UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
INSTITUTO DE BIOCIÊNCIAS – RIO CLARO

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA, EVOLUÇÃO E BIODIVERSIDADE

SEEKING THE BURNING QUESTION: A TRAIT-BASED PLANT RESPONSE TO FIRE IN A TROPICAL SAVANNA

HELOIZA LOURENÇO ZIRONDI

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Tese apresentada ao Instituto de Biociências do Câmpus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Doutora em Ecologia, Evolução e Biodiversidade.
Orientadora: Profa. Assoc. Alessandra Tomaselli Fidelis
Coorientador: Dr. Mark Ooi

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E dizer qual grão vai crescer e qual não vai, fale comigo. "

- William Shakespeare, *Macbeth*, 1:3

RESUMO

O regime de fogo influencia as respostas das plantas e seus atributos em ambientes pirofíticos. Além disso, diferentes regimes de fogo podem levar a mudanças na resposta das plantas, na diversidade e na dinâmica da comunidade. Diante disso, este estudo teve como objetivo avaliar como o regime de fogo afeta os atributos das plantas em uma savana tropical. Para isso, a pesquisa foi estruturada em objetivos principais: i) Avaliar como a frequência do fogo afeta o trade-off na alocação de recursos entre estruturas vegetativas e reprodutivas em espécies rebrotadoras clonais e não-clonais; ii) Examinar como as respostas germinativas a fatores relacionados ao fogo (choque térmico e fumaça) são afetadas quando as sementes ficam armazenadas *in situ*; iii) Explorar a relação entre a tolerância das sementes a altas temperaturas e seus atributos em espécies do Cerrado. Para responder à primeira questão, foram medidos atributos de sementes e plantas em áreas com diferentes frequências de fogo no Cerrado. Os experimentos de enterrio para o segundo objetivo foram conduzidos em duas áreas de Cerrado no Sudeste e Centro do Brasil, enquanto dados de viabilidade provenientes de diferentes regiões do Cerrado foram utilizados para o terceiro objetivo. Os resultados ressaltaram a importância dos atributos funcionais para compreender as respostas das plantas ao fogo no Cerrado. Dentre os principais achados, destacam-se: (i) a eficácia das estratégias de sobrevivência e regeneração adotadas pelas espécies do Cerrado, que apresentam poucos trade-offs na alocação de recursos, sendo essas relações fortemente influenciadas pelo regime de fogo; (ii) o papel decisivo de fatores como o ambiente, o tempo de permanência no solo e a época de dispersão, que afetam diretamente os atributos germinativos e a resposta das sementes à exposição a altas temperaturas e à fumaça; e (iii) a identificação de atributos das sementes que, em conjunto com outros fatores, determinam a tolerância térmica, evidenciando que essa adaptação aos efeitos do fogo é tanto espécie-específica quanto dependente das condições ambientais. Esperamos que os resultados desta pesquisa possam fornecer informações fundamentais sobre os atributos reprodutivos e a resposta e estratégias das plantas ao regime de fogo, contribuindo na conservação da diversidade do Cerrado e de outras savanas tropicais.

Palavra-chave: fogo e ecologia, atributos de plantas, sementes, germinação, Cerrado.

ABSTRACT

The fire regime influences plant responses and their traits in fire-prone environments. Additionally, different fire regimes can lead to changes in plant responses, biodiversity, and community dynamics. Given this, the aim of this study was to evaluate how the fire regime affects plant traits in a tropical savanna. To achieve this, the research was structured around three main objectives: i) Assess how fire frequency influences the trade-off in resource allocation between vegetative and reproductive structures in clonal and non-clonal resprouting species; ii) Examine how germination responses to fire-cues (heat shock and smoke) are affected when seeds remain stored *in situ*; iii) Explore the relationship between seed tolerance to high temperatures and seed traits in Cerrado species. To address the first objective, seed and plant traits were measured in areas with different fire frequencies in the Cerrado. Burial experiments for the second objective were conducted in two Cerrado areas in southeastern and central Brazil, while viability data for species from different regions of the biome were used for the third objective. The results highlighted the importance of functional traits in understanding plant responses to fire in the Cerrado. Key findings include: (i) the effectiveness of survival and regeneration strategies employed by Cerrado species, which show few trade-offs in resource allocation, with these relationships being strongly influenced by fire regimes; (ii) the critical role of factors such as environment, time in the soil, and dispersal season, which directly affect germination traits and seed responses to high temperatures and smoke exposure; and (iii) the identification of seed traits that, in combination with other factors, determine heat tolerance, emphasizing that this adaptation to fire is both species-specific and condition-dependent. We expect that the results of this research will provide essential insights into reproductive traits and plant responses to the fire regime, contributing to a better understanding of plant strategies in post-fire environments and to the conservation of biodiversity in the Cerrado and other tropical savannas.

Keywords: fire ecology, plant traits, seeds, germination, Cerrado

SUMÁRIO

INTRODUÇÃO GERAL

A importância dos atributos em ecologia de comunidades

Atributos são características dos organismos (fisiológicas, morfológicas e fenológicas) que podem ser mensuradas ao nível do indivíduo (Violle *et al.* 2007). Em plantas, os atributos influenciam suas funções, fitness e respostas a fatores bióticos e abióticos (Garnier *et al.* 2016; Funk *et al.* 2017; de Bello *et al.* 2021). Portanto, o conhecimento sobre esses atributos pode ajudar a elucidar processos ecológicos, respostas a distúrbios e padrões nas comunidades vegetais (Garnier *et al.* 2016; Funk *et al.* 2017; Larson *et al.* 2020). Por exemplo, plantas mais altas e com folhas maiores possuem vantagens competitivas na aquisição de luz, o que confere maior sucesso no estabelecimento e ambientes onde a luz é um fator limitante, como florestas (Díaz *et al.* 2016). Esses atributos (altura da planta e área foliar), juntamente com a área foliar específica (SLA), conteúdo de nitrogênio e seed mass, estão entre os mais utilizados para entender padrões nas estratégias ecológicas e funções das plantas (Levine 2016; Moles 2018).

Além dos atributos vegetativos, os atributos reprodutivos também são de grande relevância, pois atributos de sementes e germinação podem fornecer informações essenciais sobre processos como recrutamento, estabelecimento de plântulas, funções das plantas e estratégias ecológicas (Jiménez-Alfaro *et al.* 2016; Saatkamp *et al.* 2019). Além disso, os atributos das sementes geralmente não estão relacionados aos atributos das folhas ou outros atributos vegetativos, tornando-os uma importante fonte de informações para compreender os atributos das plantas em sua totalidade (Laughlin 2014; Pierce *et al.* 2014; Jiménez-Alfaro *et al.* 2016). Por exemplo, o tamanho (peso) e a forma das sementes já foram associadas à persistência inicial das plantas, aos processos de dispersão, às respostas a distúrbios e à competição (Westoby *et al.* 2002; Fenner & Thompson 2005; Pérez-Harguindeguy *et al.* 2016). Conforme observado por Leger et al. (2019), uma gramínea perene tem maiores chances de sobreviver à primeira estação de crescimento quando as sementes são provenientes de populações com sementes maiores e emergência mais rápida.

Portanto, existe uma diversidade de atributos das plantas a serem explorados e o aprofundamento do conhecimento sobre eles e suas interações pode melhorar a compreensão das funções e padrões das plantas (Levine 2016; Funk *et al.* 2017; Moles 2018).

Atributos de plantas em ecossistemas inflamáveis

O fogo e um fator determinante em diversos sistemas ao redor do globo, que influência direta e indiretamente organismos, processos, composição e dinâmica das comunidades vegetais (Whelan 1995; Bond & van Wilgen 1996; Bowman *et al.* 2009). Além de alterar o ciclo de nutrientes, o fogo afeta as interações entre plantas (Bond & van Wilgen 1996; Keeley *et al.* 2011) e consequentemente pode afetar o fitness das espécies em diferentes níveis, seja alterando a alocação de recursos em plantas rebrotadoras (Bond & van Wilgen 1996) ou influenciando os atributos da progênie por meio de efeitos na planta-mãe e no ambiente (Donohue 2009).

Dessa forma, plantas de ecossistemas inflamáveis exibem atributos morfológicos, anatômicos e fisiológicos relacionados ao fogo, permitindo-lhes sobreviver, reproduzir e persistir nesses ambientes (Keeley *et al.* 2011; Simon & Pennington 2012). Essas plantas possuem características relacionadas à persistência, como cascas espessas (Gignoux *et al.* 1997; Chiminazzo *et al.* 2023), gemas e meristemas protegidos que conferem aos indivíduos a capacidade de rebrota (Clarke *et al.* 2013; Pausas *et al.* 2018; Chiminazzo *et al.* 2021). Em locais onde o fogo é recorrente, muitas espécies são rebrotadoras e podem apresentar crescimento clonal, o que, por sua vez, influencia a resposta das plantas ao distúrbio (Lamont *et al.* 2011; Clarke *et al.* 2013). O fogo pode impactar a clonalidade (crescimento lateral) ao alterar condições como níveis de luminosidade e disponibilidade de nutrientes, aumentando a fertilidade do solo e estimulando o crescimento clonal (Brewer & Platt 1994). Plantas clonais também possuem vantagens em ambientes pós-fogo quando comparadas a plantas não clonais, pois o crescimento clonal pode originar muitos indivíduos que, embora geneticamente idênticos, têm o potencial de serem fisicamente e fisiologicamente independentes, proporcionando uma fonte adicional de recursos (Suzuki & Stuefer 1999; Duchoslavová & Jansa 2018; Herben & Klimešová 2020).

No Cerrado, um hotspot de biodiversidade (Myers *et al.* 2000), onde o fogo está presente há pelo menos 4 milhões de anos (Simon *et al.* 2009), as plantas possuem atributos adaptados ao fogo que influenciam sua persistência e estratégias de regeneração no ambiente pós-fogo, principalmente para espécies estrato herbáceo (Coutinho 1990).

Por exemplo, em relação à persistência, sabe-se que a maioria das plantas do Cerrado tem a capacidade de rebrotar após o fogo, pois os meristemas estão protegidos nos caules ou em órgãos subterrâneos (Coutinho 1982, 1990; Appezzato-da-Glória 2003; Pilon *et al.* 2021, Zupo et al. 2021). Grande parte dessas plantas tem sua parte aérea completamente consumida durante o fogo e depende da capacidade de rebrota para sobreviver no ambiente, algumas apresentando crescimento clonal (Coutinho 1982, 1990; Hoffmann 1998; Appezzato-da-Glória 2003; Pilon *et al.* 2021).

As plantas também apresentam atributos de regeneração em ambientes inflamáveis, incluindo o estímulo da germinação ocasionado pela fumaça, a quebra de dormência física por choques térmicos (Auld & Ooi 2009; Moreira *et al.* 2010a; Soós *et al.* 2019) e a tolerância das sementes a altas temperaturas (Ribeiro *et al.* 2013; Fichino *et al.* 2016; Daibes *et al.* 2019; Zirondi *et al.* 2019; Zupo *et al.* 2021). Apesar de estudos demonstraram que as respostas aos fatores do fogo, como fumaça e temperatura, para espécies do Cerrado não possuírem um padrão (Daibes *et al.* 2022), sendo a maioria das respostas espécie-especificas (ver Zirondi et al. 2019; Zupo et al. 2021; Motta et al. 2024), um fator que se destaca é a tolerância das sementes às altas temperaturas. A tolerância das sementes a altas temperaturas podem estar associada a certos atributos, como o tamanho das sementes (Ribeiro *et al.* 2015), o tipo de dormência (Ramos *et al.* 2016) e o conteúdo de água das sementes (Tangney *et al.* 2019). Esses atributos, entre outros, permitem a sobrevivência das plantas e o sucesso nos processos de recrutamento após o fogo.

Por fim, outros fatores ambientais podem se somar aos atributos e alterar as respostas das sementes ao fogo. No Cerrado, Dairel et. al (2024) evidenciou um aumento significativo na germinação pós-fogo de sementes do banco de sementes em áreas antes excluídas do fogo. Ainda assim, faltam estudos sobre como o tempo de armazenamento *in situ* poderia afetar as respostas dos atributos aos fatores do fogo como exposição à fumaça e altas temperaturas.

