, and T. Zupo and M. N. Rissi for collaborating in all stages of this work. To P. Maldonado, L. E. Commander and, especially, the blind reviewers, we are grateful for comments on preliminary versions of the manuscript. We also thank V. R. Pivello and S. T. Meirelles for their insights regarding Cerrado fine-scale variability. The first author received a scholarship from CAPES. This study was supported by FAPESP (2015/06743-0), CNPq (476334/2011-0; 455183/2014-7), and Fundação Grupo Boticário (0153_2011_PR; 0106_2011_PR). F. A. O. Silveira (482720) and A. Fidelis (306170/2015-9) receive scholarships from CNPq.

References

Andrade LAZ, Miranda HS (2014) The dynamics of the soil seed bank after a fire event in a woody savanna in central Brazil. *Plant Ecology* **215,** 1199-1209. doi: 10.1007/s11258-014-0378-z

Archibald S, Lehmann CER, Gómez-Dans JL, Bradstock RA (2013) Defining pyromes and global syndromes of fire regimes. *Proceedings of the National Academy of Sciences* **10**, 6442-6447. doi: 10.1073/pnas.1211466110

Auld TD (1986) Population dynamics of the shrub *Acacia suaveolens* (Sm.) Willd.: fire and the transition to seedlings. *Australian Journal of Ecology* **11**, 373-385. doi: 10.1111/j.1442-9993.1986.tb01407.x

Auld TD, O'Connell MA (1991) Predicting patterns of post-fire germination in 35 eastern Australian Fabaceae. *Australian Journal of Ecology* **16**, 53-70. doi: 10.1111/j.1442-9993.1991.tb01481.x

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**, 1-48. doi: 10.18637/jss.v067.i01

Bewley JD, Bradford KJ, Hilhorst HWM, Nonogaki H (2013) ‘Seeds: Physiology of development, germination and dormancy’ 3rd edn. (Springer Science, New York, USA). doi: 10.1007/978-1-4614-4693-4_1

BFG The Brazilian Flora Group (2015) Growing knowledge: an overview of seed plant diversity in Brazil. *Rodriguésia* **66**, 1085-1113. doi: 10.1590/2175-7860201566411

Bradstock RA, Auld TD (1995) Soil temperatures during experimental bushfires in relation to fire intensity: consequences for legume germination and fire management in South-Eastern Australia. *Journal of Applied Ecology* **32**, 76-84. doi: 10.2307/2404417

Carrington ME (2010). Effects of soil temperature during fire on seed survival in Florida sand pine scrub. *International Journal of Forestry Research* article ID 402346. doi: 10.1155/2010/402346

Cheney NP, Gould JS, Catchpole WR (1993) The influence of fuel, weather and fire shape variables on fire-spread in grasslands. *International Journal of Wildland Fire* **3**, 31-44. doi: 10.1071/WF9930031

Coutinho LM (1978) Aspectos ecológicos do fogo no cerrado. I – A temperatura do solo durante as queimadas. *Revista Brasileira de Botânica* **1**, 93-96.

Coutinho LM (1982) Ecological effects of fire in Brazilian Cerrado. In ‘Ecology of Tropical Savannas’. (Eds BJ Huntley, BH Walker) pp. 273-291 (Springer-Verlag, Berlin-Heidelberg). doi: 10.1007/978-3-642-68786-0_13

Daibes LF, Zupo TZ, Silveira FAO, Fidelis A (2017) A field perspective on effects of fire and temperature fluctuation on Cerrado legume seeds. *Seed Science Research* **27**, 74-83. doi: 10.1017/S096025851700006X

Daldegan GA, Carvalho Júnior OA, Guimarães RF, Gomes RAT, Ribeiro FF, Mcmanus C (2014) Spatial patterns of fire recurrence using remote sensing and GIS in the Brazilian savanna: Serra do Tombador Nature Reserve, Brazil. *Remote Sensing* **6**, 9873-9894. doi: 10.3390/rs6109873

Damgaard C (2014) Estimating mean plant cover from different types of cover data: a coherent statistical framework. *Ecosphere* **5**. doi: 10.1890/ES13-00300.1

Daniell JW, Chappell WE, Couch HB (1969) Effect of sublethal and lethal temperatures on plant cells. *Plant Physiology* **44**, 1684-1689. doi: 10.1104/pp.44.12.1684

Dayamba SD, Savadogo P, Zida D, Sawadogo L, Tiveau D, Oden PC (2010) Fire temperature and residence time during dry season burning in a Sudanian savanna-woodland of West Africa with implication for seed germination. *Journal of Forestry Research* **21**, 445-450. doi: 10.1007/s11676-010-0095-y

Daubenmire R (1968). Ecology of fire in grasslands. *Advances in Ecological Research* **5**, 209-266. doi: 10.1016/S0065-2504(08)60226-3

Dodonov P, Xavier RO, Tiberio FCS, Lucena IC, Zanelli CB, Matos DMS (2014) Driving factors of small-scale variability in a savanna plant population after a fire. *Acta Oecologica* **56**, 47-55. doi: 10.1016/j.actao.2014.02.003

Fichino BS, Dombroski JRG, Pivello VR, Fidelis A (2016) Does fire trigger seed germination in the Neotropical Savannas? Experimental tests with six Cerrado species. *Biotropica* **48**, 181-187. doi: 10.1111/btp.12276

Flannigan MD, Krawchuk MA, Groot WJ, Wotton BM, Gowman LM (2009) Implications of changing climate for global wildland fire. *International Journal of Wildland Fire* **18**, 483-507. doi: 10.1071/WF08187

Fowler NL (1988) What is a safe site?: Neighbor, litter, germination date, and patch effects. *Ecology* **69**, 947-961.

Fundação Grupo Boticário (2011) 'Plano de Manejo da Reserva Natural Serra do Tombador'. (Supervisor G. A. Gatti) Available at http://www.fundacaogrupoboticario.org.br [verified 28 April 2017]

Furley PA (1999) The nature and diversity of neotropical savanna vegetation with particular reference to the Brazilian cerrados. *Global Ecology and Biogeography* **8**, 223-241. doi: 10.1046/j.1466-822X.1999.00142.x

Gorgone-Barbosa E, Pivello VR, Bautista S, Zupo T, Rissi MN, Fidelis A (2015) How can an invasive grass affect fire behavior in a tropical savanna? A community and individual plant level approach. *Biological Invasions* **17**, 423-431. doi: 10.1007/s10530-014-0740-z

Grubb PJ (1977) The maintenance of species-richness in plant communities: the importance of the regeneration niche. *Biological Reviews* **52**, 107-145. doi: 10.1111/j.1469-185X.1977.tb01347.x

Harper JL, Williams JT, Sagar GR (1965) The behaviour of seeds in soil: I. The heterogeneity of soil surfaces and its role in determining the establishment of plants from seed. *Journal of Ecology* **53**, 273-286. doi: 10.2307/2257975

Herranz JM, Ferrandis P, Martínez Sánchez JJ (1998) Influence of heat on seed germination of seven Mediterranean Leguminosae species. *Plant Ecology* **136**, 95-103. doi: 10.1023/A:1009702318641

Hiers JK, O'Brien JJ, Mitchell RJ, Grego JM, Loudermilk EL (2009) The wildland fuel cell concept: an approach to characterize fine-scale variation in fuels and fire in frequently burned longleaf pine forests. *International Journal of Wildland Fire* **18**, 315-325. doi: 1049-8001/09/030315

Hobbs RJ, Atkins L (1988) Spatial variability of experimental fires in south-west Western Australia. *Australian Journal of Ecology* **13**, 295-299. doi: 10.1111/j.1442-9993.1988.tb00977.x

Jaureguiberry P, Díaz S (2015) Post-burning regeneration of the Chaco seasonally dry forest: germination response of dominant species to experimental heat shock. *Oecologia* **177**, 689-699. doi: 10.1007/s00442-014-3161-x

Kauffman JB, Cummings DL, Ward DE (1994) Relationships of fire, biomass and nutrient dynamics along a vegetation gradient in the Brazilian Cerrado. *Journal of Ecology* **82**, 519-531. doi: 10.2307/2261261

Keeley JE, Parker VT, Vasey MC (2016) Resprouting and seeding hypotheses: a test of the gap-dependent model using resprouting and obligate seeding subspecies of *Arctostaphylos*. *Plant Ecology* **217**, 743-750. doi: 10.1007/s11258-015-0551-z

Krawchuk MA, Moritz MA, Parisien MA, van Dorn J, Hayhoe K (2009) Global Pyrogeography: the current and future distribution of wildfire. *PLoS ONE* **4**. doi: 10.1371/journal.pone.0005102

Lakon G (1949) The topographical tetrazolium method for determining the germination capacity of seeds. *Plant Physiology* **24**, 389-394. doi: 10.1104/pp.24.3.389

Lamont BB, Witkowski ETF, Enright NJ (1993) Post-fire litter microsites: safe for seeds, unsafe for seedlings. *Ecology* **74**, 501-512. doi: 10.2307/1939311

Lehmann CER, Anderson TM, Sankaran M, Higgins SI, Archibald S, Hoffmann WA, Hanan NP, Williams RJ, Fensham RJ, Felfili J, Hutley LB, Ratnam J, San Jose J, Montes R, Franklin D, Russell-Smith J, Ryan CM, Durigan G, Hiernaux P, Haidar R, Bowman DMJS, Bond WJ

(2014) Savanna vegetation-fire-climate relationships differ among continents. *Science* **343**, 548-552. doi: 10.1126/science.1247355

Le Stradic S, Silveira FAO, Buisson E, Cazelles K, Carvalho V, Fernandes GW (2015) Diversity of germination strategies and seed dormancy in herbaceous species of *campo rupestre* grasslands. *Austral Ecology* **40**, 537-546. doi: 10.1111/aec.12221

Liu Y, Stanturf J, Goodrick S (2010) Trends in global wildfire potential in a changing climate. *Forest Ecology and Management* **259**, 685-697. doi: 10.1016/j.foreco.2009.09.002

Loudermilk EL, O'Brien JJ, Mitchell RJ, Cropper Jr WP, Hiers JK, Grunwald S, Grego J, Fernandez-Diaz JC (2012) Linking complex forest fuel structure and fire behaviour at fine scales. *International Journal of Wildland Fire* **21**, 882-893. doi: 10.1071/WF10116

Miranda AC, Miranda HS, Dias IFO, Dias BFS (1993) Soil and air temperatures during prescribed cerrado fires in Central Brazil. *Journal of Tropical Ecology* **9**, 313-320. doi: 10.1017/S0266467400007367

Miranda HS, Sato MN, Neto WN, Aires FS (2009) Fires in the cerrado, the Brazilian savanna. In 'Tropical Fire Ecology: Climate change, land use, and ecosystem dynamics'. (Ed MA Cochrane) pp. 427-450 (Springer-Verlag, Berlin-Heidelberg). doi: 10.1007/978-3-540-77381-8_15

Moreira B, Pausas JG (2012) Tanned or burned: The role of fire in shaping physical seed dormancy. *PLoS ONE* **7**. doi:10.1371/ journal.pone.0051523

Myers N, Mittermeier RA, Mittermeier CG, Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature* **403**, 853-858. doi: 10.1038/35002501

O'Brien JJ, Loudermilk EL, Hornsby B, Hudak AT, Bright BC, Dickinson MB, Hiers JK, Teske C, Ottmar RD (2016 a) High-resolution infrared thermography for capturing wildland fire behaviour – RxCADRE 2012. *International Journal of Wildland Fire* **25**, 62-75. doi: 10.1071/WF14165

O'Brien JJ, Loudermilk EL, Hiers JK, Pokswinski S, Hornsby B, Hudak A, Strother D, Rowell E, Bright BC (2016 b) Canopy- derived fuels drive patterns of in-fire energy release and understory plant mortality in a longleaf pine (*Pinus palustris*) sandhill in northwest Florida, USA. *Canadian Journal of Remote Sensing* **42**, 489-500. doi: 10.1080/07038992.2016.1199271

Odion DC, Davis FW (2000) Fire, soil heating and the formation of vegetation patterns in Chaparral. *Ecological Monographs* **70**, 149-169. doi: 10.2307/2657171

Oliveira-Filho AT, Ratter JA (2002) Vegetation physiognomies and woody flora of the Cerrado biome. In 'The cerrados of Brazil: ecology and natural history of a neotropical savanna'. (Eds PS Oliveira PS, RJ Marquis) pp. 91-120 (Columbia University Press, New York, USA). doi: 10.7312/oliv12042.9

Ojeda F, Pausas JG, Verdú M (2010) Soil shapes community structure through fire. *Oecologia* **163**, 729-735. doi: 10.1007/s00442-009-1550-3

Ooi MKJ, Denham AJ, Santana VM, Auld TD (2014) Temperature thresholds of physically dormant seeds and plant functional response to fire: variation among species and relative impact of climate change. *Ecology and Evolution* **4**, 656-671. doi: 10.1002/ece3.973

Parr CL, Lehmann CER, Bond WJ, Hoffmann WA, Andersen AN (2014) Tropical grassy biomes: misunderstood, neglected, and under threat. *Trends in Ecology and Evolution* **29**, 205-213. doi: 10.1016/j.tree.2014.02.004

Paula S, Pausas JG (2008) Burning seeds: germinative response to heat treatments in relation to resprouting ability. *Journal of Ecology* **96**, 543-552. doi: 10.1111/j.1365-2745.2008.01359.x

Ramos DM, Liaffa ABS, Diniz P, Munhoz CBR, Ooi MKJ, Borghetti F, Valls JFM (2017) Seed tolerance to heating is better predicted by seed dormancy than by habitat type in Neotropical savanna grasses. *International Journal of Wildland Fire* **25**, 1273-1280. doi: 10.1071/WF16085

Ramos-Neto MB, Pivello VR (2000) Lightning fires in a Brazilian savanna national park: Rethinking management strategies. Environmental Management **26**, 675-684. doi: 10.1007/s002670010124

R Core Team (2016) ‘R: A language and environment for statistical computing’. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Ribeiro LC, Barbosa ERM, van Langevelde F, Borghetti F (2015) The importance of seed mass for the tolerance to heat shocks of savanna and forest tree species. *Journal of Vegetation Science* **26**, 1102-1111. doi: 10.1111/jvs.12314

Ribeiro LC, Pedrosa M, Borghetti F (2013) Heat shock effects on seed germination of five Brazilian savanna species. *Plant Biology* 15, 152-157. doi: 10.1111/j.1438-8677.2012.00604.x

Rissi MN, Baeza MJ, Gorgone-Barbosa E, Zupo T, Fidelis A (2017) Does season affect fire behaviour in the Cerrado? *International Journal of Wildland Fire* **26,** 427-433. doi: 10.1071/WF14210

Salazar A, Goldstein G, Franco AC, Miralles-Wilhelm F (2011) Timing of seed dispersal and dormancy, rather than persistent soil seed-bank, control seedling recruitment of woody plants in Neotropical savannas. *Seed Science Research* **21**, 103-116. doi: 10.1017/S0960258510000413

Santana VM, Baeza MJ, Blanes MC (2013) Clarifying the role of fire heat and daily temperature fluctuations as germination cues for Mediterranean Basin obligate seeders. *Annals of Botany* **111**, 127-134. doi: 10.1093/aob/mcs238

Schmidt IB, Fidelis A, Miranda HS, Ticktin T (2017) How do the wets burn? Fire behavior and intensity in wet grasslands in the Brazilian savanna. *Brazilian Journal of Botany* **40**, 167-175. doi: 10.1007/s40415-016-0330-7

Westerling AL, Hidalgo HG, Cayan DR, Swetnam TW (2006) Warming and earlier spring increase Western U.S. forest wildfire activity. *Science* **313**, 940-943. doi: 10.1126/science.1128834

Whelan RJ (1995) ‘The ecology of fire’. (Cambridge University Press, Cambridge, UK).

Whitmore TC (1989) Canopy gaps and the two major groups of forest trees. *Ecology* **70**, 536-538. http://www.jstor.org/stable/1940195

Wikum DA, Shanholtzer GF (1978) Application of the Braun-Blanquet cover-abundance scale for vegetation analysis in land development studies. *Environmental Management* **2**, 323-329. doi: 10.1007/BF01866672

Wiggers MS, Kirkman LK, Boyd RS, Hiers JK (2013) Fine-scale variation in surface fire environment and legume germination in the longleaf pine ecosystem. *Forest Ecology and Management* **310**, 54-63. doi: 10.1016/j.foreco.2013.07.030

Williams PR, Congdon RA, Grice AC, Clarke, PJ (2003) Fire-related cues break seed dormancy of six legumes of tropical eucalypt savannas in north-eastern Australia. *Austral Ecology* **28**, 507-514. doi: 10.1046/j.1442-9993.2003.01307.x

Supplementary Material

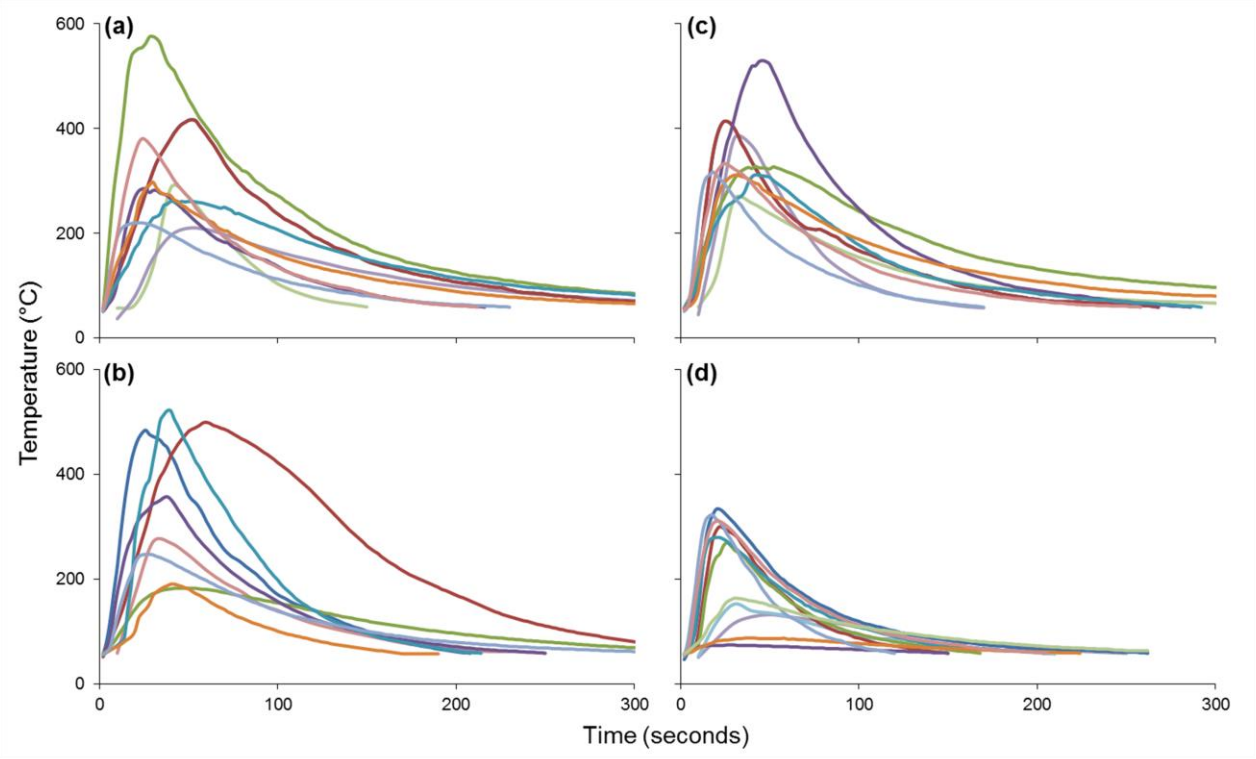


Fig. S1. Fire temperature and residence time in four 12-m long transects (a, b, c, and d) established at soil-level in a Cerrado open savanna in Central Brazil (Experiment 1). Each line shows the fire curve of temperature (°C) per time (s) for a sensor in a meter of transects. The constraint in number of curves per transect (*n* should be = 12 for each transect) is due to sensor failures in fire measurements at some subplots.