Influência do regime de fogo nos atributos das plantas

As plantas não são adaptadas ao fogo em si, mas aos regimes de fogo (Keeley *et al.* 2011). Assim, a ocorrência de certo atributo depende da pressão exercida por características do regime de fogo (ex. época, intensidade e frequência do fogo) (Bond & van Wilgen 1996; Keeley *et al.* 2011). Dentre essas, a frequência do fogo é uma característica importante do regime de fogo, pois atua como um filtro, selecionando espécies adaptadas a intervalos específicos de retorno do fogo. Por exemplo, plantas que necessitam de mais tempo para crescer e atingir a maturidade (como as germinadoras) só sobreviveriam em áreas com baixa frequência de fogo (Bond & van Wilgen 1996; Keeley *et al.* 2011). Por outro lado, plantas com alta capacidade de rebrota conseguem persistir em habitats com maior frequência de fogo (Bond & Midgley 2001; Pausas *et al.* 2004).

Os atributos de sementes e germinação também são afetados por mudanças na frequência do fogo, como a morfologia das sementes (Saracino *et al.* 2017), sua persistência e dormência (Ooi 2012; Jiménez-Alfaro *et al.* 2016). Gomez-Gonzalez et al. (2011) observaram que uma espécie de Asteraceae apresentou sementes mais pubescentes, arredondadas e com pericarpos mais espessos em áreas com maior frequência de fogo. Assim, diferentes regimes de fogo podem levar a populações com diferentes atributos das plantas e na frequência com que esses atributos aparecem na comunidade. Contudo, há ainda lacunas de conhecimento sobre como o regime de fogo afeta esses atributos em espécies de savanas abertas.

Alguns fatores influenciam os regimes de fogo, como a presença de espécies invasoras, atividades humanas e mudanças climáticas (Brooks *et al.* 2004; Bond & Parr 2010; Pivello 2011; Stevens *et al.* 2016; Alvarado *et al.* 2018). No Cerrado, o regime de fogo - principalmente a estação e a frequência do fogo - vem sendo alterados devido a mudanças no uso da terra (agricultura e pastagem) e políticas de supressão de fogo (Coutinho 1990; Fidelis & Pivello 2011; Pivello 2011; Schmidt & Eloy 2020). A política de supressão de fogo, conhecida como "política de fogo zero", tem mudado recentemente (Schmidt *et al.* 2018; Fidelis *et al.* 2018), mas já resultou em consequências negativas, como a perda de riqueza de espécies herbáceas e o adensamento em algumas áreas (Durigan & Ratter 2016; Abreu *et al.* 2017). Em geral, alterações nos regimes de fogo em savanas tropicais têm grandes impactos sobre as interações entre espécies vegetais, os processos ecossistêmicos, a dinâmica e a biodiversidade da comunidade. Portanto, compreender como mudanças nos regimes de distúrbios afetam os

ecossistemas de savanas é de suma importância para entender os fatores que influenciam sua distribuição (Hoffmann *et al.* 2012).

Importância ecológica

As informações sobre atributos de persistência e regeneração são essenciais para compreender como o fogo afeta os ciclos de vida das plantas e, consequentemente, a montagem das comunidades e os processos ecológicos em ambientes inflamáveis (Jiménez-Alfaro *et al.* 2016; Larson & Funk 2016; Saatkamp *et al.* 2019). Essas informações são fundamentais para garantir a conservação da biodiversidade, pois, ao entender como o fogo impacta as comunidades vegetais, é possível planejar ações que assegurem sua preservação, especialmente em relação às espécies presentes no estrato herbáceo. O estrato herbáceo apresenta alta diversidade e concentra grande número de espécies relevantes em áreas abertas do Cerrado, contribuindo significativamente para o armazenamento de carbono no subsolo e fornecendo diversos serviços ecossistêmicos para a sociedade (Castro & Kauffman 1998; Fidelis *et al.* 2013; Honda & Durigan 2016; Buisson *et al.* 2019, 2022).

As sementes desempenham um papel crucial como fonte de propágulos na restauração de ecossistemas. Entender os requisitos para a germinação pode melhorar esse processo e ser determinante para o sucesso da restauração (Jiménez-Alfaro *et al.* 2016). Por exemplo, a restauração no Cerrado por meio de semeadura direta tem enfrentado desafios devido a fatores como disponibilidade de sementes viáveis, presença de espécies invasoras e características do solo (Silva & Vieira 2017; Pellizzaro *et al.* 2017; Pilon *et al.* 2019; Sampaio *et al.* 2019).

Embora a semeadura direta tenha se mostrado eficiente para a recuperação de algumas áreas, especialmente com o uso de espécies arbóreas e arbustivas (Silva & Vieira 2017; Pellizzaro *et al.* 2017), ainda há lacunas no conhecimento sobre os requisitos de germinação de espécies herbáceas para a restauração de ecossistemas abertos no Cerrado. Além disso, faltam estudos sobre como sementes do banco de sementes do solo respondem a estímulos de fogo após serem armazenadas *in situ.* Esse conhecimento seria valioso para a restauração

de ecossistemas abertos do Cerrado. Ao compreender como esses atributos influenciam a comunidade vegetal, seria possível melhorar o sucesso da germinação e do estabelecimento de plantas em áreas de restauração. Também permitiria entender as mudanças no ambiente pós-fogo, preservar espécies e desenvolver planos de manejo do fogo voltados para a conservação da diversidade vegetal.

Portanto, considerando o fogo como um distúrbio recorrente no Cerrado, este estudo buscou investigar como os atributos das plantas foram afetados pelo fogo em uma savana tropical. Além de avaliar o trade-off na alocação de recursos em plantas rebrotadoras e como variam conforme a frequência do fogo, o estudo também avaliou os impactos nos atributos das sementes e respostas ao fogo ao longo do tempo, quando armazenadas no solo, e a relação entre os atributos e a tolerância às altas temperaturas em espécies do Cerrado.

Objetivos gerais

O objetivo geral deste estudo foi analisar como o regime de fogo afeta os atributos das plantas de espécies de savanas tropicais. Incluindo principalmente atributos de germinação e sementes e como o regime de fogo influenciaram esses atributos. Assim, o estudo se concentrou em três perguntas principais:

1. Existe trade-off na alocação de recursos entre estruturas vegetativas e reprodutivas (sementes) de plantas rebrotadoras do Cerrado? E como a frequência de fogo e o histórico do fogo afetariam esse trade-off, principalmente em plantas clonais versus não-clonais?

   Hipótese: Tanto plantas clonais quanto não-clonais teriam seus atributos reprodutivos (e.g., peso e forma das sementes) negativamente impactados pelo aumento da frequência de fogo e pelo tempo decorrido desde a última queima. Esses efeitos ocorreriam devido à alocação contínua de recursos para a rebrota e à menor ciclagem e disponibilidade de nutrientes em áreas com um maior tempo desde a última queima.

No entanto, espera-se que plantas clonais sejam menos afetadas, pois possuem a vantagem de armazenar maiores reservas em órgãos subterrâneos, conferindo-lhes maior resiliência em comparação às espécies não-clonais.

2. As respostas germinativas aos estímulos de fogo de sementes nativas são afetadas quando as sementes são armazenadas *in situ*?

   Hipótese: Sementes armazenadas no solo apresentariam uma diminuição na longevidade e viabilidade, além de alterações nas respostas aos fatores de fogo (e.g., choque térmico e fumaça), dependendo do período de armazenamento, já que sementes enterradas no solo seriam afetadas pelas condições ambientais (e.g., mudanças de temperatura, sazonalidade, predação, etc.) vivenciadas no campo (*in situ*).

3. Existe uma relação entre a tolerância das sementes a altas temperaturas e os atributos das sementes para espécies do Cerrado?

   Hipótese: Sementes com maior tolerância a altas temperaturas apresentariam tamanhos menores, formas mais arredondadas e menor conteúdo de água. Além disso, esperava-se encontrar correlações mais fortes entre a tolerância das sementes a altas temperaturas e seus atributos em espécies de ecossistemas abertos do Cerrado, uma vez que o fogo é mais frequente nessas áreas.

Metodologia geral e dificuldades encontradas

Os experimentos e coletas referentes aos objetivos 1 e 2 foram realizados em áreas de *campo sujo* (Figura 1), caracterizadas pelo domínio de estrato herbáceo e graminóide rico em espécies, com árvores e arbustos esparsos na vegetação (Coutinho 1978; Oliveira-Filho &

Ratter 2002). Isso porque o presente estudo visou focar em espécies que compõe o estrato herbáceo do Cerrado, incluindo herbáceas não-graminóides, gramíneas e arbustos.

Figura 1. Exemplos de fisionomia d*e campo sujo,* caracterizadas pelo domínio de estrato herbáceo e graminóide rico em espécies, com árvores e arbustos esparsos na vegetação.

As coletas das plantas e atributos para o Objetivo 1 foram realizados na Reserva Natural Serra do Tombador (RNST, 13° 35-38' S e 47° 45'-51' W, 560–1118 m a.n.m.), localizada no Brasil Central (Goiás, Figura 2).

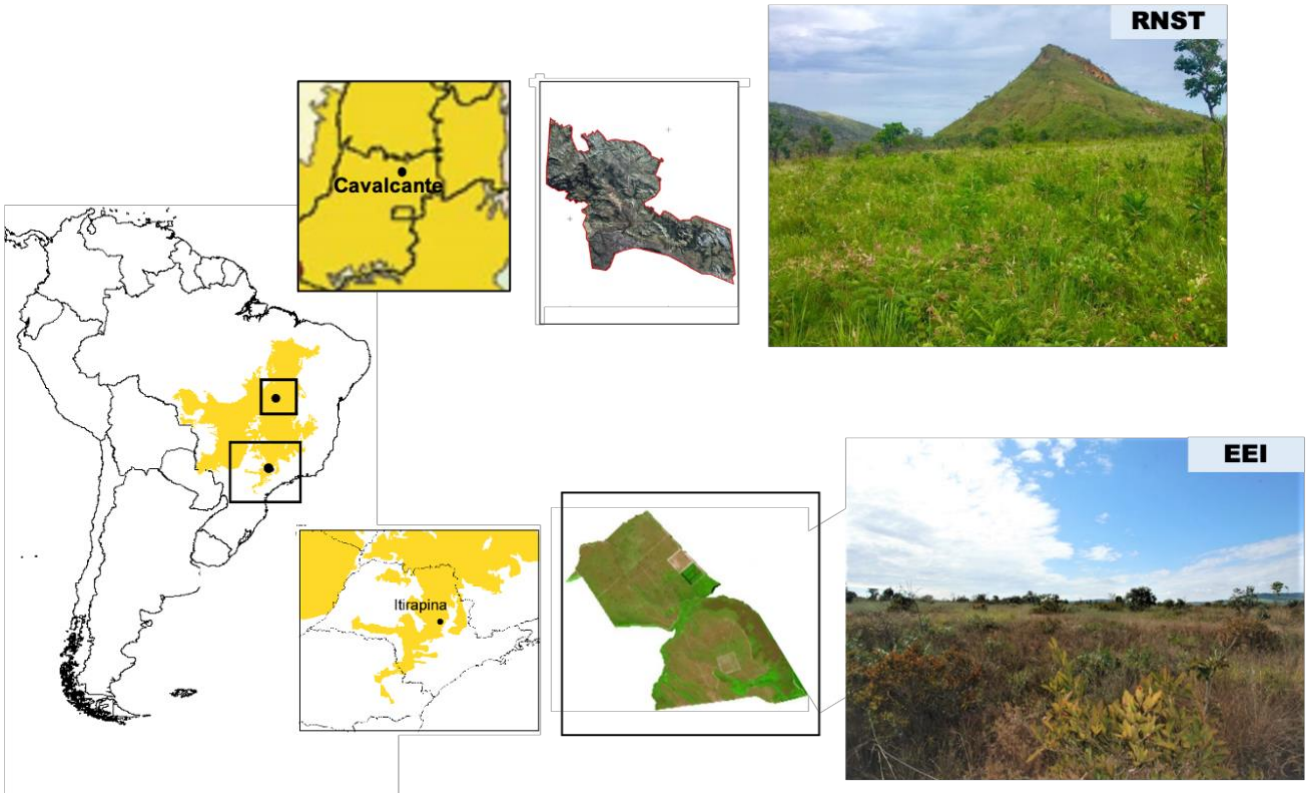

Figura 2. Localização das áreas de estudo: Reserva Natural Serra do Tombador (RNST), Cavalcante-GO, e Estação Ecológica de Itirapina (EEI), SP. A área destacada em amarelo representa a região do Cerrado. Figura de Zanzarini (2019).

A RNST possui experimentos de queimas controladas desde 2013 (Figura 3) e implementação do Manejo integrado do fogo (MIF) a partir de 2021. Antes do estabelecimento dos experimentos a recorrência do fogo era a cada 2-3 anos (Daldegan et al., 2014). Com isso, a RNST oferece locais com diferentes regimes de fogo, permitindo a investigação de seus efeitos sobre a vegetação.