CAPÍTULO 3

Gap-simulated temperature fluctuation does not break physical dormancy in Cerrado savannas and forest legume seeds

A seed is an end and a beginning; it is the bearer of the essentials of inheritance; it symbolizes multiplication and dispersal, continuation and innovation, survival, renewal and birth.

Walter Heydecker

Gap-simulated temperature fluctuation does not break physical dormancy in Cerrado savannas and forest legume seeds

L. Felipe Daibes, Nathalia Bonani, Fernando A.O. Silveira, Alessandra Fidelis

Abstract

Seed germination seems to be enhanced, mostly in physically dormant seeds, by daily temperature fluctuation in vegetation gaps. However, such patterns remain controversial regarding Neotropical seeds, so we aimed to investigate the role of gap-simulated temperature fluctuation, under dry conditions, on seeds of 15 legume species from Cerrado savannas and forest. We *a priori* hypothesized that temperature fluctuation could enhance germination of physically dormant (PY) seeds while seed germination of intermediate (IN) and/or non-dormant (ND) species could be positively affected in the savannas, while depleted in forest-occurring species. We conducted the gap-simulated temperature fluctuation treatment in germination chambers for 90 days and kept controls under laboratory conditions for the same period. Differences among treatments were tested using GLMMs with a binomial distribution and replicates as random effects. Temperature fluctuation did not enhance germination in any of the species, irrespective of their vegetation type of occurrence. Instead, six species have shown a reduction on their germination percentage. Four out of six IN/ND species also delayed germination times (t50) after treatment. All species kept the same seed viability by the end of the tests, except for two forest species which showed a decrease. In conclusion, temperature fluctuation does not break PY, but rather delays seed germination in legumes from the Cerrado mosaic.

Keywords: Cerrado, gap, Leguminosae, regeneration, seed dormancy, tropical savannas

Introduction

Daily fluctuations in soil temperature expose seeds to a thermal regime which might affect germination and thus, influence seedling emergence (Thompson *et al.*, 1977; Vázquez-Yanes &

Orozco-Segovia, 1982; Fenner & Thompson, 2005). In fire-prone ecosystems, a relatively higher temperature fluctuation might be reached in the vegetation gaps, usually formed from the removal of aboveground vegetation in the post-fire environment (Auld & Bradstock, 1996; Fidelis *et al.*, 2012).

The increased amplitude of temperature fluctuation often results in breaking of physical dormancy (PY; seed coat impermeability) in species from Mediterranean and Australian ecosystems (Auld & Bradstock, 1996; Baeza & Roy, 2008; Santana *et al.*, 2010; 2013). A threshold of temperature seems to be required to disrupt the seed coat of PY seeds in fire-prone ecosystems, allowing their imbibition and thus germination to occur (Morrison *et al.*, 1998; Moreira & Pausas, 2012; Ooi *et al.*, 2014). Therefore, PY species can be classified according to their functional dormancy-breaking mechanism, as *obligate* or *facultative pyrogenic* (Ooi *et al.*, 2014).

Whether formed by fires or occurring in patches among vegetation, gaps serve as open niches and opportunities for recruitment of gap-colonizer species (Grubb, 1977, Ooi *et al.*, 2014). Detecting gaps would thus be important for the early establishment of seedlings, occupying vacant spaces and avoiding competition (Jones *et al.*, 1997; Verdú & Traveset, 2005; Castro, 2006; Ooi, 2010). Hence, not only dormancy-breaking, but also germination time would be crucial for a rapid establishing in the open environment (Fenner & Thompson, 2005).

Within this context, species from open savannas (fire-prone) should thus take advantage from gap-occurring temperatures in relation to species from forest (fire-free) environment, occurring under a closed canopy. In addition, higher amplitude of temperature was recorded in the soil surface in open savanna gaps compared to shaded conditions (see Daibes *et al.*, 2017). The Cerrado vegetation comprises a savanna-forest mosaic (Oliveira-Filho & Ratter, 2002) and there has been some discussion about the environmental filters that drive the establishment in one or another environment (Hoffmann, 1996; 2000; Salazar *et al.*, 2012; Ribeiro & Borghetti, 2014). Although the open savannas are frequently disturbed by fires (Coutinho, 1982), fire-related heat shocks might have little to no effect on PY-breaking in Cerrado species (20-30% of increasing, see Ribeiro *et al.*, 2013; Daibes *et al.*, 2017). Therefore, here we focused on the effects of gap-simulated temperature fluctuations.

Field experiments have shown that gap-occurring temperature fluctuation could break PY in some legume seeds from Cerrado open savannas (see Daibes *et al*., 2017), while permeable seeds could have their germination decreased. However, few species have been addressed and this subject remains controversial (Zupo *et al.*, 2016). Regarding forest species, PY does not seem to be broken under dry gap-occurring temperatures (Geisler *et al.*, 2017), which could be a limiting factor for their establishment in the savannas.

We studied species showing not only PY, but also intermediate (IN) and non-dormant (ND) seeds (Baskin & Baskin, 2014; Dayrell *et al.*, 2017) throughout the savanna-forest mosaic. Hence, we aimed to investigate the role of temperature fluctuation on 15 legume species typically occurring in Cerrado savanna-forest ecotones that differ in dormancy classes. We simulated temperature fluctuation as previously recorded under natural field conditions (Daibes *et al.*, 2017) to test whether this environmental cue could help explaining the mechanism underlying PY-breaking and seed germination. We *a priori* hypothesized that temperature fluctuation could break PY and positively affect seed germination of IN and ND species in the Cerrado savannas. Instead, forest-occurring species could have their germination (percentage and time) decreased. As a consequence, this could help us to understand the factors driving species establishment in these environments.

Materials and methods

Seed collection and storage

Seeds were collected throughout the mid-late dry season, between July and October in the years of 2014 or 2015 (Table 1). The collections were made from three to 10 mother-plants in 15 legume species (shrubs and trees) across different Cerrado savannas and forest formations, in

Northern, Central and Southeastern Brazil (Table 1). Two of the tree study species, *Copaifera langsdorffii* and *Senegalia polyphylla*, occur in the seasonal forest, but also in closed Cerrado formations (*cerrado sensu stricto* and *cerradão*), and were then grouped with the forest species (see Mendonça *et al.*, 2008).

The Cerrado savanna-forest mosaic is characterized by highly seasonal rainfall, with a dry season from May to September and the precipitation concentrated during the rainy season (1300-2000 mm/year, Eiten, 1972). Seeds were screened in the laboratory and stored under refrigerated conditions (5°C) until the beginning of the experiments in December 2015.

Temperature fluctuation experiment

To determine the effects of temperature fluctuation on PY alleviation, we performed the following treatments: (1) Control: seeds were kept under room temperature for 90 days before being set to germinate (see germination procedures below); (2) Gap-simulated temperature fluctuation, under dry conditions, using germination chambers programmed to daily simulate a range of temperatures from 18 to 55°C (Fig. 1). Those temperatures were recorded in an open Cerrado area in Central Brazil (Daibes *et al*. 2017). Seeds were exposed to temperature fluctuations for 90 days before being tested for germination (see Fig. 1). This 90-day interval corresponds to the time that seeds from most of the study species experience temperature fluctuation cycles at the soil surface during the dry season, before the onset of the rainy season in October.

The experimental design consisted of four replicates of 25 seeds per treatment each (4 x 25 = 100 seeds/treatment/species), except for *Erythrina speciosa* and *Peltophorum dubium,* for which we had only 10 seeds/replicate each, due to the limited number of seeds. Four independent germination chambers were used to simulate temperature fluctuations, assuring independency among replicates (Morrison & Morris, 2000).

Germination experiments

After the application of treatments, seeds were set to germinate under a constant temperature (27°C, 12 h light/12h dark), considered to be the optimum conditions for germination and also the average temperature in the soil surface in the Cerrado (Fichino *et al.*, 2016; Daibes *et al.*, 2017). Germination counting was made three times a week for one month and seeds were considered to be germinated upon radicle emergence (Bewley *et al.*, 2013). At the end of the trials, ungerminated seeds were mechanically scarified with sandpaper and set to germinate for an additional week. Seeds that germinated within this period were counted as viable and such procedure helped us to assure that seeds had no other type of dormancy in addition to PY. Seed viability of the fewer remaining seeds was assessed by using tetrazolium tests (1%, pH7) and seeds showing embryos stained red were considered viable (Lakon, 1949; Hilhorst, 2011).

Based on seed germination of the control treatments, we classified seeds according to dormancy classes (see Baskin & Baskin, 2014; Dayrell *et al.*, 2017) in the following categories: (1) PY: when germinating (non-dormant) seeds were <30% of viable seeds, assuring that the remaining viable ones were not imbibed (water-impermeable seed coats); (2) IN: intermediate dormancy, species with germination between 30 and <70% of viable seeds; (3) ND: non-dormant, species with germination >70% of viable seeds within 30 days (Table 1).

Data analyses

Germination data was evaluated separately for each species, considering seed germination and seed viability separately as predicted variables as a function of the different treatments (control and temperature fluctuation). We fitted GLMMs with a binomial distribution and taking the replicates as random effects in the analyses (see Zuur *et al.*, 2009). All analyses were performed using the *lme4* package (Bates *et al.*, 2015). Significance level was considered as $\alpha \leq 0.05$.

We performed accelerated failure time (AFT) models for estimating time (t50) for 50% of germination (Klein & Moeschberger, 2003). This model allows estimates of germination curves based on the probability of germinating-or-not of individual seeds, so that it is possible to extrapolate germination time beyond the length of the experiment. This analysis assumes that ungerminated seeds are still viable after the trials. *Plathymenia reticulata* was excluded from this analysis because of its low seed viability (see Results). Since this is a parametric modeling, we run different data distribution for our germination dataset: exponential, Weibull, log-normal, and log-logistic. We used AIC criterion to assure which distribution better fitted our data. As the log-normal distribution had the lowest AIC value, we used it to perform the models with the *survreg* function using the *Survival* package (Therneau, 2015). We used the *predict* function to estimate t50 both for control and temperature fluctuation treatments. Estimates of time were made separately for each replicate and compared statistically using a linear model. All analyses were conducted using the software R version 3.5.2 (R Core Team, 2016).

Table 1 Vegetation type, dormancy class (PY = physically dormant, IN = intermediate dormancy, ND = non-dormant), growth form, site of collection and collection date (month and year). PEJ = *Parque Estadual do Jalapão* (TO 10°22’S 46°40’W); RNST = *Reserva Natural Serra do Tombador* (GO 13°39’S 47°49’W); PEPF = *Parque Estadual Porto Ferreira* (SP 21°50’S 47°26’W); ITI = private property, Itirapina (SP 22°15’S 47°53’W); RCL = *Campus* Unesp, Rio Claro (SP 22°23’S 47°32’W). * = Species also occurring in Cerrado woody savannas.

Species	**Vegetation type**	**Dormancy class**	**Growth form**	**Site of collection**	**Collection date**
Albizia niopoides (Spruce ex Benth.) Burkart	Forest (seasonal forest)	ND	tree	PEPF	October 2015
Chamaecrista viscosa (Kunth) H.S.Irwin & Barneby	Cerrado (open savannas)	PY	shrub	PEJ	July 2014
Copaifera langsdorffii Desf.	Forest* (transitional forest)	ND	tree	PEPF	August 2015
Erythrina speciosa Andrews	Forest (seasonal forest)	PY	tree	RCL	September 2015
Mimosa claussenii Benth.	Cerrado (open savannas)	PY	shrub	RNST	October 2014
Mimosa oligosperma Barneby	Cerrado (open savannas)	PY	shrub	RNST	October 2015
Mimosa somnians Humb. & Bonpl. ex Willd.	Cerrado (open savannas)	PY	subshrub	PEJ	July 2014
Mimosa spixiana Barneby	Cerrado (open savannas)	IN	shrub	PEJ	July 2014
Peltophorum dubium (Spreng.) Taub.	Forest (seasonal forest)	PY	tree	PEPF	October 2015
Plathymenia reticulata Benth.	Cerrado (woody savannas)	ND	tree	ITI	September 2015
Schizolobium parahyba (Vell.) Blake	Forest (seasonal forest)	PY	tree	PEPF/RCL	August 2015
Senegalia polyphylla (DC.) Britton & Rose	Forest* (transitional forest)	ND	tree	PEPF	October 2015
Senna cana (Nees & Mart.) H.S.Irwin & Barneby	Cerrado (open savannas)	PY	shrub/tree	RNST	October 2014
Senna silvestris (Vell.) H.S.Irwin & Barneby	Cerrado (open savannas)	IN	shrub/tree	RNST	October 2014
Stryphnodendron obovatum Benth.	Cerrado (woody savannas)	IN	tree	ITI	September 2015

Results

The temperature fluctuation treatment did not break PY in any of the study species, regardless of the environment (Fig. 2). Instead, we found a significant decrease in germination percentage in four species from the Cerrado savannas: one PY (*Mimosa claussenii*, from 20±7 to 10±4%; *P*=0.04) and three IN or ND seeds [*Plathymenia reticulata* (from 36±17 to 11±5%; *P*=0.001), *Senna silvestris* (from 35±13 to 14±11%; *P*=0.006) and *Stryphnodendron obovatum* (from 26±7 to 8±3%; *P*=0.001)]. The other five savanna species (*Chamaecrista viscosa*, *Mimosa oligosperma*, *Mimosa somnians*, *Mimosa spixiana*, and *Senna cana*) were unaffected by temperature fluctuation, maintaining germination percentage equal to the controls (Fig. 2).

A single forest species, *Peltophorum dubium*, tended to have a positive effect on germination (from 5±10% to 24±23%) after temperature fluctuation, although no significant difference was found. Two other forest species showed a reduction in germination percentage with the temperature fluctuation: *Albizia niopoides* (from 55±9 to 25±11%; *P*<0.001) and *Copaifera langsdorffii* (from 59±11 to 23±10%; *P*<0.001; Fig. 2). The remaining forest species (*Erythrina speciosa*, *Schizolobium parahyba* and *Senegalia polyphylla*) showed no difference in seed germination between the treatments (Fig. 2).

Two forest species (*C. langsdorffii* and *P. dubium*) had a significant decrease in viability after temperature fluctuation treatments (Table 2). Seed viability in *C. langsdorffii* was reduced by 20% in relation to the control (from 72±10 to 53±6%; *P*=0.004), while in *P. dubium*, seed viability decreased nearly by 30% after temperature fluctuation (from 59±8 to 31±16%; *P*=0.02; Table 2). All other species, both from Cerrado and forest, showed no difference in seed viability among treatments (*P*>0.05; Table 2). *P. reticulata* seeds had low viability (<40%) in both treatments (Table 2).

Germination times (t50) predicted by the AFT model in the temperature fluctuation showed a two-three folded delay when compared to controls in four out of six IN/ND species. From those, two species were from the Cerrado savannas (*S. silvestris* and *S. obovatum*) and two from forest (*A. niopoides* and *C. langsdorffii*; see Table 3). *M. spixiana* and *S. polyphylla* showed no significant differences in t50 between the two treatments (Table 3).

Table 2 Seed viability (mean ± SD) of Cerrado savannas and forest legume seeds in control (untreated seeds) and after gap-simulated temperature fluctuation (90 days). Asterisks indicate significant differences in relation to the control (baseline) in the GLMM analysis (binomial distribution, replicates as random effects). * = *P* ≤0.05; ** = *P* ≤0.01.

Species	**Control**	**Temp. fluc**
Albizia niopoides	71 ± 11	59 ± 16
Chamaecrista viscosa	93 ± 7	84 ± 16
Copaifera langsdorffii	72 ± 10	53 ± 6 **
Erythrina speciosa	73 ± 10	40 ± 37
Mimosa claussenii	75 ± 18	67 ± 14
Mimosa oligosperma	85 ± 19	89 ± 22
Mimosa somnians	87 ± 12	90 ± 7
Mimosa spixiana	96 ± 5	97 ± 4
Peltophorum dubium	59 ± 8	31 ± 16 *
Plathymenia reticulata	39 ± 13	30 ± 13
Schizolobium parahyba	97 ± 4	92 ± 9
Senegalia polyphylla	84 ± 3	82 ± 8
Senna cana	63 ± 6	74 ± 16
Senna silvestris	74 ± 18	50 ± 32
Stryphnodendron obovatum	74 ± 8	66 ± 11

Table 3 Germination time for 50% (t50), as predicted by AFT (accelerated failure time) model in gap-simulated temperature fluctuation for legume species possessing intermediate or non-dormant seeds. Significant *P*-values (<0.05) highlighted in bold.

	t50 (days ± SD)		
Species	**Control**	**Temp. fluc**	***P*-value**
Albizia niopoides	27 ± 6	83 ± 37	**0.026**
Copaifera langsdorffii	25 ± 3	48 ± 13	**0.012**
Mimosa spixiana	40 ± 11	48 ± 36	0.695
Senegalia polyphylla	5 ± 1	7 ± 1	0.057
Senna silvestris	46 ± 19	110 ± 29	**0.032**
Stryphnodendron obovatum	91 ± 27	249 ± 75	**0.007**

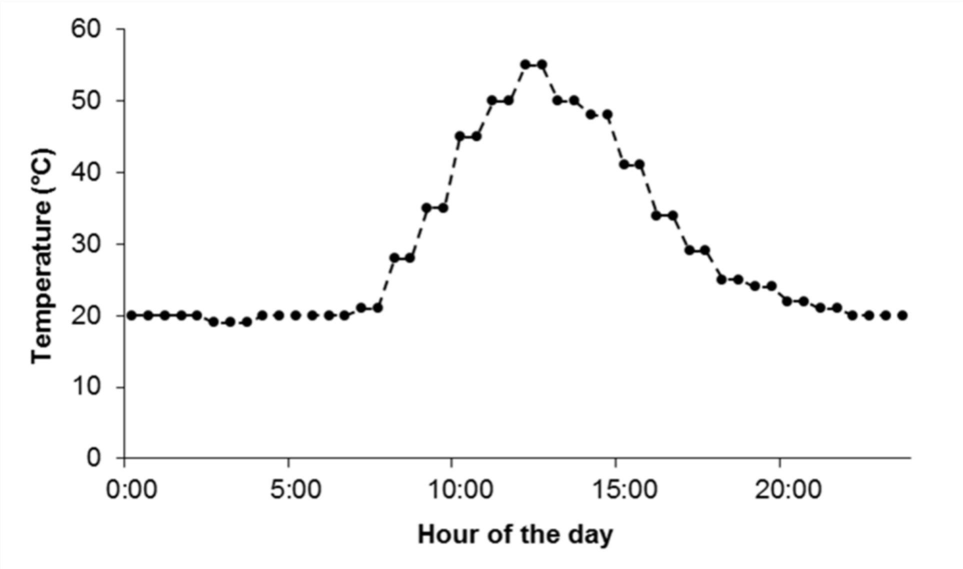


Fig. 1 Gap-simulated temperatures as daily reproduced in the germination chambers during the experiment for 90-days. The simulation follows original data recorded under field conditions in the Cerrado open savannas, Central Brazil (see Daibes *et al.*, 2017).

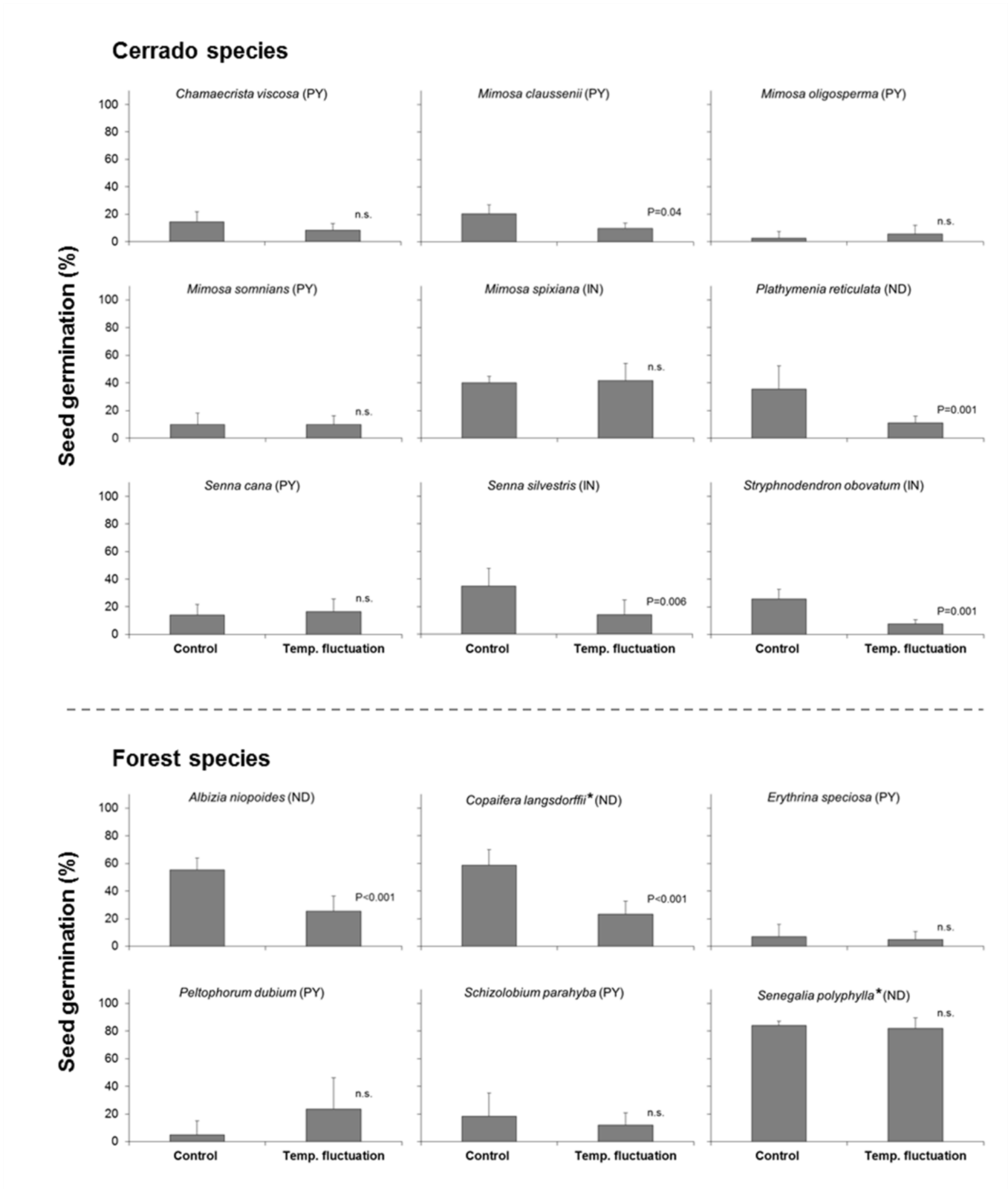


Fig. 2 Seed germination (mean ± SD) of Cerrado savannas and forest legume seeds in control (untreated seeds) and after gap-simulated temperature fluctuation (90 days). Dormancy class shown in parenthesis: PY = physical dormancy; IN = intermediate dormancy; ND = non-dormant. Significant *P*-values are shown in the charts; n.s. = not significant (P>0.05). * = Species also occurring in Cerrado closed formations.

Discussion

Contrary to what has been found in other ecosystems, our results showed that gap-simulated temperature fluctuation did not alleviate PY, neither in Cerrado savannas nor in forest legume species. This counters our initial hypothesis that gap-simulated temperature fluctuations would break PY in savanna species and strongly contrasts to studies with Australian legumes (Auld & Bradstock, 1996; Santana *et al.*, 2010) and Mediterranean species (Baeza & Roy, 2008; Santana *et al.*, 2013). These fire-prone ecosystems show PY-breaking mediated by fire-related temperatures and/or temperature fluctuation (Ooi et al., 2014). In the Cerrado savannas, our data indicate dormancy breaking does not seem bounded nor to fire (Ribeiro et al., 2013; Fichino et al., 2016; Daibes et al., 2017) and, according to our results, neither to temperature fluctuation.

However, we found that temperature fluctuation caused a delay in seed germination times for intermediate and/or non-dormant seeds, both from savanna and forest vegetation. A delaying on germination time has rarely been reported in the literature, but it was recently suggested for a single non-dormant open savanna species, *Bauhinia dumosa* (Zupo *et al.*, 2016). We argue that such delay would be important for preventing seed recruitment under stressful conditions of drought and temperatures daily rising >50°C (Daibes *et al.*, 2017), therefore spreading germination in time (Rolston, 1978; Fenner & Thompson, 2005).

Rather, gaps could work better as a germination cue detecting extremely high temperatures and water deficit conditions, which could be hazardous for seedling establishment. Gap conditions could also kill a portion of seeds exposed in the Cerrado open savannas, while some seeds might have PY-breaking associated to such exposure (Daibes *et al.*, 2017). Nevertheless, the experiments performed by Daibes *et al.* (2017) were subjected to a broad of unmonitored conditions in the gaps, such as solar radiation and moisture availability, which are hard to keep under control. Therefore, we highlight the importance of fixing and simulating the environmental factors under laboratory conditions, so we can untangle processes that otherwise would remain misunderstood. By simulating the gap-occurring temperatures in the laboratory, we can assure that PY break in the Cerrado savannas is not likely to be a function of dry temperature fluctuations alone.

Some seeds might persist in the soil, ungerminated but still viable, until specific environmental conditions to come (Ooi *et al.*, 2004). This could thus be a strategy to avoid unfavorable conditions, supporting the idea that seedling establishment of both savanna and forest species seems to benefit from a less stressful environment (Hoffmann, 1996; Salazar *et al.*, 2012). The 90-days exposure to temperature fluctuation seems to have resulted in drying of the seed tissues and could, thus, retard imbibition and/or germination process. Delaying on germination time could prevent seed germination until their incorporation into the soil seed bank (Williams, 1983). On the other hand, seeds buried in the soil were recognized to be gradually recruited with the onset of the rainy season, in a more stochastic process (Salazar *et al.*, 2011; Andrade & Miranda, 2014). Because Cerrado has a highly marked seasonality and a relatively high precipitation during the rainy season (Eiten, 1972; Oliveira-Filho & Ratter, 2002), water supply in the soil could also be important to underlie PY-breaking process in this ecosystem.

Wet conditions seem to break PY in some tropical species, in combination with enhanced or fluctuating temperatures (Moreno-Casasola *et al.*, 1994; van Klinken *et al.*, 2006). For instance, the forest trees *Peltophorum dubium* and *Mimosa bimucronata* seem to show PY alleviation under wet heat shocks (40 and 50°C), as well as when set to germinate under wet alternation (mostly 20/40°C), with less effect from the dry shocks (Geisler *et al.*, 2017). *Schizolobium parahyba* also seems to have its PY-breaking process mediated by wet alternating temperatures (20/40°C, see Souza *et al.*, 2012). The same pattern occurs in other legume trees, including *Senna* and *Cassia* species (de Paula *et al.*, 2012; Rodrigues-Junior *et al.*, 2014), which also occurs in the Cerrado savanna-forest mosaic.

Savanna species are less studied, but in some *Mimosa* species high constant temperatures seem to be enough to break PY (Silveira & Fernandes, 2006; Dayrell *et al.*, 2015). Moreover, a species-rich ecosystem usually shows a variety of regeneration niches, occupied by different species to avoid competition (Grubb, 1977). How savanna legume seeds behave for colonizing open sites remains not fully explained, and might be dependent of a broad of environmental combinations, less explored in the literature. Intra-specific variation (Liyanage & Ooi, 2015) could allow seeds to colonize a range of possibilities in the heterogeneous and frequently disturbed open savannas (see Williams *et al.*, 2005; Scott *et al.*, 2010). Therefore, other factors could account for dormancy-breaking of the remaining proportions of seeds within a given population and intraspecific variation would help explaining PY-breaking mechanisms and seed germination in the savanna-forest legumes.

In conclusion, legumes from the Cerrado savanna-forest mosaic do not seem to fit the mechanisms for PY-breaking and the temperature-mediated classifications proposed for other fire-prone ecosystems (Moreira & Pausas, 2012; Ooi *et al.*, 2014). There is still much to be discovered about the mechanisms underpinning PY-breaking in the Cerrado. Also, understanding spread of germination as a function of soil heating is indispensable for the ecology of regeneration and species assembling under increased temperatures (Ooi *et al.*, 2009; Gremer & Venable, 2014). However, gap-occurring temperatures were rather here reported to delay germination time, whether preventing establishment under hazardous conditions, both for savanna and forest species.

Acknowledgments

We thank J.S. Nunes and all the fellows from the Laboratory of Vegetation Ecology (LEVeg/Unesp) for helping with laboratory work. To P.R.M. Souza Filho we thank for helping with the AFT models. To T.M. Zupo we thank for friendly reviewing the manuscript. We thank the *Instituto Florestal do Estado de São Paulo* for the collection permit (COTEC 260108 – 008.322/2015). We also thank the people who have helped during seed collection, especially A.L.T. De Lucca, D.E. Escobar, E.P. Dickfeldt, G.P. Sabino. To L.M. Borges and M.F. Simon we thank for helping with taxonomic identification, particularly *Mimosa*. L.F. Daibes received scholarship from the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES). This study was supported by *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP 2015/06743-0), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 455183/2014-7), and *Fundação Grupo Boticário* (0153_2011_PR). F.A.O. Silveira (482720) and A. Fidelis (306170/2015-9) receive scholarships from CNPq.

References

Andrade, L.A.Z. and Miranda, H.S. (2014) The dynamics of the soil seed bank after a fire event in a woody savanna in central Brazil. *Plant Ecology* **215,** 1199-1209.

Auld, T.D. and Bradstock, R.A. (1996) Soil temperatures after the passage of a fire: Do they influence the germination of buried seeds? *Australian Journal of Ecology* **21,** 106-109.

Baeza, M.J. and Roy, J. (2008) Germination of an obligate seeder (*Ulex parviflorus*) and consequences for wildlife management. *Forest Ecology and Management* **256,** 685-693.

Baskin, C.C. and Baskin, J.M. (2014) *Seeds: ecology, biogeography, and evolution of dormancy and germination.* 2nd edition, Elsevier BV: Academic Press.

Baskin, J.M. and Baskin, J.M. (2000) Evolutionary considerations of claims for physical dormancy-break by microbial action and abrasion by soil particles. *Seed Science Research* **10,** 409-413.

Bates, D., Maechler, M., Bolker, B. and Walker, S. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67,** 1-48.

Bewley, J.D., Bradford, K., Hilhorst, H.W.M. and Nonogaki, H. (2013) *Seeds: Physiology of development, germination and dormancy.* 3rd edition, New York, USA: Springer Science.

Castro, J. (2006) Short delay in timing of emergence determines establishment success in *Pinus sylvestris* across microhabitats. *Annals of Botany* **98,** 1233-1240.

Coutinho, L.M. (1982) Ecological effects of fire in Brazilian Cerrado. In: Huntley BJ, Walker BH, eds. *Ecology of tropical savannas.* Springer-Verlag. 273-291.

Daibes, L.F., Zupo, Z., Silveira, F.A.O and Fidelis, A. (2017) A field perspective on effects of fire and temperature fluctuation on Cerrado legume seeds. *Seed Science Research* **27,** 74-83.

Dayrell, R.L.C., Garcia, Q.S., Negreiros, D., Baskin, C.C., Baskin, J.M. and Silveira, F.A.O. (2017) Phylogeny strongly drives seed dormancy and quality in a climatically buffered hotspot for plant endemism. *Annals of Botany* **119,** 267-277.

Dayrell, R.L.C., Gonçalves-Alvim, S.J., Negreiros, D., Fernandes, G.W. and Silveira, F.A.O. (2015) Environmental control of seed dormancy and germination of *Mimosa calodendron* (Fabaceae): implications for ecological restoration of a highly threatened environment. *Brazilian Journal of Botany* **38,** 395-399.

de Paula, A.S., Delgado, C.M.L., Paulilo, M.T.S. and Santos, M. (2012) Breaking physical dormancy of *Cassia leptophylla* and *Senna macranthera* (Fabaceae: Caesalpinioideae) seeds: water absorption and alternating temperatures. *Seed Science Research* **22,** 259-267.

Eiten, G. (1972) The Cerrado vegetation of Brazil. *The Botanical Review* **38,** 201-341.

Fenner, M. and Thompson, K. (2005) *The Ecology of Seeds.* Cambridge University Press.

Fichino, B., Dombrovski, J.R.G., Pivello, V.R. and Fidelis, A. (2016) Does fire trigger seed germination in the Neotropical savannas? Experimental tests with six Cerrado species. *Biotropica* **48,** 181-187.

Fidelis, A., Blanco, C.C., Müller, S.C., Pillar, V.D., Pfadenhauer, J. and Otto, W. (2012) Short-term changes caused by fire and mowing in Brazilian *campos* grasslands with different long-term fire histories. *Journal of Vegetation Science* **23,** 552-562.

Geisler, G.E., Pinto, T.T., Santos, M. and Paulilo M.T.S. (2017) Seed structures in water uptake, dormancy release, and germination of two tropical forest Fabaceae species with physically dormant seeds. *Brazilian Journal of Botany* **40,** 67-77.

Gremer, J.R. and Venable, D.L. (2014) Bet hedging in desert winter annual plants: optimal germination strategies in a variable environment. *Ecology Letters* **17,** 380-387.

Grubb, P.J. (1977) The maintenance of species-richness in plant communities: the importance of the regeneration niche. *Biological Reviews* **52,** 107-145.

Hoffmann W.A. (1996) The effects of fire and cover on seedling establishment in a Neotropical savana. *Journal of Ecology* **84,** 383-393.

Hoffmann, W.A. (2000) Post-establishment seedling success in the Brazilian Cerrado: a comparison of savanna and forest species. *Biotropica* **32,** 62-69.

Hilhorst, H.W.M. (2011) Standardazing seed dormancy research. In: Kermode AR, ed. *Seed Dormancy: Methods and Protocols,* Methods in Molecular Biology, vol. 773. Springer Science+Business Media.

Jones, R.H., Allen, B.P. and Sharitz, R.R. (1997) Why do early-emerging tree seedlings have survival advantages?: a test using *Acer rubrum* (Aceraceae). *American Journal of Botany* **84,** 1714-1718.

Klein, J.P. and Moeschberger, M.L. (2003) *Survival Analysis: Techniques for censored and truncated data.* Statistics for Biology and Health. New York: Springer.

Lakon, G. (1949) The topographical tetrazolium method for determining the germination capacity of seeds. *Plant Physiology* **24,** 389-394.

Liyanage, G.S. and Ooi, M.K.J. (2015) Intra-population level variation in thresholds for physical dormancy-breaking temperature. *Annals of Botany* **116,** 123-131.

Mendonça, R.C., Felfili, J.M., Walter, B.M.T., Silva Júnior, M.C., Rezende, A.B., Filgueiras, T.S., Nogueira, P.E., Fagg, C.W. (2008) Flora vascular do bioma Cerrado: *Checklist* com 12.356 espécies. In: Sano SM, Almeida SP, Ribeiro, JF, eds. *Cerrado: ecologia e flora.* Embrapa Cerrados, Brasília, DF: Embrapa Informação Tecnológica.