Figura 3. Experimento de queima controlada sendo realizado na Reserva Natural Serra do Tombador (RNST) em uma parcela de 30x30 metros.

Inicialmente, a proposta era selecionar áreas que refletissem um gradiente de frequências de fogo. No entanto, essa abordagem não foi viável, pois as espécies avaliadas não estavam presentes em todos os níveis desse gradiente. Diante disso, para avaliar se as diferentes frequências de fogo afetam a alocação de recursos entre estruturas vegetativas e reprodutivas em espécies rebrotadoras, as coletas foram realizadas em áreas com frequências de fogo opostas: Alta (11 a 16 queimas nos últimos 20 anos) e Baixa (0 a 2 queimas nos últimos 20 anos, Figura 4). Além disso, dentro de cada extremo de frequência de fogo, foram selecionadas áreas com diferentes tempos desde a última queima, permitindo avaliar o efeito do tempo pós-fogo nas respostas das espécies (Figura 4).

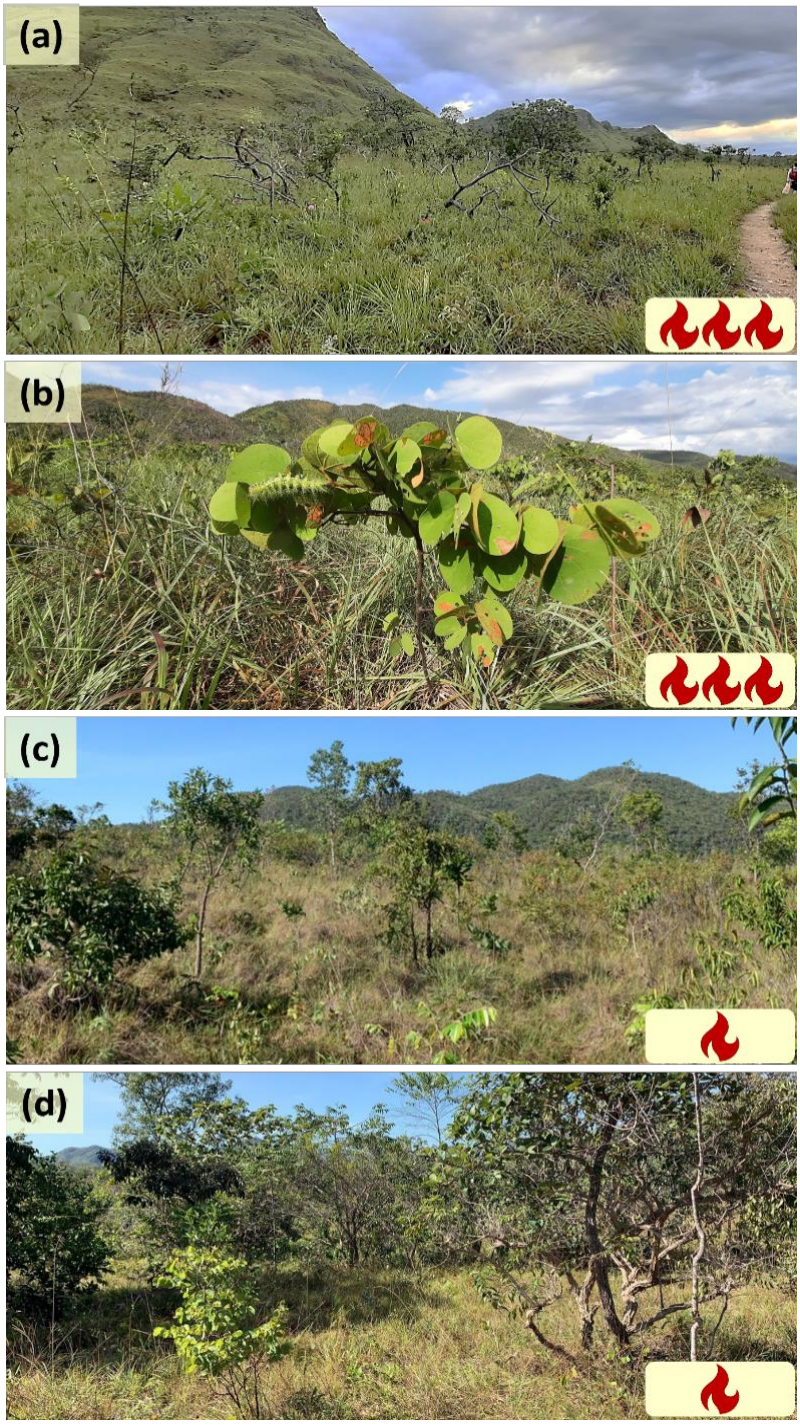

Figura 4. Áreas experimentais com diferentes frequências de fogo e tempo depois da última queima. (a) alta frequência de fogo, recentemente queimada (High_RB); (b) alta frequência de fogo, longo tempo desde a última queima (High_LTB); (c) baixa frequência de fogo, recentemente queimada (Low_RB); (d) baixa frequência defogo, longo tempo desde a última queima (Low_LTB).

Além disso, a seleção de espécies para o Objetivo 1 foi ajustada, uma vez que a proposta inicial era incluir pelo menos nove espécies. No entanto, a escolha final foi condicionada à disponibilidade de espécies que estivessem em fase reprodutiva e com maior ocorrência nas quatro áreas de estudo. Dessa forma, foram selecionadas quatro espécies, sendo duas clonais e duas não-clonais (Figura 5). Foram medidos atributos vegetativos e reprodutivos nessas quatro espécies nativas. Os atributos avaliados estão listados na Tabela 1

e foram obtidos de indivíduos em fase reprodutiva, com as medições realizadas ao final da estação seca, fora do período de crescimento, garantindo assim a padronização das condições metabólicas (Saura-Mas & Lloret 2009).

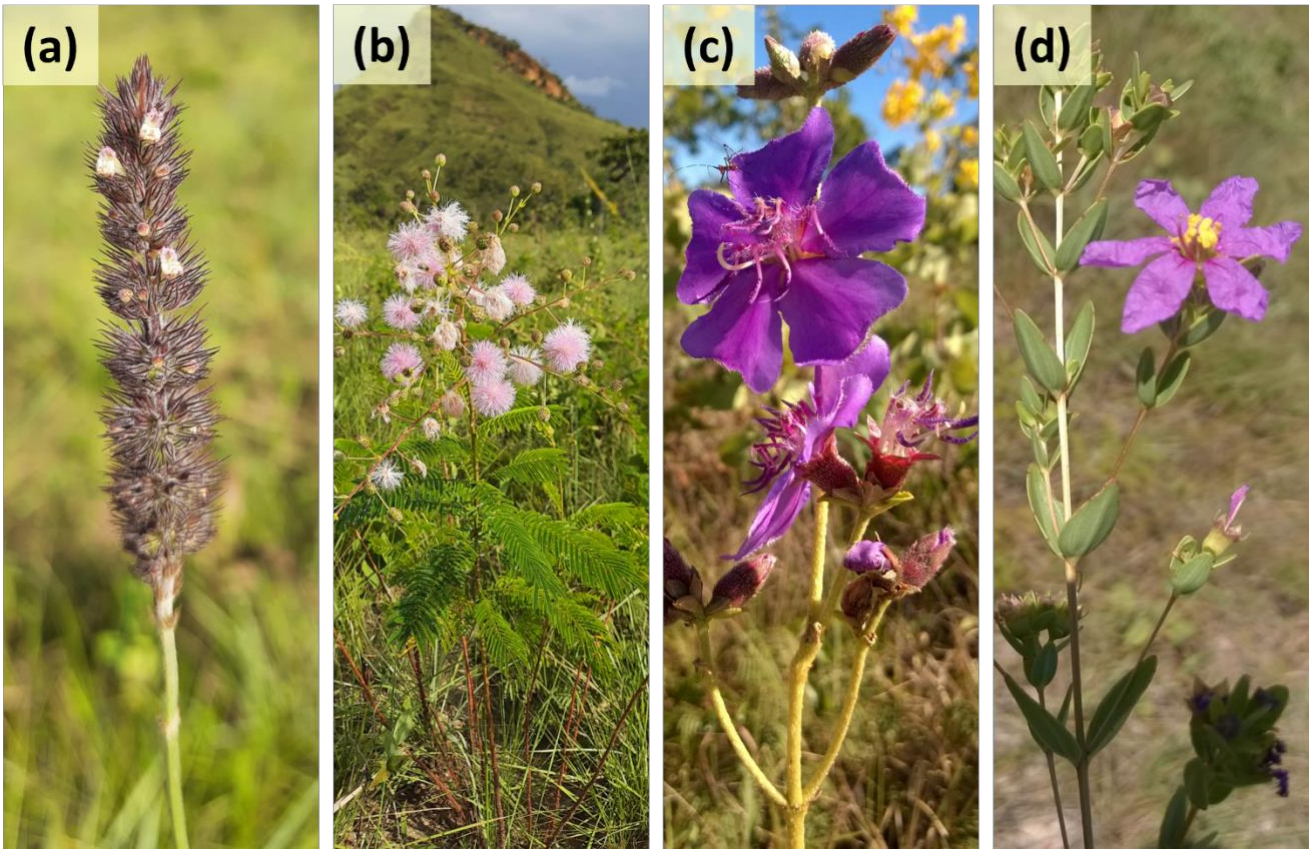

Figura 5. Espécies selecionadas para o Objetivo 1, que avaliou a alocação de recursos entre estruturas vegetativas e reprodutivas em plantas rebrotadoras do Cerrado. As espécies clonais incluem: (a) *Gymneia chapadensis* e (b) *Mimosa leiocephala*, enquanto as espécies não-clonais são: (c) *Tibouchina melastomoides* e (d) *Diplusodon punctatus*.

Tabela 1. Atributos de sementes, germinação e plantas que foram medidos, juntamente com suas respectivas significâncias funcionais, métodos e referências.

Atributos	Unidades de medida	Significado funcional	Método	Adaptado de (Referências)	Objetivo
Peso da semente (tamanho)	mg	Relacionado ao estabelecimento de plântulas; Distância de dispersão, persistência no solo, tempo de	Peso seco de sementes ou grupo de sementes	(Cornelissen *et al.* 2003; Pérez-Harguindeguy *et al.* 2016a)	1,3

		germinação e disponibilidade de recursos			
Forma da semente	razão largura:comprimento, mm	Enterramento e persistência no solo; tolerância a altas temperaturas	Análise de imagens usando o software especializado	(Pérez-Harguindeguy *et al.* 2016b; Dayrell *et al.* 2023)	3
Número de frutos por indivíduo	Contagem	Alocação de recursos para reprodução	Contado por indivíduo *in situ*	(Cornelissen *et al.* 2003; Pérez-Harguindeguy *et al.* 2016b)	1
Peso do fruto	mg	Alocação de recursos para reprodução	Peso seco do fruto		1
Número de sementes	Contagem	Alocação de recursos para reprodução	Contado por indivíduo	(Pérez-Vich *et al.* 1998; Diederichsen & Raney 2006; Li *et al.* 2006)	1
Número de sementes cheias	Contagem /Porcentagem	Alocação de recursos para reprodução	Análise de imagem usando X-ray; Contadas por indivíduo	(Alencar *et al.* 2012; Tonguç *et al.* 2012)	1
Tipo de dormência	Categórico	Afeta o tempo de germinação e a resposta ao fogo	Classificação segundo protocolo	(Baskin & Baskin 2014)	2,3
Germinabilidade	Porcentagem	Capacidade de colonização, vigor da semente (geralmente resultado da influência de outros atributos)	Experimentos de germinação por 30 dias	(Zirondi *et al.* 2019b)	2,3
Viabilidade	Porcentagem	Efeitos ambientais, disponibilidade de recursos, sobrevivência do propágulo, persistência	Teste de viabilidade-solução de tetrazólio a 1%	(Zirondi *et al.* 2019b)	2,3
Tempo médio de germinação (MGT)	Dias	Capacidade competitiva, capacidade de germinação em condições pós-fogo, tempo de recrutamento	Calculado a partir da fórmula MGT	(Ranal & Santana 2006; Ranal *et al.* 2009)	2