Moreira, B. and Pausas, J.G. (2012) Tanned or burned: the role of fire in shaping physical seed dormancy. *PLoS ONE* **7,** e51523.

Moreno-Casasola, P., Grime, J.P. and Martínez, M.L. (1994) A comparative study of the effects of fluctuations in temperature and moisture supply on hard coat dormancy in seeds of coastal tropical legumes in Mexico. *Journal of Tropical Ecology* **10,** 67-86.

Morrison, D.A., McClay, K., Porter, C. and Rish, S. (1998) The role of the lens in controlling heat-induced breakdown of testa-imposed dormancy in native Australian legumes. *Annals of Botany* **82,** 35-40.

Morrison, D.A. and Morris, E.C. (2000) Pseudoreplication in experimental designs for the manipulation of seed germination treatments. *Austral Ecology* **25,** 292-296.

Oliveira-Filho, A.T. and Ratter, J.A. (2002) Vegetation physiognomies and woody flora of the Cerrado biome. In: Oliveira PS, Marquis RJ, eds. *The cerrados of Brazil: ecology and natural history of a neotropical savanna.* New York, USA: Columbia University Press, pp. 91-120.

Ooi, M.K.J. (2010) Delayed emergence and post-fire recruitment success: effects of seasonal germination, fire season and dormancy type. *Australian Journal of Botany* **58,** 248-256.

Ooi, M.K.J., Auld, T.D. and Denham, A. (2009) Climate change and bet-hedging: interactions between increased soil temperatures and seed bank persistence. *Global Change Biology* **15,** 2375-2386.

Ooi, M.K.J., Auld, T.D. and Whelan, R.J. (2004) Delayed post-fire seedling emergence linked to season: a case study with *Leucopogon* species (Epacridaceae). *Plant Ecology* **174,** 183-196.

Ooi, M.K.J., Denham, A.J., Santana, V.M. and Auld, T.D. (2014) Temperature thresholds of physically dormant seeds and plant functional response to fire: variation among species and relative impact of climate change. *Ecology and Evolution* **4,** 656-671.

R Core Team. (2016) *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/

Ribeiro, L.C. and Borghetti, F. (2014) Comparative effects of desiccation, heat shock and high temperatures on seed germination of savanna and forest tree species. *Austral Ecology* **39,** 267-278.

Ribeiro, L.C., Pedrosa, M. and Borghetti, F. (2013). Heat shock effects on seed germination of five Brazilian savanna species. *Plant Biology* **15,** 152-157.

Rodrigues-Junior, A.G, Faria, J.M.R., Vaz, T.A.A., Nakamura, A.T. and José, A.C. (2014) Physical dormancy in *Senna multijuga* (Fabaceae: Caesalpinioideae) seeds: the role of seed structures in water uptake. *Seed Science Research* **24,** 147-157.

Rolston, M.P. (1978) Water impermeable seed dormancy. *The Botanical Review* **44,** 365-396.

Salazar, A., Goldstein, G., Franco, A.C. and Miralles-Wilhelm, F. (2011) Timing of seed dispersal and dormancy, rather than persistent soil seedbanks, control seedling recruitment of woody plants in Neotropical savannas. *Seed Science Research* **21,** 103-116.

Salazar, A., Goldstein, G., Franco, A.C. and Miralles-Wilhelm, F. (2012) Differential seedling establishment of woody plants along a tree density gradient in Neotropical savannas. *Journal of Ecology* **100,** 1411-1421.

Santana, V.M., Baeza, M.J. and Blanes, M.C. (2013) Clarifying the role of fire heat and daily temperature fluctuations as germination cues for Mediterranean Basin obligate seeders. *Annals of Botany* **111,** 127-134.

Santana, V.M., Bradstock, R.A., Ooi, M.K.J., Denham, A.J., Auld, T.D. and Baeza, M.J. (2010) Effects of soil temperature regimes after fire on seed dormancy and germination in six Australian Fabaceae species. *Australian Journal of Botany* **58:** 539-545.

Scott, K., Setterfield, S., Douglas, M. and Andersen, A. (2010) Soil seed banks confer resilience to savanna grass-layer plants during seasonal disturbance. *Acta Oecologica* **36,** 202-210.

Silveira, F.A.O. and Fernandes, G.W. (2006) Effect of light, temperature and scarification on the germination of *Mimosa foliolosa* (Leguminosae) seeds. *Seed Science and Technology* **34,** 585-592.

Souza, T.V, Voltolini, C.H., Santos, M. and Paulilo, M.T.S. (2012) Water absorption and dormancy-breaking requirements of physically dormant seeds of *Schizolobium parahyba* (Fabaceae–Caesalpinioideae). *Seed Science Research* **22,** 169-176.

Therneau, T. (2015). *A package for Survival Analysis in S*. version 2.38 URL: http://CRAN.R-project.org/package=survival

Thompson, K., Grime, J.P. and Mason, G. (1977) Seed germination in response to diurnal fluctuations of temperature. *Nature* **267,** 147-149.

van Klinken, R.D., Flack, L.K. and Pettit, W. (2006) Wet-season dormancy release in seed banks of a tropical leguminous shrub is determined by wet heat. *Annals of Botany* **98,** 875-883.

Vázquez-Yanes, C. and Orozco-Segovia, A. (1982) Seed germination of a tropical rainforest pioneer tree (*Heliocarpus donnell-smithii*) in response to diurnal fluctuation of temperature. *Physiologia Plantarum* **56,** 295-298.

Verdú, M. and Traveset, A. (2005) Early emergence enhances plant fitness: a phylogenetically controlled meta-analysis. *Ecology* **86,** 1385-1394.

Wiliams, E.D. (1983) Effects of temperature fluctuation, red and far-red light and nitrate on seed germination of five grasses. *Journal of Applied Ecoogy* **20,** 923-935.

Williams, P.R., Congdon, R.A., Grice, A.C. and Clarke, P.J. (2005) Germinable soil seed banks in a tropical savanna: seasonal dynamics and effects of fire. *Austral Ecology* **30,** 79-90.

Zupo, T., Baeza, M.J. and Fidelis, A. (2016) The effect of simulated heat-shock and daily temperature fluctuations on seed germination of four species from fire-prone ecosystems. *Acta Botanica Brasilica* **30,** 514-519.

Zuur A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A. and Smith G.M. (2009) *Mixed effects models and Extensions with Ecology in R.* Statistics for Biology and Health. New York: Springer.

CAPÍTULO 4

Seed germination is unlinked to fire in the Brazilian savanna-forest mosaic

Viver de noite
Me fez senhor do fogo
A vocês eu deixo o sono
O sonho, não
Esse eu mesmo carrego
Paulo Leminski

Seed germination is unlinked to fire in the Brazilian savanna-forest mosaic

L. Felipe Daibes, Juli G. Pausas, Jessika da Silva Nunes, Nathalia Bonani, Fernando A.O. Silveira, Alessandra Fidelis

Abstract

Heat shocks have been argued as a major factor breaking dormancy and enhancing seed germination in fire-prone ecosystems across the globe. Propagule tolerance to high temperatures is also an important trait to warrant seed supply after fire. However, patterns involving seed-tolerance and physical dormancy (PY) breaking are variable and controversial in tropical savannas from Africa and South America. Therefore, we aimed to investigate the role of fire on seed germination and seed mortality of 46 legume species from the Brazilian savanna-forest mosaic. We applied heat shock treatments (100°C 1-min, 100°C 3-min, 200°C 1-min), conducted seed germination tests and assessed seed viability by the end of the experiments. GLMMs and a phylogenetical comparison were employed to analyze the data. We found little effect of the fire-related temperatures in PY-breaking. On the other hand, seeds mainly resisted the 100°C 1-min treatment. Seed mortality was significantly higher only at the most severe conditions. Seed mass was important to explain seed mortality only in the 200°C treatment, and this trait could be influenced by phylogeny among the tree species. The small shrub seeds could thus be relatively more sensitive to hotter fires, but the presence of PY was also an important trait to predict seed survival under a severe condition within this growth-form. Those results suggest that larger-seeded forest trees could have helped to colonize the Cerrado savannas and a trade-off between growth-form, phylogeny and seed traits could help to understand seed mortality and seed regeneration in this ecosystem.

Keywords: tropical savanna, fire ecology, regeneration, seed traits, seed mass, seed shape, physical dormancy

Introduction

Heat shocks consist one of the most outstanding mechanisms underlying seed recruitment in flammable ecosystems, by disrupting the seed coat of physically dormant (PY; water-impermeable) seeds (Morrison et al. 1998; Moreira and Pausas 2012; Ooi et al. 2014). Legume seeds are some of the best examples of propagules possessing PY (Baskin and Baskin 2014; Rubio de Casas et al. 2017), frequently broken by fire in Australian and Mediterranean vegetation (Auld and O'Connell 1991; Herranz et al. 1998; Williams et al. 2003; Reyes and Trabaud 2009; Moreira et al. 2010). However, dormancy-breaking by fire is highly variable and controversial in tropical savannas both from Africa (e.g. Gashaw and Michelsen 2002; Dayamba et al. 2008) and South America (e.g. Fichino et al. 2016; Daibes et al. 2017). In fact, there is evidence that savanna fires do not affect emergence from soil seed banks in the Cerrado (Andrade and Miranda 2014), where heat shocks also have little effect on germination under laboratory conditions (Ribeiro et al. 2013; Fichino et al. 2016).

Because germination might not be enhanced by fire, the capacity of seeds to tolerate high temperatures may be relevant in frequently burned savannas (Gashaw and Michelsen 2002; Fichino et al. 2016; Ramos et al. 2016). Those ecosystems face relatively low intense and faster fires, reoccurring each few years when compared to the Mediterranean vegetation (Whelan 1995), and such fire regime might influence on seed regeneration strategies (Pausas and Keeley 2009; 2014). Moreover, seed traits (ex. seed mass, seed shape, dormancy class) could predict seed mortality in relation to fire temperatures (Ribeiro et al. 2015; Gómez-González et al. 2016; Saracino et al. 2017). Larger seeds, for instance, seem to present higher tolerance to heating (Gashaw and Michelsen 2002; Ribeiro et al. 2015) while the smaller ones, on the other hand, could be more easily stimulated to germinate following fires (Hanley et al. 2003). However, seed morphological traits, as well as the presence of dormancy, might be related to the phylogenetical proximity of the species (Moles et al. 2005; Dayrell et al. 2017) and therefore, it is important to take phylogeny into account (see Rubio de Casas et al. 2017).

To clarify this subject, it is also crucial to establish comparisons between seed tolerance in fire-prone and fire-free ecosystems. If tropical forest seeds are less resistant, thus presenting higher mortality after heat shocks (Ribeiro and Borghetti 2014; Ribeiro et al. 2015), fire could be an environmental filter preventing the establishment of forest species in the Cerrado savannas (Hoffmann 2000; Dantas et al. 2013). Information on seed and germination traits could thus help us to describe better the regeneration patterns, and thus, understand assembly rules across different ecosystems (Jiménez-Alfaro et al. 2016). Nevertheless, the adaptive value of seed traits in response to fires still breeds much debate globally (Gómez-González et al. 2011; Bradshaw et al. 2011; Keeley et al. 2011; Jaganathan 2015; Lamont and He 2017).

Within this context, the Cerrado mosaic comprises a gradient of vegetation, with different fire frequencies, from fire-sensitive (forest) to fire-prone (savannas). Fire is infrequent in the woody savannas and absent in the forest (Oliveira-Filho and Ratter 2002), while the open savannas are frequently disturbed by surface fires (every 3-5 years) with low intensity and fast rates of spread (Coutinho 1982; Miranda et al. 2009; Rissi et al. 2017). These different fire-frequencies help to maintain the structure of such savanna-forest mosaic (Hoffmann et al. 2012; Dantas et al. 2016). In addition, fire has been present for millions of years in the Cerrado and seems to be related to the high diversification rates of the legumes (Simon et al. 2009). Therefore, Leguminosae is one of the richest taxonomic groups occurring across the Cerrado hotspot (see Myers et al. 2000), possessing >12,000 species and *ca.* 1,200 legumes among them (BFG 2015). These species also evolved several transitions from dormant to non-dormant seeds (Rolston 1978, Rubio de Casas et al. 2017), presenting enough interspecific trait variation to allow a comparative approach. In addition, recent and comprehensive phylogenies (see LPWG 2017) make possible to implement solid phylogenetic contrasts.

We aimed to untangle how fire affects seed germination and mortality for a better understanding of population persistence and regeneration strategies in this savanna-forest mosaic. Specifically, we addressed (1) The role of fire-related heat shocks on seed germination and mortality in both forest (trees) and savanna (shrubs and trees) species; and (2) The role of seed traits (seed mass, seed shape, and germinability) on predicting seed mortality, by using a phylogenetically-controlled analysis. We expected a contrasted effect between forest and savanna species, in such a way that savanna species could show some dormancy-breaking and should be more fire-resistant (less fire mortality) than the forest ones. Alternatively, seed traits could be key-drivers of seed mortality, because depending on the trait (larger seed mass, rounder shape, or the presence of PY) they could work as a physical protection to the embryo in the face of fire temperatures. In turn, seed traits could be substantially influenced by phylogeny, so we then employed phylogenetically-controlled analyses to disentangle ecological *vs* historical factors driving seed tolerance to high temperatures

Material and Methods

Seed collection

Seeds of 46 legume species were collected mostly during the years of 2014 and 2015, from savannas and forests of the Central and Southeastern Brazil (see Table S1 for details). We included species from four different subfamilies across the phylogeny of legumes (LPWG 2017, Fig. S1): Cercidoideae (2 spp.), Detarioideae (4 spp.), Caesalpinioideae (36 spp., including 22 spp. from Mimosoid clade) and Papilionoideae (4 spp.). Savanna species were collected in both open savannas (*campo sujo*; i.e., with a continuous layer of herbaceous and shrub species, facing fires each three-four years of interval; Coutinho 1982; Miranda et al. 2009) and woody savannas (cerrado *sensu stricto*, i.e., with less of the herbaceous component and dominated by tree species, infrequent fires; Eiten 1972; Oliveira-Filho & Ratter, 2002). Forest species were collected mostly from semideciduous forests and *cerradão* (forest formation in the Cerrado, both rarely affected by fire). Collection sites have a similar climate, with a marked dry season from May to September and a high precipitation level (up to 2,000 mm/year) concentrated during the wet months (Eiten 1972).

Because shrubs occurred almost exclusively in the open savannas, we categorized the study species in three growth forms: (1) savanna shrubs, (2) savanna trees, and (3) forest trees. Some species were subshrubs, functionally herbaceous but showing a woody underground organ (*Mimosa gracilis* and *M. somnians*), and were included as savanna shrubs in the analyses. Also, species described both as shrubs and/or trees, but mainly occurring in open savannas (the *campo sujo*), were included among the savanna shrubs (*Senna cana* and *S. silvestris*; see Table S1). *Senna hirsuta* was the only shrub species clearly occurring both in forest and savanna environments, and was also grouped as a savanna shrub during the analyses (see data analyses below).

Seeds of each species were pooled from three to 11 individual plants (Table S1), and kept in the laboratory (under low temperatures ~5°C) until their use in the experiments. Most seeds were kept stored for a period between four and six months, but few of them could have been store over one-to-three years before the trials (Table S1). However, we always accessed dormancy class, initial germination and total viability of the seed batch for each species (see Tables S1, S2, S3) by means of their control treatment during the experiments (see germination procedures below).

Heat shocks

We applied heat shocks in the laboratory to evaluate the effects of fire-related temperatures on seed germination and viability for all species. Seeds were sorted out into four treatments: (1) a laboratory control (untreated seeds); (2) 100°C for one minute, (3) 100°C for three minutes; (4) 200°C for one minute. We did not test temperatures below 100°C because they seem to have no effect on germination of Cerrado seeds (Ribeiro et al. 2013; Fichino et al. 2016; Zupo et al. 2016; Daibes et al. 2017); and we tested the maximum temperature of 200°C because it is the average lethal temperature for seeds when exposed in the soil surface (Rizzini 1976; Daibes et al. 2017). The duration of heat shocks was selected based on the average time of heat pulses during Cerrado fires, which have fast rates of spread (Miranda et al. 1993).

Five replicates in each treatment were separately exposed to their respective temperature/time (Morrison and Morris 2000). Number of seeds per replicate is displayed in Table S4. After treatment application, seeds were tested for germination under laboratory conditions (see germination procedures below).

Germination and viability assessments

Seeds were placed to germinate in Petri dishes upon a double layer of filter paper moistened in distilled water. They were incubated in germination chambers at a constant temperature of 27°C (12 h light), which is an average temperature recorded in the soil surface at Central Brazil (Daibes et al. 2017). This temperature was considered as an optimal condition for seed germination and used to prevent additional effect of temperature variations (see also Fichino et al. 2016). Germination was scored at each two-three day interval for one month, and seeds exhibiting radicle protrusion were considered as germinated. Germinated seeds were discarded after scoring.

By the end of the trials, non-germinated seeds were tested for seed viability. Hard seeds (remaining dormant) were mechanically scarified with sandpaper and set to germinate for an additional week. If seeds germinated within this period, they were scored as viable. Imbibed but non-germinated seeds were carefully cut with a razor blade and subjected to tetrazolium tests (1%, pH 7). Seeds with embryos stained red were counted as viable (Hilhorst 2011). Non-stained or visibly damaged seeds were scored as dead.

Seed trait measurements

We assessed three different seed traits: seed mass, seed shape and germinability. Seed dry seed mass was measured by weighing individual seeds, usually at least 20 seeds per each mother-plant collected, after oven-drying at 80°C for 48 h. The total number of individual seeds measured by species is shown in Table S1. Seed shape was measured based on seeds three main dimensions: length, breadth and width (in mm). We computed each dimension and divided them by the largest dimension of the seed, usually the length, and then calculated the variance among them (Pérez-Harguindeguy et al. 2013). Shape-values vary between zero and one; where zero represents a spherical seed and the closer the value gets to one the more flattened and/or elongated is the seed (see Thompson et al. 1993).

We assessed seed germinability based on germination of the control samples (*ca.* 100 seeds/species) in the experiments described above (untreated seeds). We obtained the total number of germinated and PY (hard) seeds by the end of the trials, so we could calculate a proportion of germinant (permeable) seeds in proportion to those which were physically dormant, but viable. Based on those levels obtained for germinability, we categorized seed dormancy in three classes (see Baskin and Baskin 2014; Dayrell et al. 2017): PY = physically dormant seeds, with less than 30% of germination among the viable seeds; IN = intermediate dormancy, with 30-70% of germination among viable seeds; and ND = non-dormant, exhibiting more than 70% of germination among viable seeds (see Table S1).

Data analyses

We first performed GLMMs to evaluate data of germination percentage and viability as a function of heat shock treatments separately for each species (see Table S1; S2). We used a binomial distribution and considered replicates as random effects (Zuur et al. 2009). Total viability was considered as the sum of seeds germinated during the trials + seeds viable in the viability tests carried out after the trials (scarification and tetrazolium).

Based on the germination and viability data, we calculated the magnitude of the effects of the heat shock treatments in relation to the controls. We obtained the size of the effect on seed germination for each species by subtracting the number of germinated seeds from a given treatment to the number of germinated seeds in the control. We also adjusted this effect size of germination in relation to the total viability of seeds in the controls, so we had a proportion of germinating seeds after the treatments: $[(\mathrm{Ngerm}_{(\mathrm{treat})} - \mathrm{Ngerm}(_{\mathrm{control}}))/\mathrm{Nviab}_{(\mathrm{control})}]$. This index can be positive (enhanced germination) or negative (decrease in germination). Such positive or negative effect was compared as a function of the interaction between growth-forms and heat shock treatments using a LMM model, considering the species as random effects.

Seed mortality was first evaluated as the difference between seed viability in the controls and the heat shock treatment $[(\mathrm{Nviab}_{(\mathrm{control})} - \mathrm{Nviab}_{(\mathrm{treat})})/\mathrm{Nviab}_{(\mathrm{control})}]$. Then, we assessed whether the heat shock increased or decreased seed mortality (i.e., positive or negative values) with a GLMM assuming a binomial error distribution. In this model, similarly to the effect size on germination, seed mortality was also evaluated as a function of the interaction between the and heat shock treatments and the growth-form categories, being considered from the less subjected to the most influenced by fires: forest trees, savanna trees, savanna shrubs. Thus, forest species and the 100°C 1-min treatment were taken as the baseline (intercept) during the analyses, and the species were considered as random effects.

Because seeds very rarely died in the 100°C 1-min treatment (see Results), we proceeded with the analysis of seed mortality in relation to seed traits (seed mass, seed shape and germinability) only for the treatments where mortality was statistically significant. Hence, we conducted GLMMs with a binomial distribution, considering that a seed could be either dead or viable, in relation to the initial viable seeds in the controls. When relevant, we also conducted this analysis separately considering (1) all the study species and (2) considering the different growth-forms separately: trees alone (grouping forest and savanna trees), and savanna shrubs alone. Forest and savanna trees were grouped in this analysis because this growth-form showed less effect from heat shocks (see Results). All analyses (and the following below) were performed in R software version 3.2.5 (R Core Team 2016), using the interface of R Studio (RStudio Core Team 2016). All mixed-effects models were performed using the *lme4* package (Bates et al. 2015).

In order to assess the phylogenetical comparison on seed mortality and seed traits, we performed a comparative analysis using phylogeny as an effect, with the function *compar.gee* in the package *APE* (Paradis et al. 2004). To do so, we had a previously reconstructed phylogenetic tree for legumes based on *matk839c* (Simon et al. 2009), kindly provided by Dr. M.F. Simon (Embrapa/Cenargen, Brazil). We removed species or genera of our study species originally present in the tree, and added missing species with the Mesquite software version 3.2 (Maddison and Maddison 2017). Resolution in the phylogenetic tree was established according to published phylogenies on *Mimosa*, *Senna* and *Chamaecrista* (Marazzi et al. 2006; Conceição et al. 2009; Simon et al. 2011). Branch lengths were calibrated based on the initial times dated in the original tree and therefore an average estimation was made for the added species using the Phylocom software and the *bladj* function (Webb et al. 2008). Graphical plotting was performed with the package *adephylo* (Jombart et al. 2010).

Results

Overall, seed germination was unaffected by treatments irrespective of the heat shock treatment or the growth-form (Fig. 1a; Table 1). When looking separately at each species, heat shocks had a positive effect on seed germination of six savanna shrubs, out of the 46 study species (four *Mimosa* and two *Senna*; Table S2). The magnitude of the effect in relation to the controls was never greater than 30%. On the other hand, seed mortality was significantly higher both in the 100°C 3-min and 200°C 1-min (Fig. 1b; both P <0.001, see Table 1). Additionally, the interaction between savanna shrubs and 200°C was statistically significant (P = 0.004; Table 1) suggesting that savanna shrubs had the highest seed mortality (Fig. 1b).

However, seeds both from forest and savanna species mostly resisted the 100°C 1-min treatment (Fig. 1b; Table S3), showing that they can be fire-tolerant under this condition. Considering the seed traits, no significant effect was detected regarding mortality in the 100°C 3-min treatment (Fig. 2a; Table 2). Under the hottest condition (200°C), on the other hand, seed mortality was negatively related to the seed mass, with larger seed mass showing less mortality when considering all species together (P <0.001; Fig. 2b; Table 2). Seed mass was lower for the savanna shrubs (0.003 to 0.063 g) than both savanna and forest trees (which varied from 0.01 to 3.45 g; Fig. 2c; Table S4). Because shrubs had smaller seed mass, it could explain their higher mortality in comparison to tree species under 200°C.

When we evaluated trees alone, in the hottest condition, the effect of seed mass on seed mortality was significant only in the phylogenetic-based analysis (Table 2). That was due to the presence of medium-to-large seeds in the basal *Hymenaea* and *Copaifera* (Detarioideae) species (Table S4; Fig. S1; Fig. S2a), which were nearly unaffected by heating (Table S3; Fig. S1; Fig. S2b). Other more derived groups of trees (Caesalpinioideae including Mimosoid clade) showed smaller seeds and had higher mortality (Fig. S1; Fig. S2).

The relationship between seed shape and seed mortality was never significant, regardless the treatment (Table 2). Shape varied from spherical to elongate-flattened seeds both in savanna shrubs (0.05 to 0.2; see Table S4), as well as in savanna and forest trees (0.04 to 0.28). Germinability had a positive effect on seed mortality under 200°C 1-min, but only when considering savanna shrubs alone (P = 0.006; Table 2; Fig. 3). Dormancy class varied from dormant (PY) to non-dormant (ND) seeds, showing some intermediate dormant (IN) species both among shrubs and trees. Grouping species by their dormancy classes, we see that more non-dormant shrub seeds (IN and/or ND) had higher proportions of mortality than the PY ones (P = 0.004; Fig. 3). Regarding trees alone, seed mortality was not different among IN/ND and PY species (Fig. 3).

Table 1 Coefficients of the GLMM models for seed germination and seed mortality in relation to the heat shock treatments (100°C 1-min, 100°C 3-min, 200°C 1-min) and growth-form categories (forest trees, savanna trees, savanna shrubs). The 100°C 1-min treatment and forest trees category were considered as the baseline (intercept) in the analysis.

	Germination			**Mortality**		
	Estimate	s.e.	*P*-value	Estimate	s.e.	*P*-value
Intercept	0.012	0.065	--	-4.051	0.481	--
treatment (100°C 3-min)	-0.166	0.069	n.s.	**1.971**	**0.206**	**<0.001**
treatment (200°C 1-min)	-0.105	0.069	n.s.	**2.170**	**0.204**	**<0.001**
savanna trees	-0.076	0.101	n.s.	1.168	0.732	n.s.
savanna shrubs	-0.037	0.081	n.s.	0.987	0.588	n.s.
treat 100 3-min * savanna trees	0.041	0.110	n.s.	-0.075	0.295	n.s.
treat 100 3-min * savanna shrubs	0.190	0.087	n.s.	-0.431	0.242	n.s.
treat 200 1-min * savanna trees	-0.106	0.107	n.s.	-0.478	0.288	n.s.
treat 200 1-min * savanna shrubs	-0.012	0.086	n.s.	**0.688**	**0.239**	**0.004**

Table 2 Coefficients of the GLMM models and phylogenetical comparisons of how the seed traits [seed mass (mass), seed shape (shape), and germinability (germ)] explain seed mortality in the heat shock treatments that killed a significant proportion of the seeds (100°C 3-min and 200°C 1-min). Trees and shrubs are separately shown for the 200°C 1-min treatment due to the significant interaction on seed mortality (treatment x growth-form) as reported in Table 1.

		GLMM model			**Phylogenetical comparison**		
		Estimate	s.e.	*P*-value	Estimate	s.e.	*P*-value
All species							
100°C 3-min	Intercept	-4.141	1.393	--	-0.369	0.867	--
	mass	-0.427	0.289	n.s.	0.255	0.172	n.s.
	shape	0.006	0.071	n.s.	0.001	0.042	n.s.
	germ	0.009	0.012	n.s.	-0.009	0.007	n.s.
All species							
200°C 1-min	Intercept	-4.229	1.007	--	-1.526	0.926	--
	mass	**-0.731**	**0.211**	**<0.001**	-0.188	0.190	n.s.
	shape	0.011	0.054	n.s.	0.009	0.037	n.s.
	germ	0.017	0.009	n.s.	0.006	0.006	n.s.
Forest and savanna trees							
200°C 1-min	Intercept	-4.055	1.157	--	-3.899	1.041	--
	mass	-0.500	0.351	n.s.	**-0.696**	**0.269**	**0.032**
	shape	0.049	0.076	n.s.	0.004	0.055	n.s.
	germ	0.009	0.011	n.s.	0.006	0.008	n.s.
Savanna shrubs							
200°C 1-min	Intercept	-2.917	2.284	--	-2.722	1.673	--
	mass	-0.114	0.495	n.s.	-0.041	0.323	n.s.
	shape	0.117	0.115	n.s.	0.190	0.080	n.s.
	germ	**0.034**	**0.012**	**0.006**	0.024	0.010	n.s.

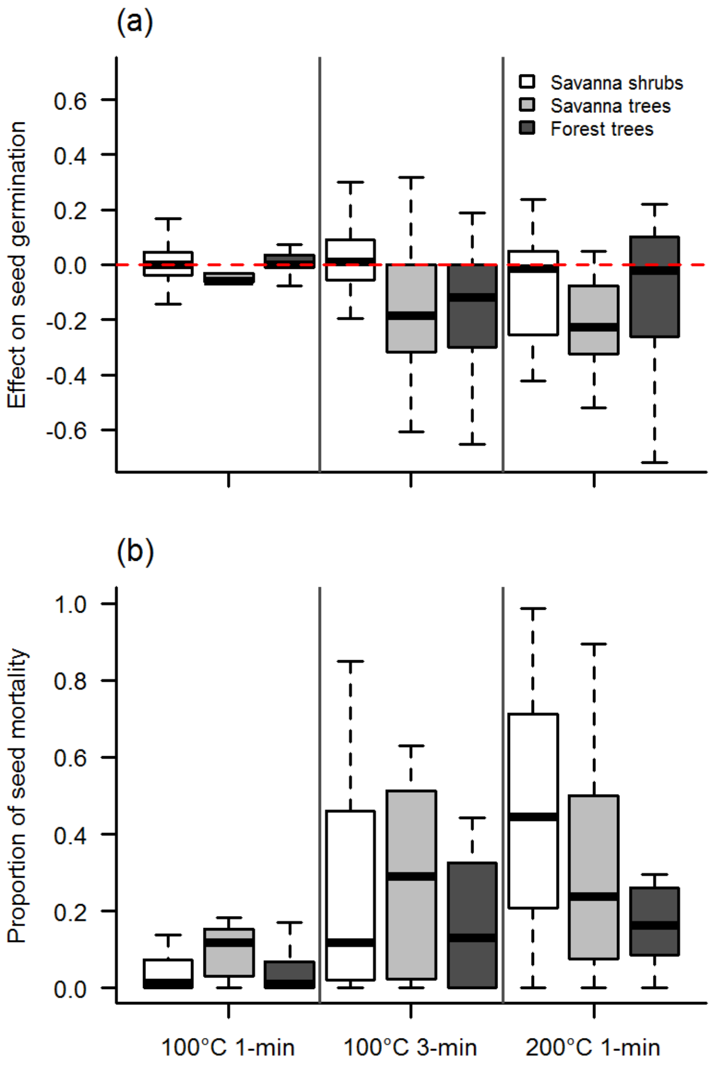


Fig. 1 Relation between **(a)** seed germination (effect size in relation to controls) and **(b)** seed mortality (dead seeds in relation to controls) under different heat shock treatments for the different growth-forms (forest trees, savanna trees, savanna shrubs). See Table 1 for statistical tests.

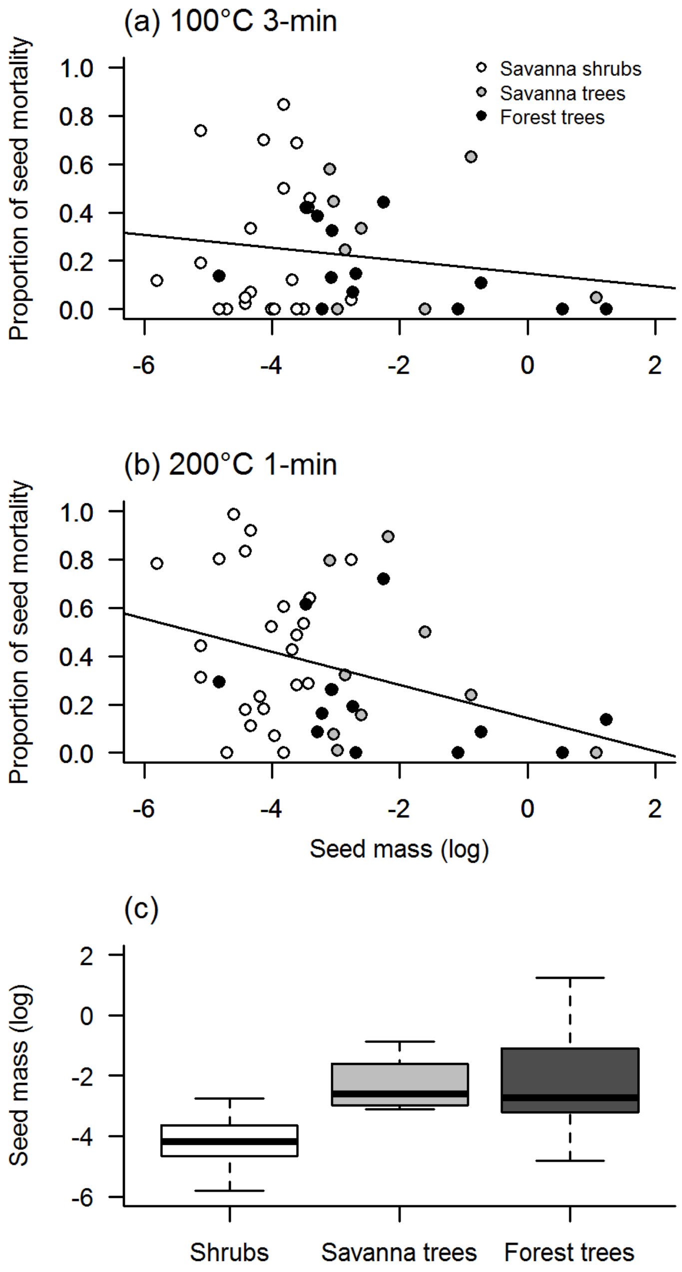


Fig. 2 (a) Proportion of seed mortality (dead seeds relative to controls) in relation to seed mass (log) in the 100°C 3-min treatment. **(b)** Proportion of seed mortality in relation to seed mass in the 200°C 1-min treatment. **(c)** Seed mass (log) for the different growth-forms (forest trees, savanna trees, savanna shrubs). See Table 2 for statistical tests.

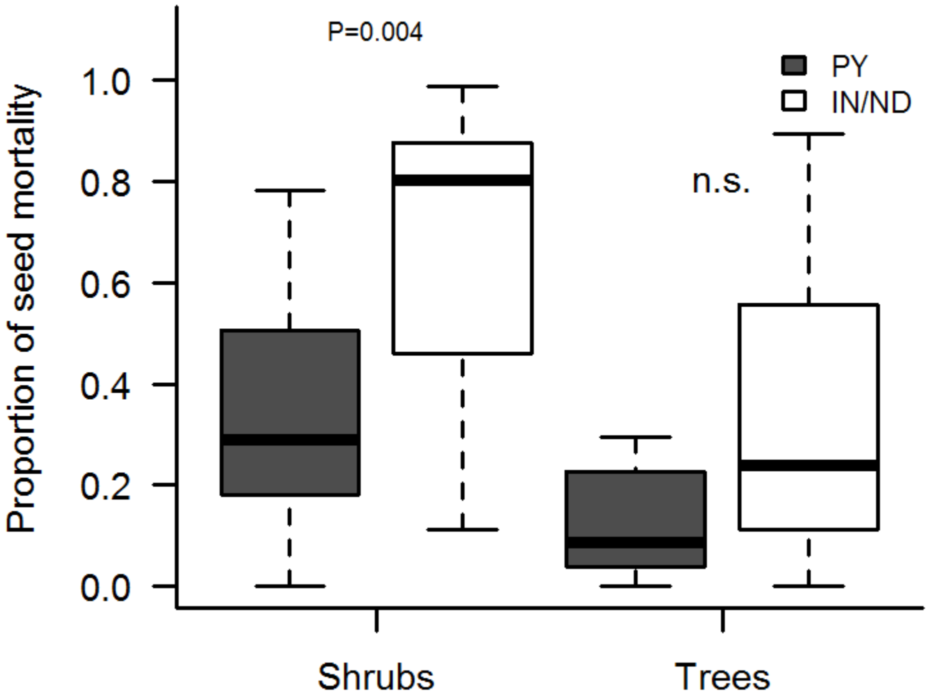


Fig. 3 Seed mortality under 200°C 1-min in relation to dormancy level (PY = physical dormancy; IN/ND = species with intermediate and/or non-dormant seeds) separately for the different growth-forms (shrubs and trees).

Discussion

Our results show that fire has a very limited role in triggering seed germination in the Cerrado. Heat shocks broke the seed dormancy in only few species (*six* out of 46) and their germination was never enhanced by more than 30% in relation to controls. This is especially relevant given that legumes are one of the clearest example of species with physical dormancy, and thus the group that is most likely to have evolved fire-stimulated germination in environments with recurrent fires. This limited role played by fire in the recruitment of Cerrado species strongly contrast with those from Mediterranean vegetation, where fire plays a key role in alleviating seed dormancy and stimulating germination (e.g. Herranz et al. 1998; Reyes and Trabaud 2009; Moreira et al. 2010).