Sincronia (Z)	Quantitativo (0 a 1)	Sincronia de germinação, estabelecimento em ambientes pós-fogo	Calculado a partir da fórmula Z	(Ranal & Santana 2006; Ranal *et al.* 2009)	2
Resposta ao choque de temperatura	Porcentagem	Resposta ao fogo, capacidade de germinação após choque térmico (ligada à espessura do tegumento da semente e à dormência física)	Experimentos de choque térmico; exposição de sementes a 100 ℃ e 200 ℃	(Zirondi *et al.* 2019b)	2,3
Resposta de fumaça	Porcentagem	Adaptação ao fogo, capacidade de germinação após aplicação de fumaça	Sementes expostas à solução de fumaça por 24 horas	(Jäger *et al.* 1996; Moreira *et al.* 2010b; Zirondi *et al.* 2019b)	2
Carboidratos não-estruturais	mg/g	Fonte de energia e reserva	Análise em laboratório em sementes, folhas e órgãos subterrâneos	(Pérez-Harguindeguy *et al.* 2016a; Wigley *et al.* 2020)	1
Biomassa	g	Alocação de recursos	Peso seco das estruturas reprodutivas e/ou vegetativas		1
Comprimento da planta	cm	Vigor competitivo e o intervalo que as plantas têm para crescer entre os incêndios	Medido *in situ*	(Cornelissen *et al.* 2003; Pérez-Harguindeguy *et al.* 2016b)	1
Número de ramos	Contagem	Alocação de recursos para rebrote	Contado por indivíduo *in situ*	(Cornelissen *et al.* 2003; Pérez-Harguindeguy *et al.* 2016b)	1
Macronutrientes	g kg-1	Disponibilidade de recursos no solo	Análise laboratorial de N, P, Ca, K, Mg, S, P, S, K, Ca, Mg	(da Silva 2009)	1
Forma de crescimento	Categórico	Crescimento e persistência das plantas, influenciando outros atributos das plantas	Classificação segundo protocolo	(Cornelissen *et al.* 2003; Pérez-Harguindeguy *et al.* 2016b)	3

Clonalidade	Categórico	Regeneração, persistência após distúrbio (fogo)	Classificação no campo segundo protocolo	(Cornelissen *et al.* 2003; Pérez-Harguindeguy *et al.* 2016b; Klimešová *et al.* 2019)	1

Para o objetivo 2, parte dos experimentos também foram realizados em uma área de savana na Estação Ecológica de Itirapina (EEI, 22° 11'–22° 15' S, 47° 51'–48° 00' W, Figura 2). Foram coletadas sementes frescas de 13 espécies nativas, com base na disponibilidade de sementes no pico de dispersão (início da estação seca na EEI e início da estação chuvosa na RNST) (Figura 6).

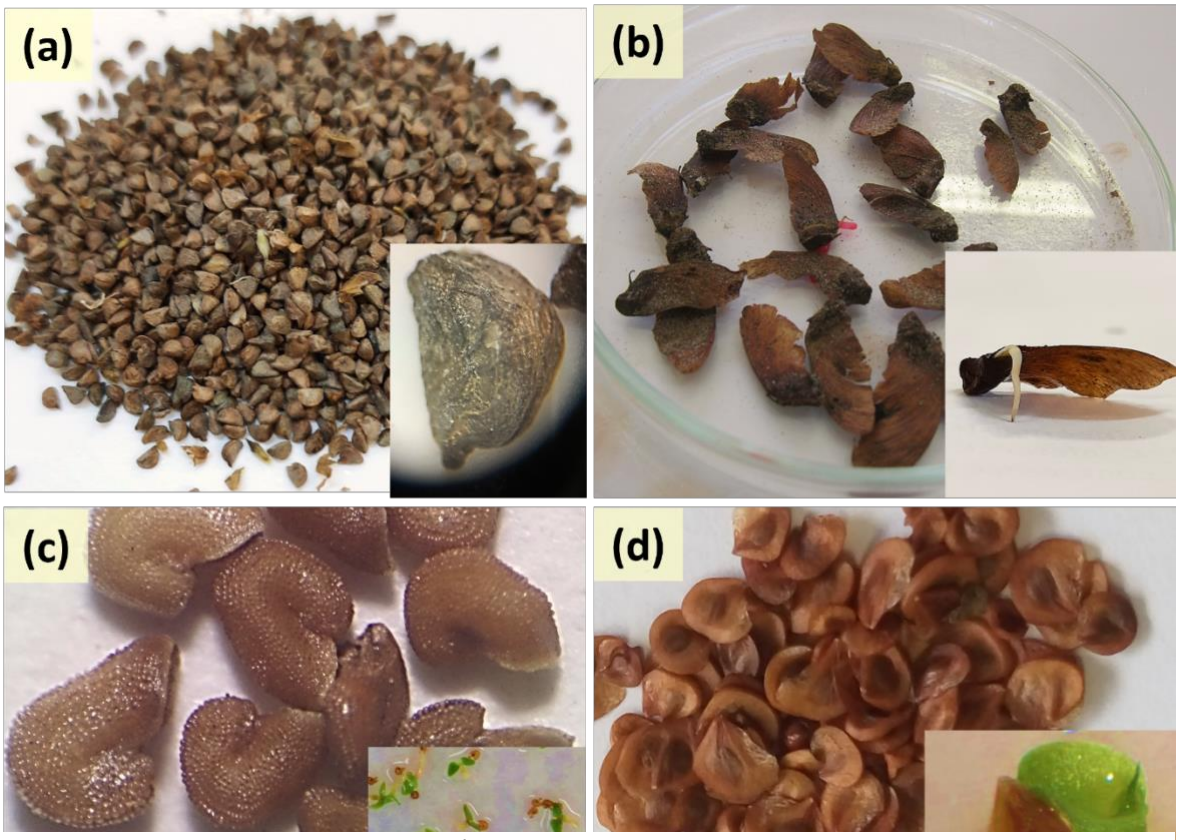

Figura 6. Sementes de quatro das 13 espécies selecionadas para os experimentos que avaliaram como as respostas germinativas a fatores relacionados ao fogo (choque térmico e fumaça) são afetadas pelo armazenamento *in situ*. As espécies que dispersam no início da estação seca incluem: (a) *Waltheria communis* e (b) *Banisteriopsis stellaris*, enquanto as espécies que dispersam no início da estação chuvosa são: (c) *Tibouchina melastomoides* e (d) *Dyckia brasiliana*.

Em ambas as áreas, foram estabelecidas seis réplicas, espaçadas por pelo menos dois metros entre si (Figura 7). Cada réplica foi marcada com um vergalhão central para identificação, e um arame fino foi utilizado para fixar os sacos contendo as amostras de cada período de enterramento, devidamente identificadas com placas de alumínio indicando o momento da remoção (T1 – apenas para a estação chuvosa, T3, T6, T9 e T12) (Figura 7). Os sensores de temperatura foram instalados tanto na superfície do solo quanto a 1 cm de profundidade, garantindo um monitoramento térmico preciso. Além disso, todas as réplicas foram posicionadas em áreas com, no mínimo, 40% de cobertura vegetal.

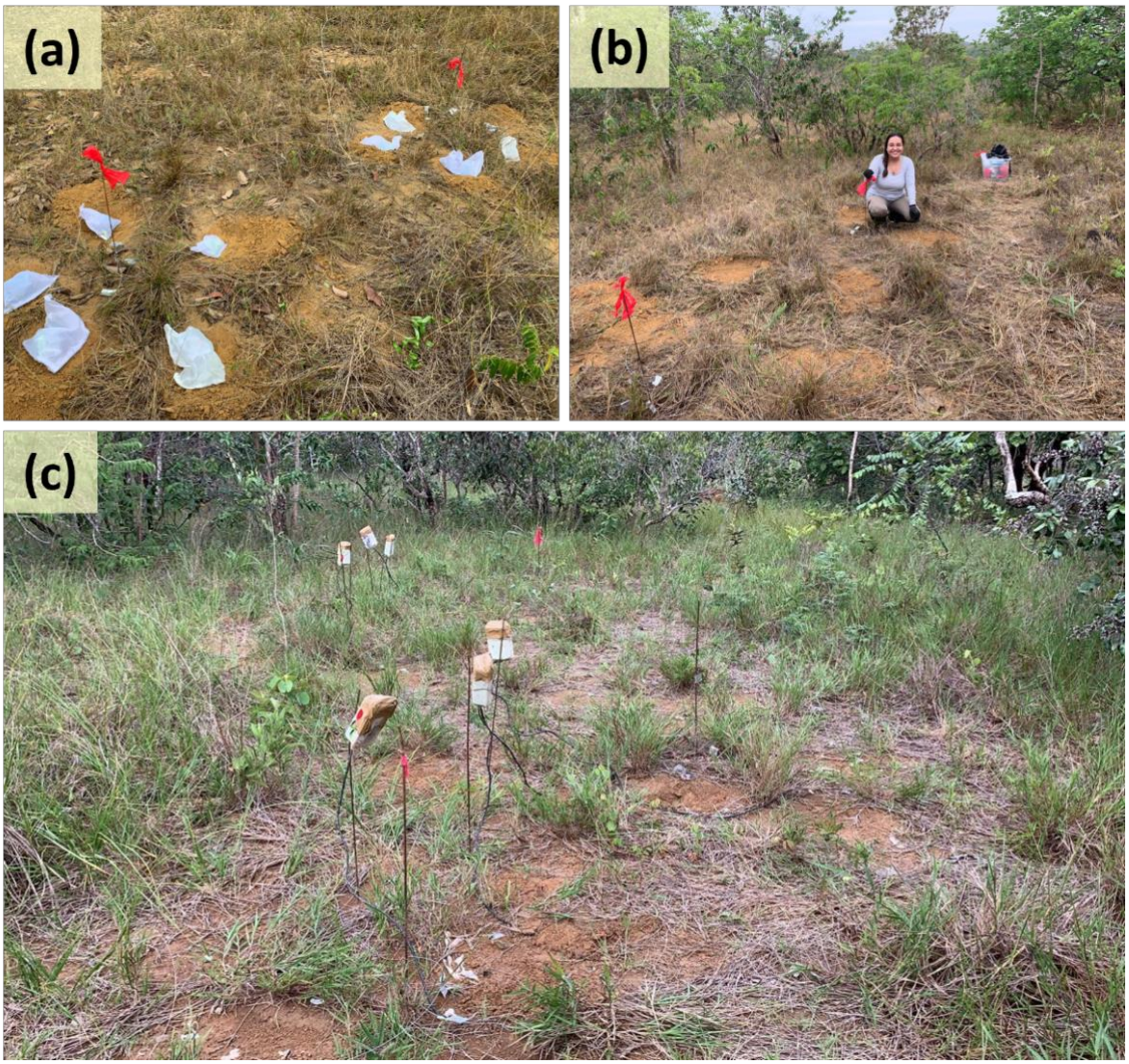

Figura 7. Montagem do experimento de campo na Reserva Natural Serra do Tombador (RNST), realizado no período de transição entre o final da estação seca e o início da chuvosa. (a) Sacos contendo as réplicas de sementes dispostos nos locais de enterramento; (b) Amostras já enterradas e sinalizadas com vergalhões; (c) Instalação de sensores de microclima para monitoramento da temperatura na superfície do solo e a 1 cm de profundidade.

Ao longo do tempo, em ambas as áreas, sensores e sacos experimentais foram perdidos devido à ação de animais locais (Figura 8). Diante disso, o número de sementes destinadas aos experimentos de choque de temperatura e fumaça (Figura 9) foi ajustado conforme a disponibilidade. Nos casos em que a quantidade de sementes não foi suficiente para todos os tratamentos, a prioridade foi dada aos experimentos de temperatura.