Fire regimes vary substantially across the globe (Archibald et al. 2013; Lehmann et al. 2014; Pausas and Ribeiro 2017), and fire frequency might play a role on the selection of regeneration strategies (Bond and Midgley 2003; Pausas and Keeley 2009; Clarke et al. 2013) in the different flammable ecosystems. In Mediterranean ecosystems, fire return intervals are longer (decades), and fires are more severe than in savannas; thus fire may kill adult individuals of many non-resprouting species, whose persistence would thus rely on recruitment from seeding, favoring regeneration at population level (Pausas et al. 2004; Paula & Pausas 2008; Pausas and Keeley 2014). In contrast, the low intensity fires that occur in the savannas would make the evolution of bud protection more likely. Therefore, basal and epicormic resprouting seems to have been selected as the major regeneration strategy in relation to fire in many savannas, including the Brazilian ones (Simon and Pennington 2012; Zupo 2017; Pausas 2017; Pausas and Keeley *in press*).

Cerrado has diversified relatively recently (4-10 Mya), and shows a number of woody lineages which have originated from the surrounding fire-free forests (Simon et al. 2009; Simon & Pennington 2012). Tropical forest tree legumes usually show relatively larger seeds than (Rubio de Casas et al. 2017), and therefore this trait could have helped these species to persist and colonize the savannas (Ribeiro et al. 2015). Once established in the savannas, frequent burning would thus have selected for individuals showing the capacity to resprout; and might also help to explain the radiation of shrubs species which diversified quickly in the last ten million years (Simon et al. 2009; Simon & Pennington, 2012); a similar process seems to have occurred in African savannas (Maurin et al. 2014; Lamont et al. 2017). Parallel to the progressive decrease in the plant size, would have occurred a reduction of seed size, as these two traits are correlated (Leishman et al. 1995; Moles et al. 2005).

However, even the small-seeded shrub species showed a capacity to survive the 100°C 1-min treatment. This shows that seeds would survive fires, mainly when incorporated to the seed bank, since soil is an excellent heat insulator (Bradstock and Auld 1995; Bond et al. 1999; Gagnon et al. 2015). Therefore, seeds stored in the Cerrado seed banks would certainly tolerate the fire passage, because belowground temperatures rarely rise above 60°C in the Cerrado (Miranda et al. 1993; Daibes et al. 2017). Conversely, the density of buried propagules of woody species is low in the Cerrado seed banks (Salazar et al. 2011), and the proportion of seeds exposed in the soil surface during fires remains unknown. Hence, seed mass would be an important driver of seed mortality only when propagules are exposed to relatively severe conditions in the savannas.

The presence of PY has shown to be an important trait to predict seed mortality among the savanna shrubs under the 200°C treatment. Despite seed dormancy is not broken by fire in the Cerrado plants, PY shrub seeds experienced less mortality than the non-dormant ones. This supports the idea that dormancy class is also important to drive propagule persistence to fire (Rolston 1978; Long et al. 2015). This physical protection could help seeds to survive a fire episode, warranting some propagule supply for post-fire colonization (Jaureguiberry & Díaz 2015). Seed shape, instead, has shown little effect on predicting mortality of seeds in our study species. This contrast to the findings that rounded seeds would be more resistant to heating (Gómez-González et al. 2016).

Nevertheless, many shrubs from Cerrado are able to resprout after fires (Appezzato-da-Glória et al. 2008; Simon et al. 2009; Dantas et al. 2013) and show post-fire flowering enhancement (Coutinho 1976; Pausas 2017). Post-fire flowering and fruiting are important for Cerrado species, and might enhance germination by stimulating fruit set (Le Stradic et al. 2015). This strategy would synchronize seed shed with the post-fire environment, and this is consistent with the idea that direct fire heat shocks should not have been selected as a factor for dormancy-breaking. Other strategies for many Cerrado species might include seed dispersion synchronized with the onset of the rainy, so they have a chance to establish under a less stressful condition, with a better water supply in the soil (Salazar et al. 2011; Ramos et al 2017). One way or another, the process of seed germination would be influenced by indirect and/or non-fire-related factors. For instance, wet heating seems to be important an important factor on PY-breaking process in a number of legume species, both in fire-prone and non-fire-prone ecosystems (Moreno-Casasola et al. 1994; van Klinken et al. 2006; Geisler et al. 2017; Wiggers et al. 2017).

Both savannas and forest are in the same climatic conditions, and relative climatic stability might have shaped seed dormancy and germination patterns (Dayrell et al. 2017). Meanwhile, seed traits could at the same time follow the phylogenetic conservatism from their respective lineage, mainly seed mass and PY (Rubio de Casas et al. 2017). Summarily, larger forest seeds could provide some protection to the embryo in face of hotter fires, but this trait seems to have emerged before the settlement of Cerrado savannas. Thus, the small seeds from savanna shrubs would have higher likelihood of surviving fire when possessing PY or, otherwise, if buried in the soil seed bank. In conclusion, we have found little evidence for fire-mediated PY-breaking and seed germination in the Cerrado mosaic. We suggest that there are trade-offs between growth-form, phylogeny and seed traits that explain seed mortality; helping thus to understand seed regeneration in the tropical savannas.

Acknowledgments

We wish to thank H.L. Zirondi for providing germination data and laboratory work and T.M. Zupo for helping in the field work and during the laboratory experiments, as well as for the friendly review of the manuscript. We thank the *Instituto Florestal do Estado de São Paulo* for the collection permit (COTEC 260108 – 008.322/2015). We also thank the people who have helped during seed collection and sending seeds to us: A.B. Giroldo, A.G. Rodrigues-Junior, A.L.T. De Lucca, C. Blanco, D.E. Escobar, E.P. Dickfeldt, G.P. Sabino, J.R. Barosela, M.B. Cunha, M.F. Simon, Y. Nunes. L.M. Borges and M.F. Simon helped in the taxonomic identification, particularly in *Mimosa*. L.F. Daibes received a scholarship from the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES), and from the CAPES/PDSE Program (PDSE 88881.131702/2016-01). This study was supported by *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP 2015/06743-0), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 455183/2014-7), and *Fundação Grupo Boticário* (0153_2011_PR). F.A.O. Silveira (482720) and A. Fidelis (306170/2015-9) receive scholarships from CNPq.

References

Andrade, L.A.Z. and Miranda, H.S. (2014) The dynamics of the soil seed bank after a fire event in a woody savanna in central Brazil. *Plant Ecology* **215,** 1199-1209.

Appezzato-da-Glória, B., Cury, G., Misaki, M.K., Rocha, R. and Hayashi, A.H. (2008) Underground systems of Asteraceae species from the Brazilian Cerrado. *The Journal of the Torrey Botanical Society* **135,** 103-113.

Archibald, S., Lehmann, C.E.R., Gómez-Dans, J.L. and Bradstock, R.A. (2013) Defining pyromes and global syndromes of fire regimes. *Proceedings of the National Academy of Sciences* **110**, 6442-6447.

Auld, T.D. and O'Connell, M.A. (1991) Predicting patterns of post-fire germination in 35 eastern Australian Fabaceae. *Australian Journal of Ecology* **16,** 53-70.

Baskin, C.C. and Baskin, J.M. (2014) *Seeds: Ecology, biogeography, and evolution of dormancy and germination.* 2nd edition, San Diego: Academic Press.

Bates, D., Maechler, M., Bolker, B. and Walker, S. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67,** 1-48.

B.F.G., The Brazilian Flora Group (2015) Growing knowledge: an overview of seed plant diversity in Brazil. *Rodriguésia* **66,** 1085-1113.

Bond, W.J., Honig, M. and Maze, K.E. (1999) Seed size and seedling emergence: an allometric relationship and some ecological implications. *Oecologia* **120,** 132-136.

Bond, W.J. and Midgley, J.J. (2003) The evolutionary ecology of sprouting in woody plants. *International Journal of Plant Sciences* **164,** 103-114.

Bradshaw, S.D., Dixon, K.W., Hopper, S.D., Lambers, H. and Turner, S.R. (2011) Little evidence for fire-adapted plant traits in Mediterranean climate regions. *Trends in Plant Science* **16,** 69-76.

Bradstock, R.A. and Auld, T.D. (1995) Soil temperatures during experimental bushfires in relation to fire intensity: consequences for legume germination and fire management in South-Eastern Australia. *Journal of Applied Ecology* **32,** 76-84.

Clarke, P.J., Lawes, M.J., Midgley, J.J., Lamont, B.B., Ojeda, F., Burrows, G.E., Enright, N.J. and Knox, K.J.E. (2013) Resprouting as a key functional trait: how buds, protection and resources drive persistence after fire. *New Phytologist* **197,** 19-35.

Conceição, A.S., Queiroz, L.P., Lewis, G.P., Andrade, M.J.G., Almeida, P.R.M., Schnadelbach, A.S. and van den Berg, C. (2009) Phylogeny of *Chamaecrista* Moench (Leguminosae-Caesalpinioideae) based on nuclear and chloroplast DNA regions. *Taxon* **58,** 1168-1180.

Coutinho, L.M. (1976) Contribuição ao conhecimento do papel ecológico das queimadas na floração de espécies do Cerrado. Tese de Livre Docência. Universidade de São Paulo, São Paulo, Brazil.

Coutinho, L.M. (1982) Ecological effects of fire in Brazilian Cerrado. In: Huntley BJ, Walker BH, eds. *Ecology of tropical savannas.* Berlin: Springer-Verlag, pp. 273-291.

Daibes, L.F., Zupo, Z., Silveira, F.A.O and Fidelis, A. (2017) A field perspective on effects of fire and temperature fluctuation on Cerrado legume seeds. *Seed Science Research* **27,** 74-83.

Dantas, V.L., Batalha, M.A. and Pausas, J.G. (2013) Fire drives functional thresholds on the savanna-forest transition. *Ecology* **94,** 2454-2463.

Dantas, V.L., Hirota, M., Oliveira, R.S. and Pausas, J.G. (2016) Disturbance maintains alternative biome states. *Ecology Letters* **19,** 12-19.

Dayrell, R.L.C., Garcia, Q.S., Negreiros, D., Baskin, C.C., Baskin, J.M. and Silveira, F.A.O. (2017) Phylogeny strongly drives seed dormancy and quality in a climatically buffered hotspot for plant endemism. *Annals of Botany* **119,** 267-277.

Dayamba, S.D., Tigabu, M., Sawadogo, L. and Oden, PC. (2008) Seed germination of herbaceous and woody species of the Sudanian savanna-woodland in response to heat shock and smoke. *Forest Ecology and Management* **256,** 462-470.

Eiten, G. (1972) The Cerrado vegetation of Brazil. *The Botanical Review* **38,** 201-341.

Fichino, B., Dombrovski, J.R.G., Pivello, V.R. and Fidelis, A. (2016) Does fire trigger seed germination in the Neotropical savannas? Experimental tests with six Cerrado species. *Biotropica* **48,** 181-187.

Fidelis, A., Daibes, L.F. and Martins, A.R. (2016) To resist or to germinate? The effect of fire on legume seeds in Brazilian subtropical grasslands *Acta Botanica Brasilica* **30,** 147-151.

Gagnon, P.R., Passmore, H.A., Slocum, M., Myers, J.A., Harms, K.E., Platt, W.J. and Paine, C.E.T. (2015) Fuels and fires influence vegetation via above- and belowground pathways in a high-diversity plant community. *Journal of Ecology* **103,** 1009-1019.

Gashaw, M. and Michelsen, A. (2002) Influence of heat shock on seed germination of plants from regularly burnt savanna woodlands and grasslands in Ethiopia. *Plant Ecology* **159,** 83-93.

Geisler, G.E., Pinto, T.T., Santos, M. and Paulilo M.T.S. (2017) Seed structures in water uptake, dormancy release, and germination of two tropical forest Fabaceae species with physically dormant seeds. *Brazilian Journal of Botany* **40,** 67-77.

Gómez-González, S., Ojeda, F., Torres-Morales, P. and Palma, J.E. (2016) Seed pubescence and shape modulate adaptive responses to fire cues. *PLoS ONE* **11,** e0159655.

Gómez-González, S., Torres-Díaz, C., Bustos-Schindler, C. and Gianoli, E. (2011) Anthropogenic fire drives the evolution of seed traits. *Proceedings of the National Academy of Sciences* **108,** 18743-18747.

Hanley, M.E., Unna, J.E. and Darvill, B. (2003) Seed size and germination response: a relationship for fire-following plant species exposed to thermal shock. *Oecologia* **134,** 18-22.

Herranz, J.M., Ferrandis, P. and Martínez-Sánchez, J.J. (1998) Influence of heat on seed germination of seven Mediterranean *Leguminosae* species. *Plant Ecology* **136,** 95-103.

Hilhorst, H.W.M. (2011) Standardazing seed dormancy research. In: Kermode AR, ed. *Seed Dormancy: Methods and Protocols*, Methods in Molecular Biology, vol. 773. Springer Science+Business Media.

Hoffmann, W.A. (2000) Post-establishment seedling success in the Brazilian Cerrado: a comparison of savanna and forest species. *Biotropica* **32,** 62-69.

Hoffmann, W.A., Geiger, E.L., Gotsch, S.G., Rossatto, D.R., Silva, L.C.R., Lau, O.L., Haridasan, M. and Franco, A.C. (2012) Ecological thresholds at the savanna-forest boundary:

how plant traits, resources and fire govern the distribution of tropical biomes. *Ecology Letters* **15,** 759-768.

Jaganathan, G.K. (2015) Are wildfires an adapted ecological cue breaking physical dormancy in the Mediterranean basin? *Seed Science Research* **25,** 120-126.

Jaureguiberry, P. and Díaz, S. (2015) Post-burning regeneration of the Chaco seasonally dry forest: germination response of dominant species to experimental heat shock. *Oecologia* **177,** 689-699.

Jiménez-Alfaro, B., Silveira, F.A.O., Fidelis, A., Poschlod, P. and Commander, L.E. (2016), Seed germination traits can contribute better to plant community ecology. *Journal of Vegetation Science* **27,** 637-645.

Jombart, T., Balloux, F. and Dray, S. (2010) *adephylo*: new tools for investigating the phylogenetic signal in biological traits. *Bioinformatics* **26,** 1907-1909.

Keeley, J.E., Pausas, J.G., Rundel, P.W., Bond, W.J. and Bradstock, R.A. (2011) Fire as an evolutionary pressure shaping plant traits. *Trends in Plant Science* **16,** 406-411.

Lamont, B.B. and He, T. (2017) Fire-proneness as a prerequisite for the evolution of fire-adapted traits. *Trends in Plant Science* **22,** 278-288.

Lamont, B.B., He, T. and Pausas, J.G. (2017) African geoxyles evolved in response to fire; frost came later. *Evolutionary Ecology* **31,** 603-617.

Lehmann, C.E.R., Anderson, T.M., Sankaran, M., Higgins, S.I., Archibald, S., Hoffmann, W.A., Hanan, N.P., Williams, R.J., Fensham, R.J., Felfili, J., Hutley, L.B., Ratnam, J., San Jose, J., Montes, R., Franklin, D., Russell-Smith, J., Ryan, C.M., Durigan, G., Hiernaux, P., Haidar, R., Bowman, D.M.J.S. and Bond, W.J. (2014) Savanna vegetation-fire-climate relationships differ among continents. *Science* **343,** 548-552.

Leishman, M.R., Westoby, M. and Jurado, E. (1995) Correlates of seed size variation: a comparison among five temperate floras. *Journal of Ecology* **83,** 517-530.

Le Stradic, S., Silveira, F.A.O., Buisson, E., Cazelles, K., Carvalho, V. and Fernandes, G.W. (2015) Diversity of germination strategies and seed dormancy in herbaceous species of *campo rupestre* grasslands. *Austral Ecology* **40,** 537-546.

L.P.W.G., The Legume Phylogeny Working Group (2017) A new subfamily classification of the Leguminosae based on a taxonomically comprehensive phylogeny. *Taxon* **66,** 44-77.

Long, R.L., Gorecki, M.J., Renton, M., Scott, J.K., Colville, L., Goggin, D.E., Commander, L.E., Westcott, D.A., Cherry, H. and Finch-Savage, W.E. (2015) The ecophysiology of seed persistence: a mechanistic view of the journey to germination or demise. *Biological Reviews* **90,** 31-59.

Maddison, W. P. and Maddison. D.R. (2017) Mesquite: a modular system for evolutionary analysis. Version 3.2. http://mesquiteproject.org

Marazzi, B., Endress, P.K., Queiroz, L.P. and Conti, E. (2006) Phylogenetic relationships within *Senna* (Leguminosae, Cassiinae) based on three chloroplast DNA regions: Patterns in the evolution of floral symmetry and extrafloral nectaries. *American Journal of Botany* **93,** 288-303.

Maurin, O., Davies, J.T., Burrows, J.E., Daru, B.H., Yessoufou, K., Muasya, A.M., van der Bank, M. and Bond, W.J. (2014) Savanna fire and the origins of the ‘underground forests’ of Africa. *New Phytologist* **204,** 201-214.

Miranda, A.C., Miranda, H.S., Dias, I.F.O and Dias, B.F.S. (1993) Soil and air temperatures during prescribed cerrado fires in Central Brazil. *Journal of Tropical Ecology* **9,** 313-320. 1993.

Miranda, H.S., Sato, M.N., Neto, W.N. and Aires, F.S. (2009) Fires in the cerrado, the Brazilian savanna. In: Cochrane MA, ed. *Tropical Fire Ecology: Climate change, land use and ecosystem dynamics.* Berlin: Springer-Praxis, pp. 427-450.

Moles, A.T., Ackerly, D.D., Webb, C.O., Tweddle, J.C., Dickie, J.B., Pitman, A.J. and Westoby, M. (2005) Factors that shape seed mass evolution. *Proceedings of the National Academy of Sciences* **102,** 10540-10544.

Moreira, B. and Pausas, J.G. (2012) Tanned or burned: the role of fire in shaping physical seed dormancy. *PLoS ONE* **7,** e51523.

Moreira, B., Tormo, J., Estrelles, E. and Pausas, J.G. (2010) Disentangling the role of heat and smoke as germination cues in Mediterranean Basin flora. *Annals of Botany* **105,** 627-635.