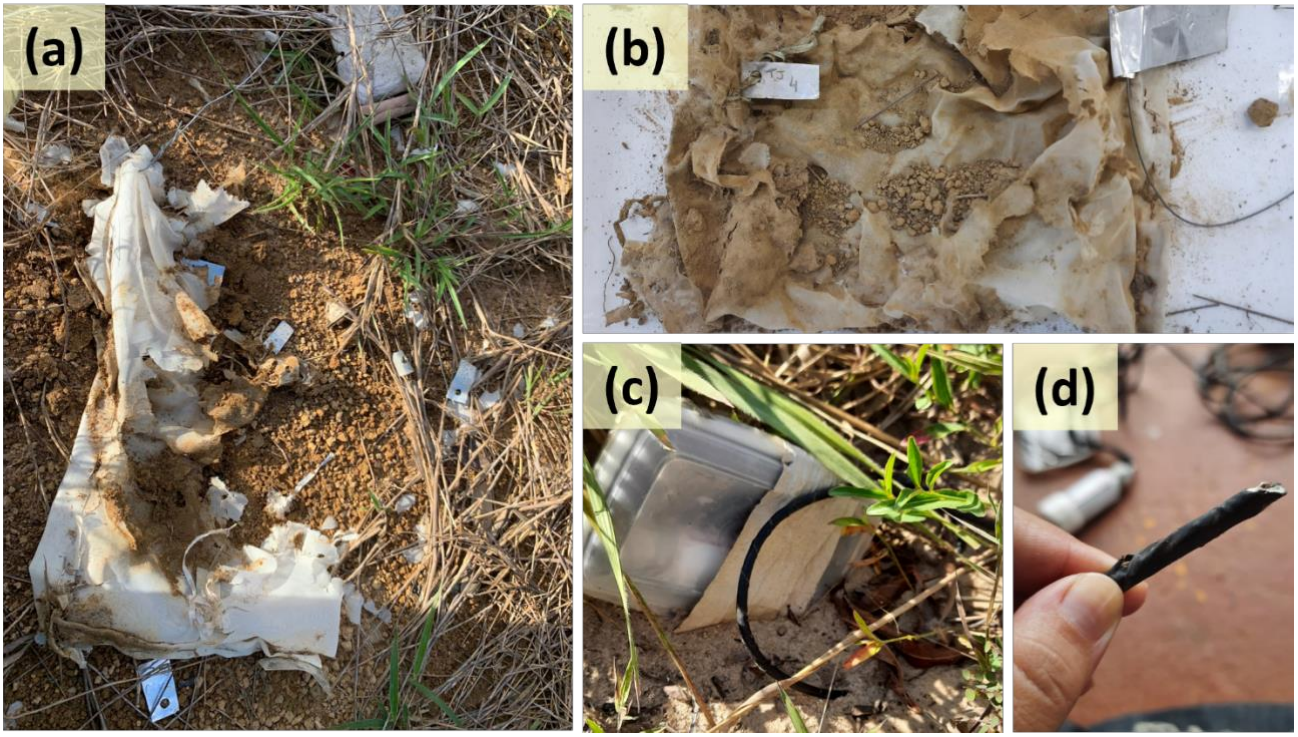

Figura 8. Problemas encontrados durante o período que as sementes ficaram em campo. Sacos com as sementes desenterrados e destruídos por cupins em a) e b). Sensores revirados, for a do lugar e danificados em c) e d).

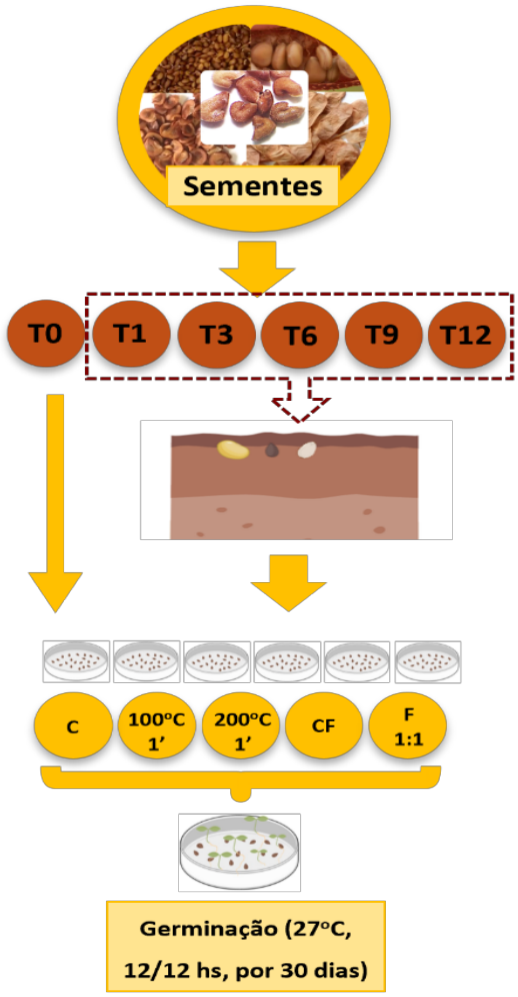

Figura 9. Esquema do experimento após a coleta das sementes em cada época de dispersão. Sementes de T0, T1, T3, T6, T9 e T12 foram expostas a experimentos de choque de temperatura e fumaça após o período enterradas (Figura 4). Nos experimentos de choque de temperatura, as sementes foram expostas a 100°C e a 200°C por um minuto, utilizando uma mufla pré-aquecida. Para o experimento de fumaça, preparamos a solução de fumaça (F) usando 5g de biomassa da área de estudo e seguindo os protocolos de Jäger et al. (1996) e Moreira et al. (2010). Em seguida, as sementes foram embebidas em solução de fumaça 1:1 por 24h, enquanto as sementes controle (CF) foram embebidas em água destilada pelo mesmo período. Após os experimentos, as sementes foram colocadas para germinar em câmaras germinadoras sob condições controladas (12h/12h de luz e escuro) a uma temperatura constante de 27°C por 30 dias. Testes de viabilidade foram realizados em sementes não germinadas, utilizando solução de tetrazólio a 1%. Por fim foram analisados os atributos germinativos incluindo Germinabilidade(%), Viabilidade (%), Tempo Médio de Germinação (MGT) e a Sincronia (Z) para todas as espécies

Quanto ao Objetivo 3, a proposta inicial era utilizar apenas os dados do *Laboratório de Ecologia da Vegetação* (LEVeg). No entanto, após uma análise preliminar dos dados realizada em conjunto com o Dr. Angelino Carta, da Universidade de Pisa, identificou-se um viés significativo nos experimentos, com uma predominância de espécies da família Fabaceae. Para obter uma representação mais abrangente da resistência das sementes às altas temperaturas das espécies do Cerrado, decidiu-se convidar pesquisadores colaboradores que possuíam dados na área. Após o recebimento dos dados, alguns ajustes foram necessários, incluindo a utilização do Heat Index (H) (ver Paula & Pausas 2008) para padronizar a estimativa da dose térmica recebida pelas sementes, dada a variação nas temperaturas e no tempo de exposição entre os experimentos. Além disso, foi preciso lidar com a ausência de alguns dados de atributos, ajustando as análises conforme necessário.

Estrutura da Tese

A tese foi estruturada em Introdução geral, três capítulos e conclusões gerais onde são abordados um resumo dos principais achados deste estudo assim como implicações ecológicas gerais do tema abordado. A introdução geral e as considerações finais foram redigidas em português, enquanto os capítulos foram redigidos em inglês, já no formato de artigos científicos e colocados na seguinte ordem:

No primeiro capítulo "**Are there trade-offs? Resource allocation between vegetative and reproductive traits in a tropical savanna",** investigamos se diferentes frequências de fogo (alta e baixa) e tempo desde a última queima afetaram a alocação de recursos entre estruturas vegetativas e reprodutivas (sementes) em plantas rebrotadoras (clonais e não-clonais).

No segundo capítulo **"In situ seed storage affects germination responses to fire-related cues in Cerrado species"**, avaliamos como o tempo de armazenamento no solo afeta as respostas germinativas aos fatores do fogo, choque de temperatura e fumaça.

No terceiro capítulo **"Seeds of Fire: Unraveling the link between heat tolerance and seed traits in Cerrado species"**, avaliamos como os atributos de sementes influenciam na tolerância das sementes às altas temperaturas.

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CAPÍTULO 1

Are there trade-offs? Resource allocation between vegetative and reproductive traits in a tropical savanna

Abstract

Resource allocation is a critical aspect of plant life strategies, influencing growth, reproduction, and survival, particularly in fire-prone ecosystems. In the Cerrado, where fire has shaped plant adaptations, resprouting plants face potential trade-offs between vegetative and reproductive traits. This study investigates whether fire regime parameters (such as fire frequency and history) affect this resource allocation between vegetative and reproductive traits in clonal and non-clonal resprouter species. Specifically, we hypothesized that increasing fire frequency would negatively impact reproductive traits due to resource allocation toward resprouting, with clonal species being less affected due to their enhanced storage capacities. We collected data and traits from four Cerrado species- two clonal (*Mimosa leiocephala*, *Gymneia chapadensis*) and two non-clonal (*Diplusodon punctatus*, *Tibouchina melastomoides*) - across areas with varying fire frequencies and histories. At a broad level, the only trade-offs were found between macronutrient in belowground organs and full seeds percentage at low fire frequency. At the species-specific level, trade-offs became evident under particular conditions. For example, in *Diplusodon punctatus*, a significant trade-off between starch allocation in belowground organs and seeds was observed in areas with low fire frequency and recent burns. Results also showed positive relationships between non-structural carbohydrates and vegetative biomass affecting seeds and fruits number. Clonality affected only positive relationships between vegetative and reproductive traits. Our findings suggest that resource allocation strategies are highly species-dependent and context-specific, influenced by life history strategies and by fire regimes parameters. These results highlight the importance of integrating fire regime dynamics into studies of plant resource allocation to better understand plant survival and growth strategies in fire-prone ecosystems.

Keywords: Resource allocation, trade-off, fire frequency, Cerrado, tropical savanna, clonality

Introduction

Resource allocation is of utmost importance during plant life history, with trade-offs occurring among growth, survival and reproduction (Reekie & Bazzaz 2000). Resources such as water, mineral nutrients, carbon and carbohydrates play crucial roles for plant metabolism and storage (Madsen 1991; Bazzaz & Grace, 1997). For instance, water and nutrients affect photosynthesis while non-structural carbohydrates (NSCs) are used for plant growth and respiration processes, thus resources allocation are directly linked with life history strategies (Bazzaz & Grace 1997; Reekie & Bazzaz 2000; Landhäusser *et al.* 2018; Wigley *et al.* 2020a). Many resources are limited to plant organisms, therefore their use for different functions or life history phases could lead to trade-offs where the allocation to one function (e.g resprout, growth and vegetative reproduction) causes a decrease in the allocation to other (e.g reproduction, seed and fruit numbers) (Reekie & Bazzaz 2000; Reekie & Avila-Sakar 2005). For example, *Sargassum thunbergii* had an increase in allocation to sexual reproduction along with the decrease of vegetative regeneration (Chu *et al.* 2011). Thus, the resource allocation towards vegetative and reproductive structures in plants is a critical aspect of their life history strategies. This balance is influenced by environmental conditions, resource availability, competition and disturbances (such as fire) leading to varying outcomes in growth and reproduction (Reekie & Bazzaz 2000; Reekie & Avila-Sakar 2005; Wigley *et al.* 2020a).

Resource allocation in plants is strongly influenced by their life-history strategies, particularly by the distinction between clonal and non-clonal species (Lamont et al. 2011; Clarke et al. 2013). Clonal plants, which propagate vegetatively via specialized structures such as rhizomes or stolons, often have a distinct advantage in storing and reallocating resources in heterogeneous environments or after disturbances (Brewer & Platt 1994; Lamont et al. 2011; Clarke et al. 2013; Herben et al. 2015; Pérez-Harguindeguy et al. 2016). Duchoslavová & Jansa (2024) highlighted various resource translocation strategies in clonal species, including an 'equalization' strategy—where resources are transferred from resource-rich to resource-poor ramets—and an 'acropetal' strategy, which prioritizes the exploration and colonization of new areas. In contrast, non-clonal plants lack such physical connections and rely primarily on resource availability and individual resource storage capacity for persistence (Clarke et al. 2013). Understanding these distinct allocation strategies is essential for

comprehending how different plant types are adapted to disturbances and environmental variability.

In flammable ecosystems, fire alters the environment in various ways, including changes in resource availability, such as light and nutrients (Bond & van Wilgen 1996; Lamont et al. 2011). These changes affect individual plants and their processes, which can, in turn, influence resource acquisition and allocation (Bond & van Wilgen 1996; Lamont et al. 2011). As an example, fire can increase nutrient input to the soil, create open spaces that enhance light availability, and trigger or intensify flowering (Coutinho 1990; Bond & van Wilgen 1996; Zirondi *et al.* 2021; Fidelis & Zirondi 2021). In addition, plants have traits and strategies related to survival and persistence under recurrent fire (Bond & van Wilgen 1996; Keeley *et al.* 2011). Among these strategies, resprouting—the ability to regenerate vegetative structures from epicormic and belowground buds after fire—plays a crucial role in plant persistence (Lamont *et al.* 2011; Clarke *et al.* 2013; Chiminazzo *et al.* 2023).

For many species, resprouting is an energetically costly process, potentially leading to trade-offs between the allocation of resources to vegetative recovery and reproductive investment, such as seed production and quality (Bond & Midgley 2003; Reekie & Avila-Sakar 2005). On the other hand, it was observed that fire increased the photosynthetic rate in herbaceous plants, and high photosynthetic rates would lead rapid accumulation of reserves (de Moraes *et al.* 2016; Scalon & Rossatto 2021) and thus resprouting may not be a costly process avoiding resources trade-off. Understanding how fire regime influences plants processes, resource allocation and consequently these trade-offs is essential for predicting plant responses to fire (Bond & van Wilgen 1996; Bond & Midgley 2003; Keeley *et al.* 2011; Lamont *et al.* 2011).