Moreno-Casasola, P., Grime, J.P. and Martínez, M.L. (1994) A comparative study of the effects of fluctuations in temperature and moisture supply on hard coat dormancy in seeds of coastal tropical legumes in Mexico. *Journal of Tropical Ecology* **10,** 67-86.

Morrison, D.A., McClay, K., Porter, C. and Rish, S. (1998) The role of the lens in controlling heat-induced breakdown of testa-imposed dormancy in native Australian legumes. *Annals of Botany* **82,** 35-40.

Morrison, D.A. and Morris, E.C. (2000) Pseudoreplication in experimental designs for the manipulation of seed germination treatments. *Austral Ecology* **25,** 292-296.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., Fonseca, G.A.B. and Kent, J. (2000) Biodiversity hotspots for conservation priorities. *Nature* **403,** 853-858.

Oliveira-Filho, A.T. and Ratter, J.A. (2002) Vegetation physiognomies and woody flora of the Cerrado biome. In: Oliveira PS, Marquis RJ, eds. *The cerrados of Brazil: ecology and natural history of a neotropical savanna.* New York, USA: Columbia University Press, 91-120.

Ooi, M.K.J., Denham, A.J., Santana, V.M. and Auld, T.D. (2014) Temperature thresholds of physically dormant seeds and plant functional response to fire: variation among species and relative impact of climate change. *Ecology and Evolution* **4,** 656-671.

Paradis, E., Claude, J. and Strimmer, K. (2004) APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20,** 289-290.

Paula, S. and Pausas, J.G. (2008) Burning seeds: germinative response to heat treatments in relation to resprouting ability. *Journal of Ecology* **96**, 543-552.

Pausas, J.G. (2017) Homage to L. M. Coutinho: fire adaptations in cerrado plants. *International Journal of Wildland Fire* **26,** 249-251.

Pausas, J.G., Bradstock, R.A., Keith, D.A., Keeley, J.E. and GCTE Fire Network. (2004) Plant functional traits in relation to fire in crown-fire ecosystems. *Ecology* **85,** 1085-1100.

Pausas, J.G. and Keeley, J.E. (2009) A burning story: the role of fire in the history of life. *BioScience* **59,** 593-601.

Pausas, J.G. and Keeley, J.E. (2014) Evolutionary ecology of resprouting and seeding in fire-prone ecosystems. *New Phytologist* **204,** 55-65.

Pausas, J.G. and Keeley, J.E. (*in press*) Epicormic resprouting in fire-prone ecosystems. *Trends in Plant Science* http://dx.doi.org/10.1016/j.tplants.2017.08.010

Pausas, J.G. and Ribeiro, E. (2017) Fire and plant diversity at the global scale. *Global Ecology and Biogeography* **26,** 889-897.

Pérez-Harguindeguy, N., Díaz, S., Garnier, E. et al. (2013) New handbook for standardized measurement of plant functional traits worldwide. *Australian Journal of Botany* **61,** 167-234.

Ramos, D.M., Diniz, P., Ooi, M.K.J., Borghetti, F. and Valls, J.F.M. (2017) Avoiding the dry season: dispersal time and syndrome mediate seed dormancy in grasses in Neotropical savanna and wet grasslands. *Journal of Vegetation Science* **28**, 798-807.

Ramos, D.M., Liaffa, A.B.S., Diniz, P., Munhoz, C.B.R., Ooi, M.K.J., Borghetti, F. and Valls, J.F.M. (2016) Seed tolerance to heating is better predicted by seed dormancy than by habitat type in Neotropical savanna grasses. *International Journal of Wildland Fire* **25**, 1273-1280.

R Core Team. (2016) *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria. R version 3.2.5. https://www.R-project.org/

Reyes, O. and Trabaud, L. (2009) Germination behaviour of 14 Mediterranean species in relation to fire factors: smoke and heat. *Plant Ecology* **202,** 113-121.

Ribeiro, L.C., Barbosa, E.R.M., van Langevelde, F. and Borghetti, F. (2015) The importance of seed mass for the tolerance to heat shocks of savanna and forest tree species. *Journal of Vegetation Science* **26,** 1102-1111.

Ribeiro, L.C. and Borghetti, F. (2014) Comparative effects of desiccation, heat shock and high temperatures on seed germination of savanna and forest tree species. *Austral Ecology* **39,** 267-278.

Ribeiro, L.C., Pedrosa, M. and Borghetti, F. (2013). Heat shock effects on seed germination of five Brazilian savanna species. *Plant Biology* **15,** 152-157.

Rissi, M.N., Baeza, M.J., Gorgone-Barbosa, E., Zupo, T. and Fidelis, A. (2017) Does season affect fire behaviour in the Cerrado? *International Journal of Wildland Fire* **26,** 427-433.

Rizzini, C.T. (1976) Influência da temperatura sobre a germinação de diásporos do cerrado. *Rodriguésia* **28,** 341-381.

Rolston, M.P. (1978) Water impermeable seed dormancy. *The Botanical Review* **44,** 365-396.

RStudio Team (2016). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA. Version 1.0.136. http://www.rstudio.com/

Rubio de Casas, R., Willis, C.G., Pearse, W.D., Baskin, C.C., Baskin, J.M. and Cavender-Bares, J. (2017) Global biogeography of seed dormancy is determined by seasonality and seed size: a case study in the legumes. *New Phytologist* **214,** 1527-1536.

Salazar, A., Goldstein, G., Franco, A.C. and Miralles-Wilhelm, F. (2011) Timing of seed dispersal and dormancy, rather than persistent soil seedbanks, control seedling recruitment of woody plants in Neotropical savannas. *Seed Science Research* **21,** 103-116.

Saracino, A., Bellino, A., Allevato, E. Mingo, A., Conti, S., Rossi, S., Bonanomi, G., Carputo, D. and Mazzoleni, S. (2017) Repeated stand-replacing crown fires affect seed morphology and germination in Aleppo pine. *Frontiers in Plant Science* **8,** 1160.

Simon, M.F., Grether, R., Queiroz, L.P., Särkinen, T.E., Dutra, V.F. and Hughes, C.E. (2011) The evolutionary history of *Mimosa* (Leguminosae): Toward a phylogeny of the sensitive plants. *American Journal of Botany* **98,** 1201-1221.

Simon, M.F., Grether, R., Queiroz, L.P., Skema, C., Pennington, R.T. and Hughes, C. (2009) Recent assembly of the Cerrado, a neotropical plant diversity hotspot, by in situ evolution of adaptations to fire. *Proceedings of the National Academy of Sciences* **106,** 20359-20364.

Simon, M.F. and Pennington, T. (2012) Evidence for adaptation to fire regimes in the tropical savannas of the Brazilian Cerrado. *International Journal of Plant Sciences* **173,** 711-723.

Thompson, K., Band, S.R. and Hodgson, J.G. (1993) Seed size and shape predict persistence in soil. *Functional Ecology* **7,** 236-241.

van Klinken, R.D., Flack, L.K. and Pettit, W. (2006) Wet-season dormancy release in seed banks of a tropical leguminous shrub is determined by wet heat. *Annals of Botany* **98,** 875-883.

Whelan, R.J. (1995) *The Ecology of Fire.* Cambridge, UK: Cambridge University Press.

Wiggers, M.S., Hiers, J.K., Barnett, A., Boyd, R.S. and Kirkman, L.K. (2017) Seed heat tolerance and germination of six legume species native to a fire-prone longleaf pine forest. *Plant Ecology* **218,** 151-171.

Williams, P.R., Congdon, R.A., Grice, A.C. and Clarke, P.J. (2003) Fire-related cues break seed dormancy of six legumes of tropical eucalypt savannas in north-eastern Australia. *Austral Ecology* **28,** 507-514.

Webb, C.O., Ackerly, D.D. and Kembel, S.W. (2008) Phylocom: software for the analysis of phylogenetic community structure and trait evolution. *Bioinformatics* **24,** 2098-2100.

Wojciechowski, M.F., Lavin, M. and Sanderson, M.J. (2004) A phylogeny of legumes based on analysis of the plastid *matK* gene resolves many well-supported subclades within the family. *American Journal of Botany* **91,** 1846-1862.

Zupo, T. (2017) Estratégias de persistência e regeneração em campo sujo de Cerrado após o fogo. *PhD Thesis.* Universidade Estadual Paulista, Rio Claro/SP, Brazil.

Zupo, T., Baeza, M.J. and Fidelis, A. (2016) The effect of simulated heat-shock and daily temperature fluctuations on seed germination of four species from fire-prone ecosystems. *Acta Botanica Brasilica* **30,** 514-519.

Zuur A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A. and Smith G.M. (2009) *Mixed effects models and Extensions with Ecology in R.* Statistics for Biology and Health. New York: Springer.

Supporting Information

Table S1 Species, environment, growth-form, seed dormancy class (PY = physical dormancy; IN = intermediate dormancy; ND = non-dormant seeds), site (see legend below), date of collection and date of the experiment setup for 46 legumes from the Brazilian savanna-forest mosaic.

Species	**Environment**	**Growth-form**	**Dorm. class**	**Site**	**Date collection**	**Date experiment**
Albizia niopoides	forest	tree	ND	PEPF	Oct/2015	Apr/2016
Anadenanthera colubrina	forest	tree	ND	RIB	Sep/2015	Apr/2016
Anadenanthera falcata	woody savanna	tree	ND	ITI	Aug/2015	Apr/2016
Bauhinia dumosa	open savanna	shrub	ND	RNST	Oct/2014	Jul/2015
Bauhinia membranacea	forest	tree	ND	PGO	Jun/2014	Apr/2016
Chamaecrista claussenii	open savanna	shrub	PY	RNST	Oct/2016	Nov/2016
Chamaecrista desvauxii	open savanna	shrub	PY	RNST	May/2014	Feb/2015
Chamaecrista ochrosperma	open savanna	shrub	PY	RNST	Jul/2014	Ago/2014
Chamaecrista viscosa	open savanna	shrub	PY	PEJ	Jul/2014	Ago/2014
Copaifera langsdorffii	forest	tree	ND	PEPF	Aug/2015	Apr/2016
Copaifera oblongifolia	woody savanna	tree	ND	CHG	Aug/2013	Nov/2016
Crotalaria sp.	open savanna	shrub	PY	CIP	Feb/2015	Jun/2015
Dalbergia miscolobium	woody savanna	tree	ND	ITI	May/2015	Jun/2015
Dimorphandra mollis	woody savanna	tree	IN	ITI	May 2015	Apr/2016
Erythrina speciosa	forest	tree	PY	RCL	Sep/2015	Apr/2016
Harpalyce brasiliana	open savanna	shrub	PY	RNST	Jul/2014	Aug/2016
Hymenaea courbaril	forest	tree	IN	PEPF/RCL	Oct/2015	Apr/2016
Hymenaea stigonocarpa	savanna	tree	ND	RNST	Oct/2015	Apr/2016
Mimosa claussenii	open savanna	shrub	PY	RNST	Oct/2014	Feb/2015
Mimosa echinocaula	open savanna	shrub	PY	PIR	Jun/2016	Nov/2016
Mimosa foliolosa	open savanna	shrub	PY	DEL	Aug/2013	Nov/2016
Mimosa gracilis	open savanna	subshrub	ND	RNST	Jul/2014	Ago/2014
Mimosa kalunga	open savanna	shrub	PY	RNST	Oct/2014	Feb/2015
Mimosa leiocephala	open savanna	shrub	PY	RNST	Oct/2014	Oct/2015
Mimosa oligosperma	open savanna	shrub	PY	RNST	Oct/2015	Apr/2016
Mimosa paludosa	open savanna	shrub	IN	ITI	Oct/2015	Apr/2016
Mimosa pteridifolia	open savanna	shrub	ND	RNST	Jul/2012	Nov/2012
Mimosa somnians	open savanna	subshrub	PY	PEJ	Jul/2014	Ago/2014
Mimosa sp1	open savanna	tree	ND	RNST	Oct/2014	Feb/2015
Mimosa sp2	open savanna	shrub	PY	RNST	Oct/2014	Feb/2015
Mimosa speciosissima	open savanna	shrub	IN	BSB	Apr/2014	Nov/2016
Mimosa spixiana	open savanna	shrub	IN	PEJ	Jul/2014	Feb/2015

Peltophorum dubium	forest	tree	PY	PEPF	Oct/2015	Apr/2016
Plathymenia reticulata	woody savanna	tree	IN	ITI	Sep/2015	Apr/2016
Schizolobium parahyba	forest	tree	PY	PEPF/RCL	Aug/2015	Apr/2016
Senegalia polyphylla	forest	tree	ND	PEPF	Oct/2015	Apr/2016
Senna alata	forest	tree	PY	BSB	Ago/2013	Nov/2016
Senna cana	open savanna	shrub/tree	PY	RNST	Oct/2014	Feb/2015
Senna corifolia	open savanna	shrub	PY	RNST	Oct/2014	Feb/2015
Senna hirsuta	savanna/forest	shrub	IN	BSB	Ago/2013	Nov/2016
Senna multijuga	forest	tree	PY	LAV	Sep/2014	Apr/2016
Senna silvestris	open savanna	shrub/tree	IN	RNST	Oct/2014	Feb/2015
Senna spectabilis	forest	tree	ND	NMG	Aug/2015	Apr/2016
Stryphnodendron adstringens	woody savanna	tree	PY	BSB	Aug/2013	Nov/2016
Stryphnodendron obovatum	woody savanna	tree	IN	ITI	Sep/2015	Apr/2016
Vachellia caven	forest	tree	PY	URU	Jan/2016	Apr/2016

PEPF: *Parque Estadual Porto Ferreira* (SP 21°50’S; 47°26’W), **RIB:** *Campus* USP, Ribeirão Preto (SP 21°10’S; 47°48’W), **ITI:** Itirapina (SP 22°15’S; 47°53’W), **RNST:** *Reserva Natural Serra do Tombador*, Cavalcante (GO 13°39’S; 47°49’W), **PGO:** Posse, Northeastern of Goiás state (GO 14°04’S; 46°28’W), **PEJ:** *Parque Estadual do Jalapão* (TO 10°22’S; 46°40’W), **CHG:** Chapada Gaúcha, Northern of Minas Gerais state (MG 15°26’S; 45°27’W), **CIP:** *Serra do Cipó* (MG 19°22’S; 43°32’W), **RCL:** *Campus* Unesp, Rio Claro (SP 22°23’S; 47°32’W), **PIR:** *Parque Estadual dos Pireneus*, Pirenópolis (GO 15°47’S; 48°50’W), DEL: Delfinópolis, Southeastern of Minas Gerais state (MG 20°20’S; 46°48’W), **BSB:** Brasília (DF 15°47’S; 47°52’W), **LAV:** *Campus* UFLA, Lavras (MG 21°13’S; 44°58’W), **NMG:** Northern of Minas Gerais state, border with Bahia (MG 14°53’S; 44°41’W), **URU:** *Espinilho* region, Uruguaiana (RS 29°45’S; 57°05’W).

Table S2 Seed germination (mean ± standard deviation) in fire-related heat shocks. Asterisks indicate significant differences in relation to the control (baseline) in the GLMM analysis (binomial distribution, replicates as random effects). * = *P* ≤0.05; ** = *P* ≤0.01; *** = *P* ≤0.001.

Species	**Control**	**100°C 1 min**	**100°C 3 min**	**200°C 1 min**
Albizia niopoides	58±12	65±13	34±26	28±34*
Anadenanthera colubrina	86±11	78±18	48±37**	24±18***
Anadenanthera falcata	65±22	63±10	49±27	44±32
Bauhinia dumosa[1]	93±8	95±4	92±6	19±27***
Bauhinia membranacea	66±12	76±16	58±7	75±12
Chamaecrista claussenii	18±18	11±4	8±5	11±9
Chamaecrista desvauxii	2±4	4±5	NA	NA
Chamaecrista ochrosperma	22±9	14±10	NA	20±16
Chamaecrista viscosa	17±12	17±7	12±9	15±6
Copaifera langsdorffii	86±4	85±10	75±9	78±4
Copaifera oblongifolia	82±13	62±18	43±40**	55±12*
Crotalaria sp.	25±7	26±14	38±22	27±17
Dalbergia miscolobium	76±21	78±10	NA	10±12***
Dimorphandra mollis	11±8	12±8	18±6	6±11
Erythrina speciosa	26±13	26±21	38±17	40±16
Harpalyce brasiliana	10±8	20±29	25±15	18±21
Hymenaea courbaril	19±14	14±15	6±9	18±15
Hymenaea sp.	93±15	92±9	89±19	96±10
Mimosa claussenii	19±13	24±17	21±10	9±12
Mimosa echinocaula	7±4	6±4	8±4	14±15
Mimosa foliolosa	2±4	2±4	6±13	2±4
Mimosa gracilis	45±20	42±19	41±16	5±12***
Mimosa kalunga	7±5	7±8	30±20**	3±5
Mimosa leiocephala[1]	4±5	7±3	19±10**	19±15**
Mimosa oligosperma	4±5	16±19	32±16**	12±11
Mimosa paludosa	15±9	NA	3±6*	10±8
Mimosa pteridifolia[1]	56±14	16±16***	NA	0±0***
Mimosa somnians	16±5	27±20	15±11	15±6
Mimosa sp1	97±3	93±3	100±0	96±2
Mimosa sp2	4±7	14±13*	4±5	8±10
Mimosa speciosissima	25±7	8±7**	0±0**	7±5**
Mimosa spixiana	37±9	41±7	40±12	5±7***
Peltophorum dubium	5±6	5±4	13±17	16±11
Plathymenia reticulata	47±17	42±21	25±23*	10±8***

Schizolobium parahyba	1±0	1±0	1±0	1±0
Senegalia polyphylla	92±8	91±10	78±19	69±15**
Senna alata	15±8	7±6	4±8	12±14
Senna cana	4±6	5±5	27±14***	25±16**
Senna corifolia	2±3	2±4	0±0	2±3
Senna hirsuta	28±11	15±8	19±20	32±23
Senna multijuga	3±4	5±7	6±7	14±12*
Senna silvestris	40±24	46±7	44±15	5±9***
Senna spectabilis	46±25	53±29	13±12**	30±26
Stryphnodendron adstringens	14±12	10±8	8±7	5±4
Stryphnodendron obovatum	25±9	24±12	8±9**	21±15
Vachellia caven	3±5	5±5	1±2	1±2

---

[1] Data provided by H.L. Zirondi et al. *unpubl. data*

Table S3 Seed viability (mean ± standard deviation) after fire-related heat shocks. Asterisks indicate significant differences in relation to the control (baseline) in the GLMM analysis (binomial distribution, replicates as random effects). * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$.