Plants have strategies that influence persistence and regeneration in the post-fire environment (Coutinho 1982, 1990). For example, regarding persistence, most of Cerrados' plants bares the capacity to resprout after fire since meristems are protected on stems or in belowground organs (Coutinho 1982, 1990; Appezzato-da-Glória 2003; Pilon *et al.* 2021, Zupo et al. 2021). Several herbaceous plants rely on their resprouting capacity (mostly from basal and belowground structures) to survive fires and persist in tropical savannas, with some showing clonal growth (Coutinho 1982, 1990; Appezzato-da-Glória 2003; Pilon *et al.* 2021).

Additionally, in the Cerrado, variations in fire regime parameters significantly affect plant traits and processes (e.g. resprouting, flowering, regeneration and recruitment), potentially leading to alterations in resource allocation strategies. For instance, different fire frequencies and time since the last fire influence bud bank size, photosynthetic rates, root proportion and biomass among others (Scalon & Rossatto 2021; Le Stradic et al. 2021; Bombo et al. 2022). Fire regime parameters affecting those traits prompts questions about their broader impact on resource allocation. Although a theoretical framework for vegetative and reproductive allocation exists across many systems, empirical evidence remains mixed and highly context-dependent (Bond & Midgley 2003; Reekie & Avila-Sakar 2005; Cozin et al. 2024), and the trade-offs between these strategies are still poorly understood in the Cerrado. Furthermore, understanding the role of clonality in mediating these trade-offs is crucial, particularly in fire-prone ecosystems where clonal and non-clonal species coexist.

In this study, we aim to investigate possible trade-offs in resource allocation of clonal and non-clonal species from Cerrado. Also, we analysed how fire frequency and time since last fire would affect these trade-offs between vegetative and reproductive allocation in resprouters from the herbaceous layer. Specifically, we hypothesize that increasing fire frequency and time since last fire would negatively impact reproductive traits in both clonal and non-clonal plants. That is due to resources continually allocated to resprouting and to the lower nutrient cycling and availability in areas with a longer time since the last fire. However, we expect clonal plants to be less affected, given their capacity to store and reallocate resources more effectively than non-clonal species. By analysing reproductive and vegetative traits under different fire regimes, this study seeks to provide new insights into the ecological strategies of resprouter plants and the possible mechanisms driving trade-offs in resource allocation in fire-prone environments.

Material and Methods

Study area

The study species were collected in September 2022 at four different areas at the Reserva Natural Serra do Tombador (RNST, 13° 35’-38’ S and 47° 45’-51’ W, 560–1118 m a.s.l.)

in Central Brazil. RNST is an 8,900-ha private protected Cerrado area with a tropical climate, experiencing dry winters and rainy summers. Average maximum temperatures range from 26 to 36 °C, minimums from 8 to 14 °C, and annual rainfall averages between 1300 and 1500 mm (Antonelli-Filho, 2011). Although RNST hosts various Cerrado vegetation types, saplings were conducted in open savannas (*campo sujo*), characterized by a species-rich herbaceous layer, dominated by C4 grasses and forbs, with scattered shrubs and small trees (Coutinho, 1978).

To evaluate if different fire regimes parameters affect resource allocation towards vegetative and reproductive structures plants were collected from areas with different fire frequencies and time since last fire (here referred as fire history) at the study site. Fire frequencies were divided into High (11 to 16 fires in the last 20 years) and Low (0-2 fires in the last 20 years). Within each extreme of fire frequency, we also selected areas with different fire histories, categorized as long time since the last burn (LTB) and recently burned (RB) areas, totalizing four different experimental areas: High_LTB, High_RB, Low_LTB, and Low_RB.

Field survey and plant material collection

To evaluate if there was a trade-off and if different fire frequencies and histories affect resource allocation towards vegetative and reproductive structures (seeds) in clonal and non-clonal plants, we measured seed and plant traits that reflect those changes in the four areas (Table 1). We collected data from four species that are dominant in the area (see Rodrigues & Fidelis 2022): two non-clonal - *Diplusodon punctatus* Pohl (Lythraceae, shrub) and *Tibouchina melastomoides* (Naudin) Cogn. (Melastomataceae, forb) – and two clonal species - *Mimosa leiocephala* Benth. (Fabaceae, shrub) and *Gymneia chapadensis* Harley (Lamiaceae, forb). The traits measured are listed in Table 1 and were obtained from reproductive individuals present in at least two of the areas (with opposite fire frequencies, see Table 2). All traits were measured at the end of the dry season, outside the growing season (Saura-Mas & Lloret 2009). Species with individuals bearing structures such as rhizomes were considered clonal (Pérez-Harguindeguy *et al.* 2016; Pausas *et al.* 2018). In each area, traits were measured from 15 individuals per species per area.

Table 1. Plant traits measured in the study species: *Diplusodon punctatus*, *Tibouchina melastomoides* (non-clonal species, shrub and forb respectively), *Mimosa leiocephala*, and *Gymneia chapadensis* (clonal species, shrub and forb respectively) and its respective functional significance, methods, and references.

Traits	Units	Functional significance	Method	Adapted from (References)
Number of fruits per ind.	Count	Resource allocation towards reproduction and propagules	Counted per individual in situ	(Cornelissen et al., 2003; Pérez-Harguindeguy et al., 2016)
Fruits weight	mg	Resource allocation towards reproduction	Dry weight of fruits	
Seed mass (size)	mg	Related to seedling establishment, Dispersal distance, persistence in soil, germination timing and availability of resources	Dry weight of seeds or group of seeds	(Cornelissen et al., 2003; Pérez-Harguindeguy et al., 2016)
Total seed number	Count	Resource allocation towards reproduction	Counted per individual	
Full seeds number	Count/percentage	Resource allocation towards reproduction	Image analysis using X-ray	
Non-structural carbohydrates (seeds and belowground organs)	mg/g	Energy provision	Lab analysis	(Pérez-Harguindeguy *et al.* 2016; Wigley *et al.* 2020b)
Biomass dry weight (vegetative and reproductive)	g	Resource allocation	Dry weight of vegetative and reproductive structures	
Plant length	cm	Competitive vigor and the interval that plants must grow between fires	Measured in situ	(Cornelissen et al., 2003; Pérez-Harguindeguy et al., 2016)
Number of stems	Count	Resource allocation towards resprouting	Counted per individual in situ	(Cornelissen et al., 2003; Pérez-Harguindeguy et al., 2016)
Macronutrients (Leaves and Belowground organs)	g kg-1	Resource availability on soil	Lab analysis. Digestion: sulfuric (N); nitroperchloric (P, Ca, K, Mg, S). Determinations: colorimetry (P); turbidimetry	(da Silva 2009)

			(S); flame photometry (K); atomic absorption spectrophotometry (Ca, Mg).	
Clonal growth	Categorical	Regeneration and persistence after disturbance (fire), influencing reproductive traits	Classification in the field according to protocol	(Cornelissen et al., 2003; Klimešová et al., 2019; Pérez-Harguindeguy et al., 2016)

Table 2. Treatments where species were present and collected from (in green).

Species	High_LTB	High_RB	Low_LTB	Low_RB
Diplusodon punctatus (DP)				
Tibouchina melastomoides (TM)				
Mimosa leiocephala (ML)				
Gymneia chapadensis (GC)				

Carbohydrates analyses

We quantified non-structural carbohydrates in the seeds and belowground organs of five individuals per area in the studied species, for seeds some pools of two or more individuals were necessary to reach the minimum weight for the analyses (~40mg). For that soon after the collection we exposed the samples to 800W in a microwave to dry and stop the metabolism (Quentin *et al.* 2015), the samples were then stored in paper bags and brought to the laboratory.

For the analysis of total carbohydrates (oligosaccharides, polysaccharides, and starch), we conducted a modified anthrone-sulfuric acid protocol (adapted from Scott & Melvin, 1953), with the procedures and steps outlined below:

Sample Preparation: Plant tissue samples were dried, ground to a fine powder, and weighed accurately (~40 mg per sample). The samples were divided into tubes for each fraction: oligosaccharides, polysaccharides, and starch.

Extraction Procedure:

1. Oligosaccharides: Three sequential extractions were performed using 4 mL of 80% ethanol in a water bath at 80°C for 20 minutes per extraction. After each extraction, the tubes

were cooled, centrifuged at 3,400-4,000g for 5-10 minutes, and the supernatant was collected. The final volume was recorded, and the extract was frozen at -20°C.

2. Polysaccharides: The residue from the oligosaccharide extraction was subjected to two extractions with 3 mL of distilled water at 60°C for 30 minutes each. After each extraction, the tubes were centrifuged as described above, the supernatant was collected, the final volume was recorded, and the extract was stored at -20°C.

3. Starch: Following polysaccharide extraction, the remaining residue was extracted with 30% perchloric acid for 2 hours at room temperature. The tubes were agitated halfway through extraction to ensure resuspension. The final supernatant was collected, volume recorded, and stored at -20°C.

Quantification using Anthrone Reagent: Total carbohydrate content was quantified spectrophotometrically at 620 nm using the anthrone-sulfuric acid method. Briefly, 1 mL of sample (or water for the blank) was mixed with 2 mL of anthrone reagent in pyrex tubes, capped with marbles, and incubated in a boiling water bath for 5 minutes. Samples were then cooled, and absorbance was read at 620 nm. If absorbance exceeded the desired range (0.1-0.8), samples were diluted accordingly. Calibration was achieved using glucose standards.

Special Handling for Samples with Interfering Compounds: For samples suspected to contain interfering proteins, a preliminary Bradford test was conducted. Samples with high protein content were desalted with ammonium sulfate and refrigerated overnight to precipitate proteins. If phenolic compounds were present, activated charcoal (1% of sample volume) was added to clarify the extract before spectrophotometric analysis.

Macronutrient

Macronutrient analyses were conducted on five samples of the belowground organs of all species and treatments where samples were available, as well as on the leaves of *Mimosa leiocephala* and *Diplusodon punctatus*, since the other species lacked live leaves at the time of collection. Unfortunately, we did not have a sufficient sample weight from seeds to perform the macronutrient analyses. The samples were sent to a specialized laboratory, where analyses followed the methodology described by da Silva (2009): Digestion methods: sulfuric acid (N); nitroperchloric acid (P, Ca, K, Mg, S). Determinations: colorimetry (P); turbidimetry (S); flame photometry (K); atomic absorption spectrophotometry (Ca, Mg).

Statistical analysis

To investigate if there were trade-offs between reproductive and vegetative traits at the broad level, we performed 22 GLMM (generalized linear mixed models) with a Gaussian distribution for continuous data and negative binomial for counting data. To facilitate the comparison across predictors measured in different units, all continuous predictor variables were standardized by centering them at their mean and scaling by their standard deviation (i.e., z-transformation). To investigate the effects of fire regime parameters in the trait's relationship, fire frequencies (High and Low) and fire history (RB and LTB) were treated as fixed factors and species as a random factor. We also included clonality (clonal vs. non-clonal plants) as a fixed factor when necessary. We extracted Marginal R^2 (Represents the proportion of variance explained by the fixed effects in the mixed-effects model) and Conditional R^2 (Represents the total proportion of variance explained by the model, including both fixed and random effects) for each model using R package *MuMIn* (Barton 2012).

To assess whether clonality influences the relationship between reproductive and vegetative traits under different fire regime parameters (fire frequency and fire history), we estimated the slopes of the predictor variables across levels of Clonality and fire regimes. Slopes were extracted from the interaction term of the generalized linear mixed model (GLMM) and represent the effect of vegetative on reproductive variables for each combination of clonality and fire frequency/history. These slopes were then visualized to compare how the strength and direction of the relationship varied among groups. In addition, for this analysis we excluded data for leaves since only two species had leaves at the sampling time.

To investigate the trade-off at the species level we did simple linear regressions (LM). All statistical analyses were performed in the R software (R Core Team 2016).

Results

Resource allocations between reproductive and vegetative traits

The results showed that trade-offs were generally not prevalent when analysing the overall output (Table 3, Figure 1). In fact, most relationships were either non-significant or

positive, suggesting that trade-offs are not consistent across species and fire regimes (Table 3, Figure S1).

However, significant trade-offs were identified for specific models under certain fire regime parameters. When analysing all species together, considering species as random factors, trade-offs were observed at the Low fire frequency, and regarding macronutrients in belowground organs (N, P, Ca and Mg) and full seeds percentage ($p<0.05$, Figure 1, Table 3). No trade-off was found when analysing at the fire history level (Table 3). However, although the models were significant, marginal R^2 values (R^2m) showed that the relationships are poorly explained by the fire regime parameters (Figure 1) and the R^2 values only increased when accounting for the species (conditional R^2) (Figure 1). Therefore, results indicate that trade-offs are highly species-dependent. That is evident when looking at the species level analysis, where other trade-offs were observed. We found trade-offs for non-structural carbohydrates allocation for *Diplusodon punctatus* (starch) and *Gymneia chapadensis* (polysaccharides) occurring only in plants from low fire frequency and/or recently burned (RB) areas (Table S1). The number of seeds were also negatively affected by vegetative traits (such as biomass, number of stems and macronutrients in B.O.) for all species ($p<0.05$), mostly in plants from recently burned areas (RB) and low fire frequency (Table S1).

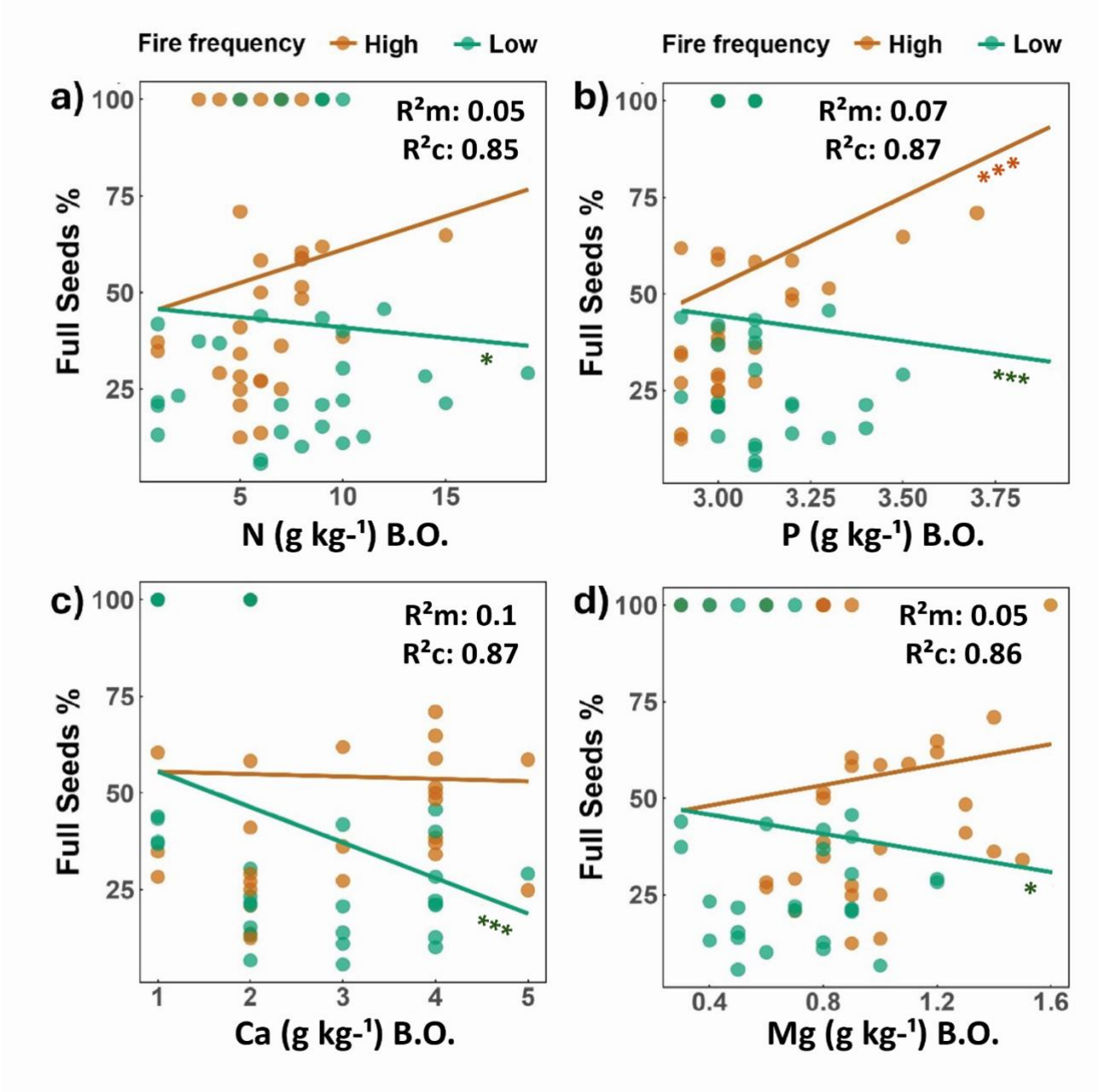

Figure 1. Figures of generalized linear mixed model (GLMM) outputs with the relationship between reproductive and vegetative traits where trade-offs are detected. Trade-offs found between full seeds % and macronutrient content in belowground organs (B.O.) under different fire frequencies. Each panel represents the relationship between full seeds % and: a) N = Nitrogen, b) P = Phosphorus, c) Ca = Calcium and d) Mg = Magnesium in B.O. Regression lines illustrate significant interactions between fire frequency (high = orange, low = green) and macronutrient content. Significant p-values are indicated, with asterisks denoting significance levels (*p < 0.05, **p < 0.01, ***p < 0.001). A negative slope in green suggests a trade-off under low fire frequency, whereas a positive slope in orange indicates a positive relationship under high fire frequency (only for phosporus). R^2m (Marginal R^2): Represents the proportion of variance explained by the fixed effects, indicating how much of the variation in the data is accounted for solely by the fixed predictors, excluding random effects. R^2c (Conditional R^2): Represents the total proportion of variance explained by the model, encompassing both fixed and random effects, and reflecting the overall fit of the model to the data.

Positive relationships (PR) between reproductive and vegetative traits, when analysing all species together, were observed between the number of reproductive structures (seeds and fruits) and vegetative biomass, influenced by both fire frequency and fire history (Table 3). Additionally, PR was found for macronutrients affecting full seed percentages, but only under high fire frequency (Table 3). At the species-specific level, results showed PR occurred between different combinations of reproductive and vegetative traits (see Table S1 for details).

Table 3. Classification of the relationship between variables based on the output of generalized linear mixed models (GLMMs). Relationships are categorized as follows: NS (No significant relationship) - Indicates no significant interaction between the tested variables; PR (Positive Relationship)- Indicates a positive association between the tested variables; TD (Trade-Off)-Indicates a trade-off between the tested variables. The levels of fire frequency are represented as High and Low, while the levels of fire history are denoted as LTB (Long Time Since Burned), areas with a long time since the last fire and RB (Recently Burned), areas that were recently burned. For interactions involving fire frequency and fire history, relationships are described for each level where applicable.

Model	Relationship	
	Fire frequency	Fire history
Full seeds percentage ~ Total vegetative aboveground weight	PR(Low)	NS
Total number of seeds ~ Total vegetative aboveground weight	PR	PR
Number of fruits ~ Total vegetative aboveground weight	PR	PR
Total reproductive weight ~ Total vegetative aboveground weight	PR	PR
Number of fruits ~ Total number of stems	NS	NS
Total number of seeds ~ Total number of stems	NS	NS
Oligosaccharides in Seeds ~ Oligosaccharides in belowground organs	NS	NS
Polysaccharides in Seeds ~ Polysaccharides in belowground organs	NS	NS
Starch in Seeds ~ Starch in belowground organs	PR	PR (LTB)
Total CHOs in Seeds ~ Total CHOs in belowground organs	NS	NS
Full seeds % ~ Nitrogen in belowground organs	TD (Low)	NS
Full seeds % ~ Phosphorus in belowground organs	PR (High) / TD (Low)	NS
Full seeds % ~ Potassium in belowground organs	PR (High)	NS
Full seeds % ~ Calcium in belowground organs	TD (Low)	NS
Full seeds % ~ Magnesium in belowground organs	TD (Low)	NS
Full seeds % ~ Sulfur in belowground organs	PR (High)	NS
Full seeds % ~ Nitrogen in leaves	NS	NS
Full seeds % ~ Phosphorus in leaves	NS	NS
Full seeds % ~ Potassium in leaves	NS	NS
Full seeds % ~ Calcium in leaves	PR(High)	NS
Full seeds % ~ Magnesium in leaves	NS	NS
Full seeds % ~ Sulfur in leaves	NS	NS

Clonal vs. Non-clonal species

Clonality significantly influenced only the positive relationships (PR) between reproductive and vegetative traits in four out of the 16 tested models (Figure 2). Two of these cases involved vegetative biomass affecting seed number and fruit number (Figure 2a, b), while the other two were related to the allocation of polysaccharides and starch between belowground organs and full seed percentages (Figure 2c, d). The results showed a stronger positive relationship between vegetative biomass and the number of seeds and fruits in non-clonal species. Consequently, non-clonal plants produced significantly more seeds and fruits than clonal species ($p < 0.05$, Figure 2a, b).

Additionally, the interaction between belowground polysaccharide content and clonality was significant ($p < 0.0001$), suggesting that the allocation of polysaccharides to seeds differed between clonal and non-clonal species (Figure 2c). A similar pattern was observed for starch allocation ($p = 0.005$, Figure 2d), indicating that clonal species tended to allocate more non-structural carbohydrates (polysaccharides and starch) to seeds.

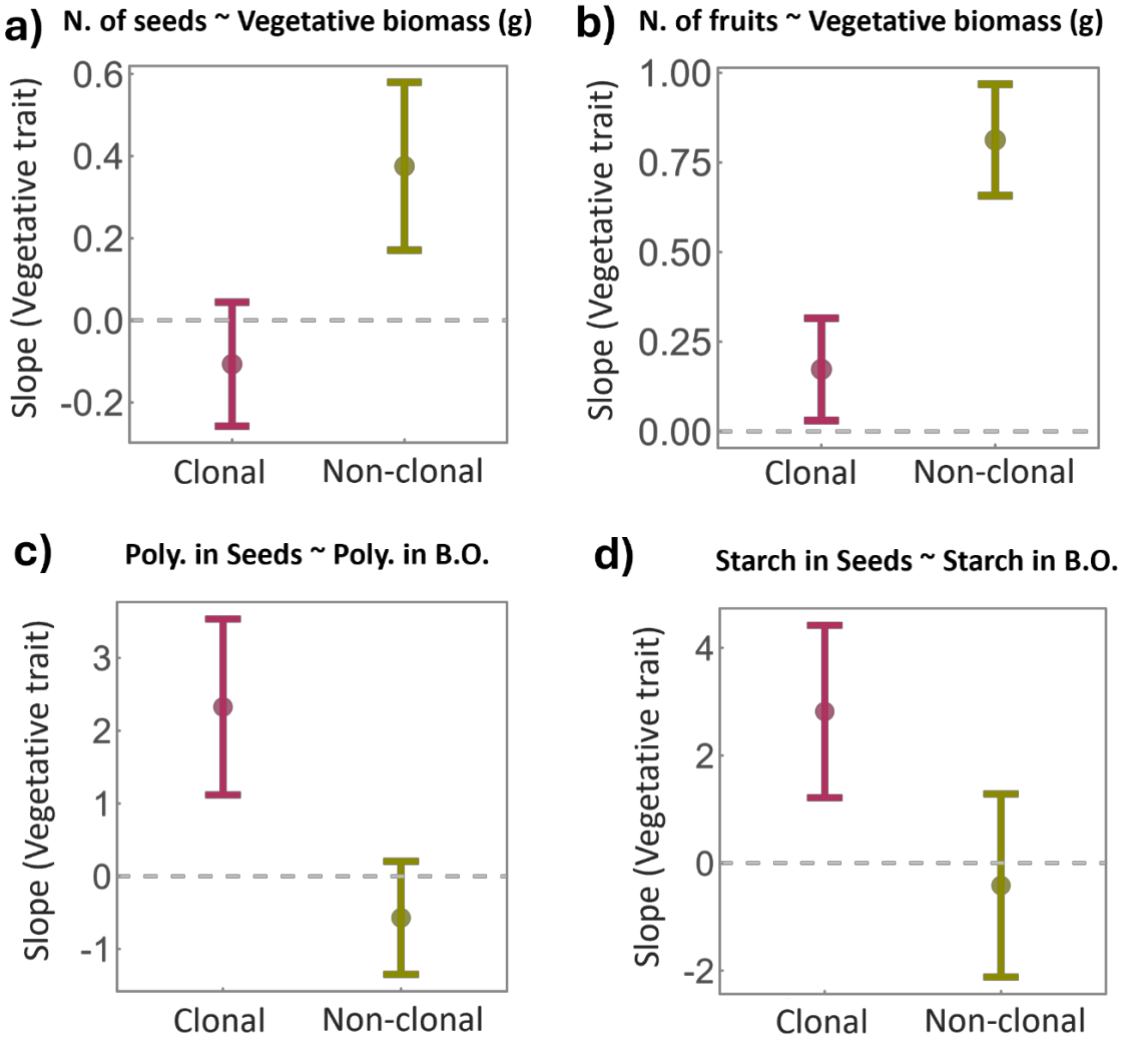

Figure 2. The figure presents the estimated slopes (β) from generalized linear mixed models (GLMMs) analysing the relationship between vegetative and reproductive traits in clonal and non-clonal species, only where a significant effect was found ($p < 0.05$). In panels (a) and (b), the interaction between vegetative biomass and clonality significantly affects the total number of seeds and fruits, respectively, with non-clonal species producing more seeds than clonal ones. Panels (c) and (d) illustrate a positive relationship in non-structural carbohydrate allocation strategies for clonal plants. Panel (c) shows the interaction between belowground (B.O.) polysaccharide content and clonality, affecting polysaccharide content in seeds, while panel (d) represents starch allocation. The dashed horizontal line at zero indicates no effect. Slopes above zero suggest a positive relationship, whereas slopes below zero suggest a trade-off.