Species	**Control**	**100°C 1 min**	**100°C 3 min**	**200°C 1 min**
Albizia niopoides	83±9	89±4	49±37*	33±36**
Anadenanthera colubrina	86±11	78±18	48±37**	24±18***
Anadenanthera falcata	65±22	63±10	49±27	44±32
Bauhinia dumosa^1	98±3	99±2	98±4	20±29***
Bauhinia membranacea	66±12	76±16	58±7	75±12
Chamaecrista claussenii	66±22	54±18	29±37	51±41
Chamaecrista desvauxii	86±9	92±18	NA	NA
Chamaecrista ochrosperma	84±8	84±7	NA	63±22**
Chamaecrista viscosa	80±27	97±4	95±5	76±24
Copaifera langsdorffii	92±8	90±6	82±9*	84±7
Copaifera oblongifolia	92±13	78±11	57±51*	74±18
Crotalaria sp.	97±3	98±3	93±10	45±29***
Dalbergia miscolobium	76±21	78±10	NA	10±12***
Dimorphandra mollis	22±10	14±9	24±8	11±12
Erythrina speciosa	80±8	100±0	84±18	88±13
Harpalyce brasiliana	51±13	23±28*	27±15	18±21***
Hymenaea courbaril	59±14	54±15	58±13	50±19
Hymenaea sp.	93±15	92±9	89±19	96±10
Mimosa claussenii	80±13	84±5	92±6	39±25***
Mimosa echinocaula	98±4	97±4	80±22	56±42*
Mimosa foliolosa	81±6	89±20	68±16	83±21
Mimosa gracilis	47±23	44±20	45±18	9±21***
Mimosa kalunga	48±14	46±15	59±25	23±18*
Mimosa leiocephala^1	74±15	85±6	83±17	57±31
Mimosa oligosperma	98±4	96±5	88±9	54±24***
Mimosa paludosa	27±13	NA	18±12	24±15
Mimosa pteridifolia^1	78±11	33±16***	NA	1±2***
Mimosa somnians	88±8	94±4	92±9	79±15
Mimosa sp1	100±0	99±2	100±0	100±0
Mimosa sp2	91±9	86±9	54±31*	64±37*
Mimosa speciosissima	44±15	21±17*	11±14**	17±8**
Mimosa spixiana	99±2	96±4	92±6	8±12***
Peltophorum dubium	71±16	65±4	61±22	50±25*
Plathymenia reticulata	70±20	61±24	29±29**	14±13***

Schizolobium parahyba	83±9	88±14	86±9	92±7
Senegalia polyphylla	92±8	91±10	78±19	69±15**
Senna alata	95±7	94±6	79±16**	85±10
Senna cana	90±9	75±26	85±11	73±20*
Senna corifolia	81±6	68±38*	25±28**	41±32*
Senna hirsuta	81±11	76±12	34±33**	56±31
Senna multijuga	87±13	75±23	78±29	64±27
Senna silvestris	84±12	91±7	82±7	14±21***
Senna spectabilis	50±24	65±27	51±33	42±34
Stryphnodendron adstringens	68±11	60±13	47±20*	65±12
Stryphnodendron obovatum	51±14	50±12	35±27	43±35
Vachellia caven	58±16	60±12	55±13	49±15

[1] Data provided by H.L. Zirondi et al. *unpubl. data*

Table S4 Number of sampled individuals, total number of seeds measured in the morphological traits (seed mass and seed shape), seed mass values (g; mean ± standard deviation), seed shape values (mean ± standard deviation) and the number of seeds per replicate in the germination tests for 46 legumes from the Brazilian savanna-forest mosaic. NA indicates non-available data.

Species	**N indiv.**	**N seeds traits**	**Seed mass**	**Seed shape**	**N seeds repl.**
Albizia niopoides	5	100	0.031±0.007	0.157±0.014	20
Anadenanthera colubrina	5	60	0.105±0.032	0.243 ± 0.024	10
Anadenanthera falcata	7	140	0.057±0.008	0.279±0.051	20
Bauhinia dumosa[1]	10	172	0.063±0.017	0.073±0.026	20
Bauhinia membranacea	NA	100	0.068±0.020	0.173±0.174	20
Chamaecrista claussenii	6	88	0.016±0.004	0.094±0.019	20
Chamaecrista desvauxii	5	98	0.005±0.001	0.203±0.011	10
Chamaecrista ochrosperma	11	136	0.015±0.003	0.101±0.017	20
Chamaecrista viscosa	5	100	0.009±0.001	0.106±0.019	20
Copaifera langsdorffii	6	110	0.480±0.093	0.039±0.012	20
Copaifera oblongifolia	NA	100	0.414±0.122	0.039±0.014	10
Crotalaria sp.	3	60	0.003±0.000	0.053±0.010	20
Dalbergia miscolobium	3	31	0.112±0.026	0.221±0.007	10
Dimorphandra mollis	5	100	0.201±0.032	0.130±0.017	20
Erythrina speciosa	6	114	0.336±0.071	0.083±0.021	10
Harpalyce brasiliana[1]	8	121	0.033±0.006	0.057±0.017	20
Hymenaea courbaril	4	47	3.447±0.711	0.052±0.023	10
Hymenaea stigonocarpa	10	119	2.946±1.036	0.083±0.031	10
Mimosa claussenii	11	200	0.030±0.006	0.114±0.019	20
Mimosa echinocaula	NA	100	0.006±0.001	0.112±0.022	20
Mimosa foliolosa	NA	100	0.022±0.005	0.086±0.027	10
Mimosa gracilis	9	78	0.008±0.003	0.120±0.043	20
Mimosa kalunga	5	100	0.018±0.003	0.053±0.019	20
Mimosa leiocephala[1]	11	216	0.027±0.004	0.078±0.025	20
Mimosa oligosperma	10	182	0.025±0.004	0.066±0.018	10

Mimosa paludosa	7	92	0.013±0.003	0.051±0.017	20
Mimosa pteridifolia[1]	11	189	0.010±0.002	0.124±0.020	20
Mimosa somnians	4	67	0.019±0.005	0.113±0.016	20
Mimosa sp1	5	89	0.051±0.009	0.176±0.027	20
Mimosa sp2	5	100	0.032±0.005	0.093±0.017	20
Mimosa speciosissima	NA	100	0.022±0.005	0.098±0.021	15
Mimosa spixiana	5	51	0.013±0.002	0.139±0.017	20
Peltophorum dubium	3	60	0.046±0.006	0.173±0.009	20
Plathymenia reticulata	8	160	0.045±0.009	0.186±0.017	20
Schizolobium parahyba	6	112	1.732±0.336	0.178±0.004	20
Senegalia polyphylla	6	120	0.047±0.013	0.171±0.015	20
Senna alata	NA	100	0.037±0.005	0.124±0.022	20
Senna cana	9	176	0.012±0.002	0.052±0.019	20
Senna corifolia	11	165	0.027±0.007	0.136±0.018	20
Senna hirsuta	NA	100	0.006±0.001	0.085±0.022	20
Senna multijuga	NA	100	0.008±0.001	0.221±0.011	20
Senna silvestris	7	120	0.012±0.003	0.152±0.014	20
Senna spectabilis	NA	100	0.040±0.006	0.131±0.011	20
Stryphnodendron adstringens	NA	100	0.048±0.019	0.101±0.024	20
Stryphnodendron obovatum	4	80	0.074±0.013	0.093±0.016	20
Vachellia caven	5	100	0.065±0.011	0.084±0.018	20

---

[1] Data provided by H.L. Zirondi et al. *unpubl. data*

Fig. S1 Phylogenetic tree and seed traits (seed mass, seed shape, permeability, and mortality at 200°C 1-min) for legume species from the Brazilian savanna-forest mosaic. *Chamaecrista desvauxii* was excluded from the figure because it was not tested under 200°C. Subfamilies are classified according to LPWG (2017). Caption needs improvement. Square colors indicate life-form: forest trees (green), savanna trees (red), and shrubs (black). Circle sizes correspond to trait values, re-scaled to vary between zero and one for all the traits.

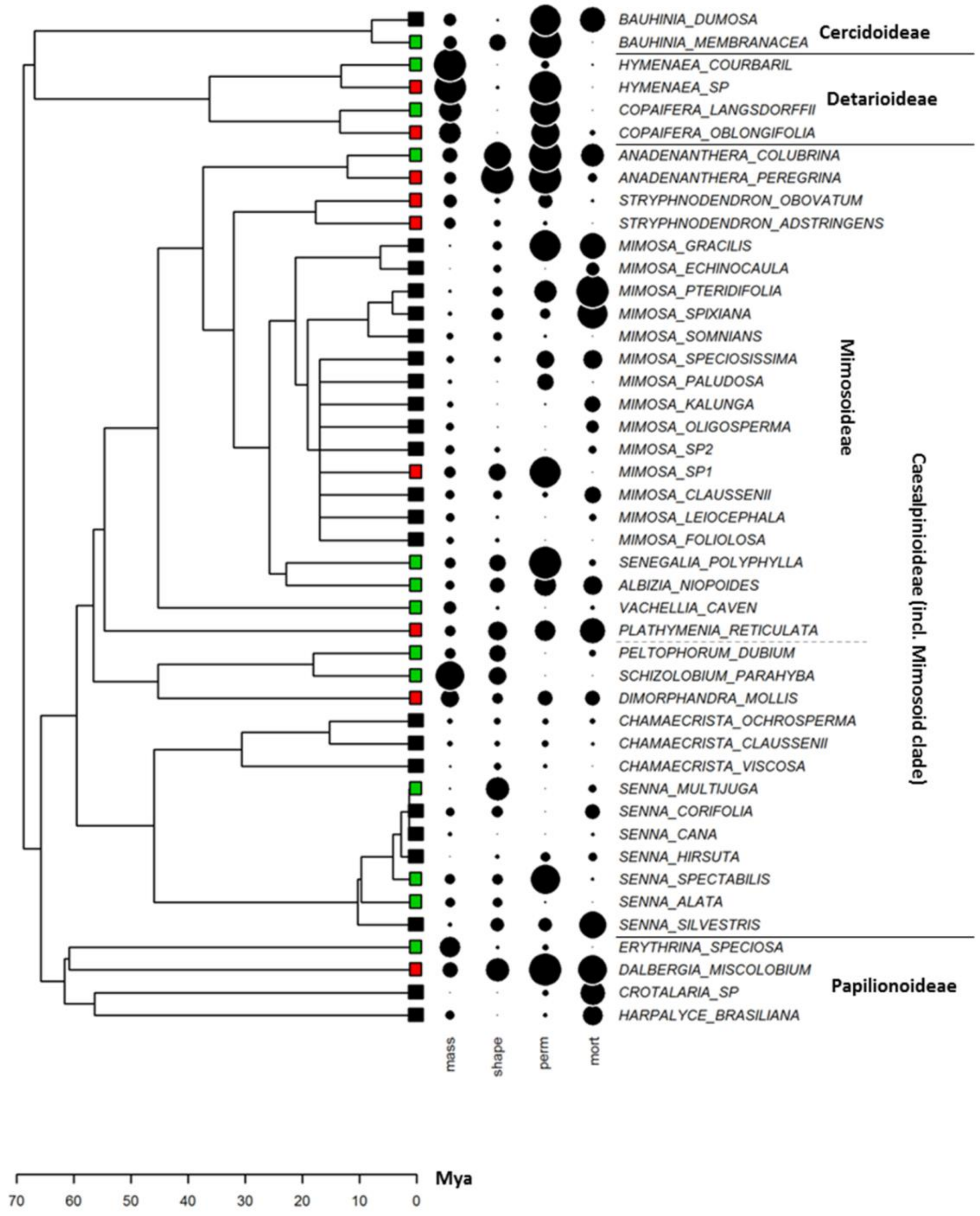
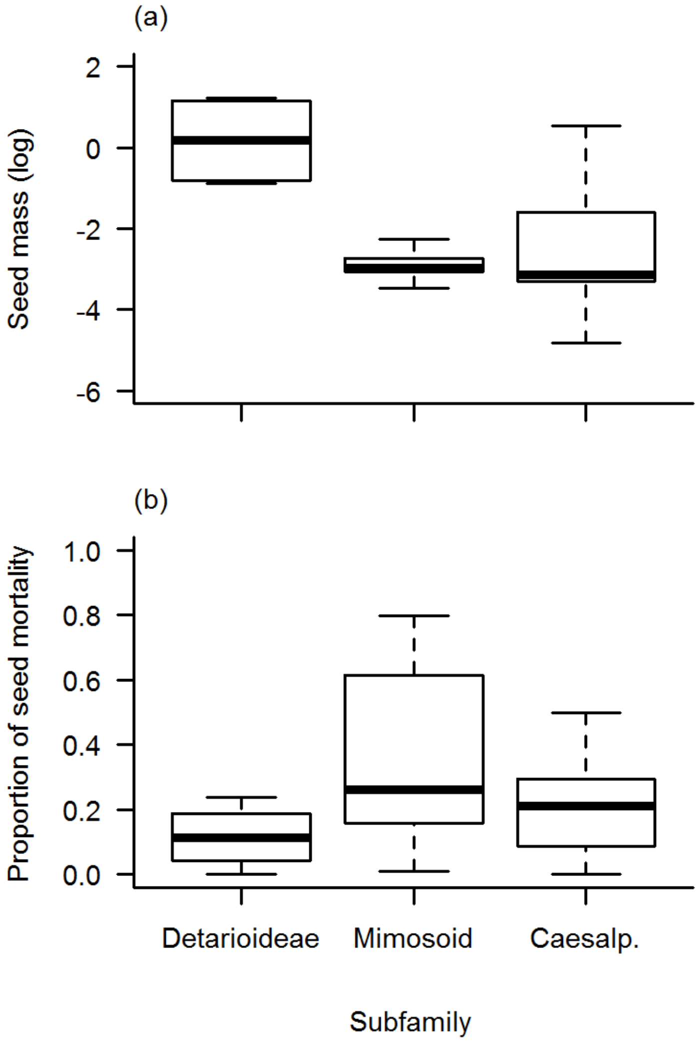


Fig. S2 Relation between (a) seed mass and (b) seed mortality of 19 tree species from the Brazilian savanna-forest mosaic as function of their legume phylogenetic groups: Detarioideae, Mimosoideae (here presented separately) and Caesalpinioideae. Cercidoideae and Papilionoideae members were excluded from the analyses because they had respectively only one and two representatives among our tree study species.



CONCLUSÃO

Neste estudo, vimos que a quebra da dormência física nas leguminosas do Cerrado não segue os mecanismos reconhecidos em outros ecossistemas inflamáveis (Auld & O’Connell, 1991; Moreira & Pausas, 2012; Ooi et al., 2014). Embora o fogo e a flutuação térmica possam quebrar a dormência em *Mimosa leiocephala* sob condições de campo, esta quebra não representa o padrão da maioria das espécies estudadas. Quando simulada em laboratório, a flutuação térmica mostra que não aumenta a germinação e, ao contrário, pode atrasar este processo em algumas espécies não-dormentes ou com dormência intermediária. Os choques térmicos, por sua vez, tiveram um efeito positivo em apenas seis das 46 espécies estudadas no total. Nestas espécies, a quebra da dormência sempre envolve uma proporção (cerca de 20%) de sementes que aumentam a germinação em comparação com o controle.

Com relação à sobrevivência das sementes ao fogo, as espécies sobrevivem quando enterradas, pois a temperatura atingida chega a menos de 60°C (ver Miranda et al., 1993). Quase todas as espécies também sobrevivem a 100°C 1-min, tanto as que ocorrem no Cerrado como espécies de floresta. Por outro lado, aquelas expostas na superfície do solo usualmente morrem devido ao contato direto com as chamas, corroborando outros estudos experimentais (Dayamba et al., 2010; Carrington 2010; Wiggers et al., 2013). Ainda assim, nossos resultados amparam a hipótese de que *gaps* maiores (maior porcentagem de solo nu) diminuem as temperaturas e tempo de residência do fogo, permitindo a sobrevivência de cerca de 40% de sementes expostas no solo nestas condições.

Assim, o tamanho relativamente pequeno das sementes dos arbustos faz com que elas sejam suscetíveis à exposição ao tratamento mais severo (200°C) aplicado em laboratório. Dentre estas sementes pequenas, a presença da dormência física também é um atributo funcional importante, que auxilia na sobrevivência das sementes. Sementes de espécies arbóreas, por outro lado, possuem tamanho maior e podem sobreviver mesmo em condições relativamente severas dos choques térmicos. A filogenia destas espécies arbóreas, tanto de Cerrado como floresta, parece influenciar também no *trade-off* entre tamanho e mortalidade das sementes. Em um contexto evolutivo, espécies arbóreas florestais, que apresentam sementes maiores, podem ter se beneficiado do tamanho da semente, permitindo a colonização de áreas de Cerrado e o surgimento de espécies próximas entre os dois ambientes.

Referências bibliográficas

AULD, T. D.; O'CONNELL, M. A. Predicting patterns of post-fire germination in 35 eastern Australian Fabaceae. Australian Journal of Ecology, 16, 53-70. 1991.

CARRINGTON, M. E. Effects of soil temperature during fire on seed survival in Florida sand pine scrub. International Journal of Forestry Research, article ID 402346. 2010.

DAYAMBA, S. D.; SAVADOGO, P.; ZIDA, D.; SAWADOGO, L.; TIVEAU, D.; ODEN, P. C. Fire temperature and residence time during dry season burning in a Sudanian savanna-woodland of West Africa with implication for seed germination. Journal of Forestry Research, 21, 445-450. 2010.

MOREIRA, B.; PAUSAS, J. G. Tanned or burned: The role of fire in shaping physical seed dormancy. Plos One, 7, 1-8. 2012.

MIRANDA, A. C.; MIRANDA, H. S.; DIAS, I. F. O.; DIAS, B. F. S. Soil and air temperatures during prescribed cerrado fires in Central Brazil. Journal of Tropical Ecology, 9, 313-320. 1993.

OOI, M. K. J.; DENHAM, A. J.; SANTANA, V. M.; AULD, T. D. Temperature thresholds of physically dormant seeds and plant functional response to fire: variation among species and relative impact of climate change. Ecology and Evolution, 4, 656-671. 2014.

WIGGERS, M. S.; KIRKMAN, L. K.; BOYD, R. S.; HIERS, J. K. Fine-scale variation in surface fire environment and legume germination in the longleaf pine ecosystem. Forest Ecology and Management, 310, 54-63. 2013.