When considering the interaction between clonality and fire regime parameters (fire frequency and fire history), we found significant effects only for positive relationships (PR) in four out of the 32 tested models—one associated with fire frequency and the others with fire history (Figure 3). On average, clonal species exhibited a 3.3-unit higher response than non-

clonal species ($p = 0.01$). At high fire frequency, an increase in belowground polysaccharide content resulted in higher polysaccharide levels in seeds (Figure 3a).

In contrast, the interaction between fire history and clonality revealed that non-clonal species had significantly higher slope estimates than clonal species ($p < 0.001$), indicating a stronger relationship between vegetative biomass and seed and fruit production in recently burned areas. Specifically, in non-clonal species, vegetative biomass had a stronger positive effect on the total number of seeds ($p = 0.018$) and fruits ($p = 0.026$), particularly in areas recently burned (RB) (Figure 3b, c). This suggests that, for non-clonal species, larger plants (with greater vegetative biomass) were associated with higher fruit production.

Regarding total carbohydrate (CHO) content in seeds, no significant differences were observed between clonal and non-clonal species. However, we found a significant PR between starch in belowground organs and seeds in areas with a long time since the last burn (LTB) (Figure 3d).

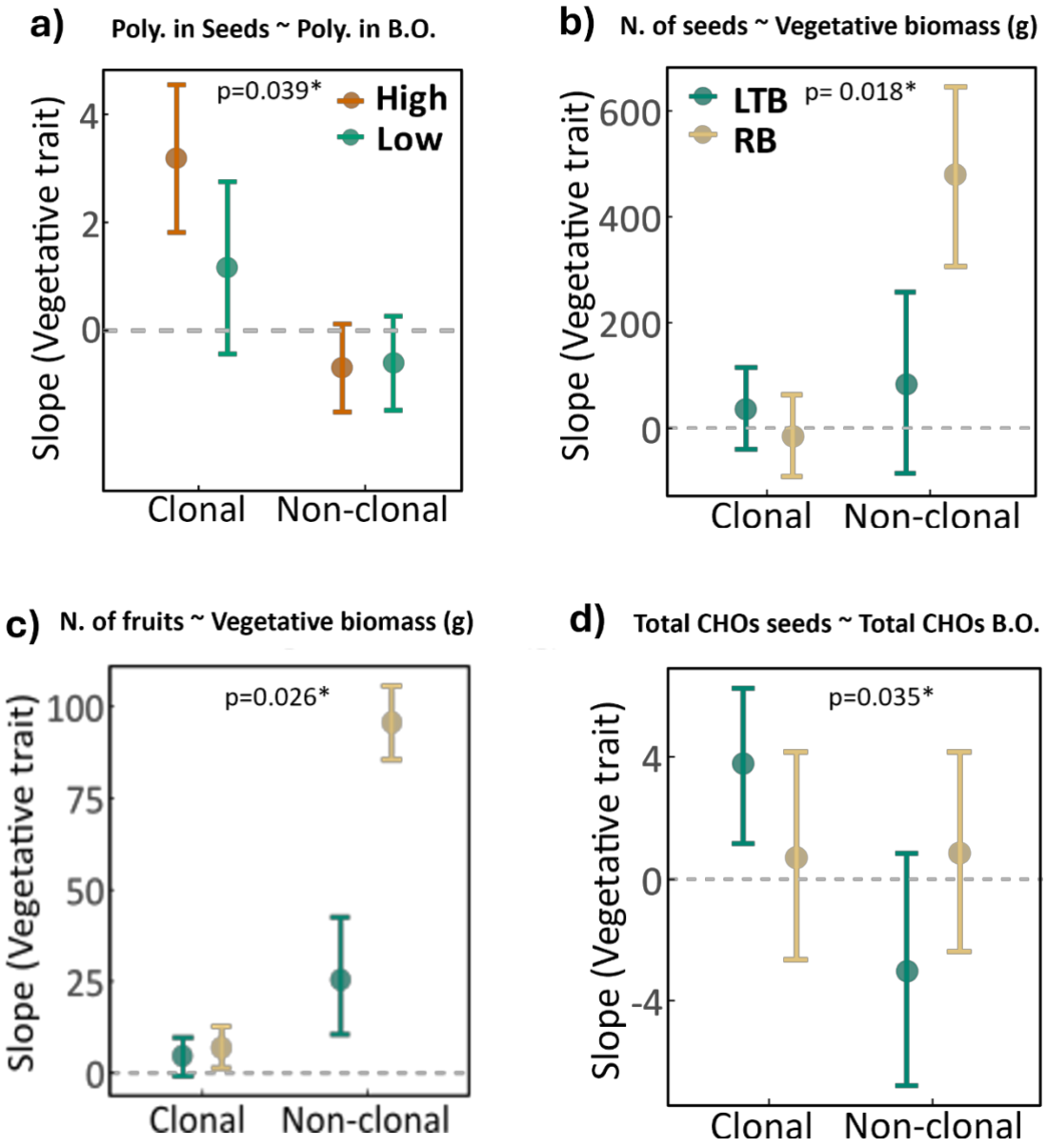

Figure 3. Figure showing slope estimates (±95% confidence intervals) from generalized linear mixed models assessing how Fire frequency (High = orange, Low = green) and Fire history (RB = yellow, LTB = dark cyan) interact with Clonality (Clonal vs. Non-clonal) to influence the relationship between vegetative and reproductive traits. Significant interactions were only found for positive relationships, specifically: (a) polysaccharides in seeds and belowground organs (B.O.) at high fire frequency for clonal plants, (b) and (c) total seed and fruit numbers in relation to vegetative biomass for non-clonal plants, and (d) starch allocation. The dashed horizontal line at zero indicates no effect. Slopes above zero suggest a positive relationship, whereas slopes below zero indicate a negative relationship. P values indicate the significance of the interaction between fire parameters and clonality.

Discussion

Contrary to our initial hypothesis, trade-offs between reproductive and vegetative traits were not widespread among Cerrado species. However, significant trade-offs emerged under specific conditions, particularly at low fire frequency, where greater macronutrient allocation in belowground organs (N, P, Ca, and Mg) negatively affected the percentage of full seeds. Additionally, species-specific trade-offs were identified, such as reduced seed numbers in plants with greater vegetative biomass or higher stem numbers, especially in recently burned areas. In contrast, we found strong evidence of positive relationships (PR) in resource allocation, where reproductive output (seed and fruit production) was positively associated with vegetative biomass across fire regimes. Notably, non-clonal species exhibited stronger PRs, suggesting distinct reproductive strategies in response to fire history. These findings challenge the classic trade-off hypothesis and highlight the roles of species identity, fire regime, and clonality in shaping reproductive and vegetative allocation patterns.

Several factors could explain the lack of trade-offs, being one possible reason for the complexity and variability of resource allocation in plants (Reekie & Bazzaz 2000; Reekie & Avila-Sakar 2005). For instance, plant structures often serve multiple functions; for example, stems support both vegetative and reproductive growth, while roots, stems, and leaves contribute to vegetative maintenance and reproduction simultaneously in vegetatively reproducing species (Reekie & Bazzaz 2000). Additionally, resource allocation patterns can vary depending on the type of resource (e.g. biomass, carbohydrates, or minerals) and these resources often limit growth and reproduction differently depending on the environment or the plant's life stage (Lamont et al. 2011). Recent evidence further complicates the trade-off hypothesis, since stored carbohydrates used for post-fire regrowth do not necessarily occur at the expense of seed production (Lamont et al. 2011). Similarly, in some cases, reproductive limitations are more frequently linked to nutrient constraints rather than resource and carbon competition with vegetative functions (Witkowski & Lamont 1996; Cruz et al. 2003; Lamont et al. 2011). Thus, trade-offs may not occur or be less detectable especially when accounting for the interactions between resource allocation, environmental variability, and multifunctional structures.

The quantification of macronutrients content in seeds was not possible in this study for testing a direct trade-off. However, since macronutrients are important for seed filling,

development and functions (Tiryakioğlu et al. 2014; de Bang et al. 2020; Hawkesford et al., 2023) we selected the percentage of full seeds to be a proxy in the trade-off of macronutrients in belowground organs affecting reproductive traits. For example, nitrogen is a key component of amino acids, which are the building blocks of proteins (Tiryakioğlu et al. 2014; Hawkesford et al., 2023) while phosphorus is essential for embryo development (Maathuis 2009). The trade-off found showed that the higher the content of some macronutrients (N, P, Ca and Mg) in belowground organs the lower the full seeds percentage in plants from low fire frequency areas. In the Cerrado, soils are inherently nutrient-poor (Coutinho 1990); however, nutrient availability is typically higher in recently burned compared to fire-excluded areas (Da Silva & Batalha 2008). Thus, under low fire frequency, where nutrient cycling is reduced, plants may prioritize storage in belowground organs to ensure survival, negatively affecting nutrient allocation to seeds.

Same principle can be applied to the species level trade-offs regarding macronutrients and non-structural carbohydrates (NCs). The trade-off between starch and polysaccharide allocation in belowground organs and seeds was significant for two species. It occurred for *Diplusodon punctatus* under low fire frequency and recently burned areas and for *Gymneia chapadensis* only at RB. In environments with limited resources and nutrient availability, resprouters tend to accumulate non-structural carbohydrates (e.g. starch and polysaccharide), as these reserves are critical for survival and vegetative regrowth (Knox & Clarke 2005; Fu et al. 2010). Starch serves as a primary storage carbohydrate that plants use to meet energy demands for growth, maintenance, resprout and reproduction (Miyanishi & Kellman 1986; Chapin et al. 1990; Pausas et al. 2018). Conversely, in seeds, starch is allocated to support seed development and provide energy reserves for germination and seedling establishment (Tetlow 2011; MacNeill et al. 2017; Aguirre et al. 2018). In areas with low fire frequency, if resource availability declines over time, plants may face a trade-off in allocating these reserves between belowground storage and reproduction. On the other hand, a similar trade-off was observed in recently burned areas, where resource availability is not necessarily limiting. However, in these environments, plants may prioritize resprouting of new stems, vegetative growth, and investment in belowground structures (Bellingham & Sparrow 2000; Bell 2001; Bond & Midgley 2003, Knox & Clarke 2005), leading to the preferential allocation of

NCs toward these functions. Consequently, this results in a reduced starch and polysaccharide content in seeds.

Other trade-offs between reproductive and vegetative variables were found at the species level under specific fire frequencies or histories. Results showed that for the two clonal species, *Gymneia chapadensis* and *Mimosa leiocephala*, trade-offs occurred only in recently burned areas (RB) and/or under high fire frequency. In contrast, for the two non-clonal species, *Diplusodon punctatus* and *Tibouchina chapadensis*, most trade-offs were observed only under low fire frequency. These findings suggest that resource allocation in clonal and non-clonal plants responds differently to fire regimes, reflecting distinct persistence and survival mechanisms. Clonal species appear to be more sensitive to high fire frequency and the immediate post-fire period, prioritizing resource allocation toward rapid resprouting to ensure survival in a highly disturbed environment (Bond & van Wilgen 1996). On the other hand, non-clonal species seem to buffer the immediate effects of fire more effectively, possibly due to a more stable resource allocation strategy over time. However, in the absence of fire or under low fire frequency, non-clonal species begin to exhibit trade-offs, likely due to prioritizing resource storage on belowground organs when the environmental resources are scarce (Knox & Clarke 2005; Fu et al. 2010). This suggests that fire frequency and history play a crucial role for individual species in shaping resource allocation strategies in clonal and non-clonal species, ultimately influencing their ecological responses in fire-prone environments.

On the positive relationships found, we observed that at high fire frequency, bigger plants produced more seeds and fruits as well as a positive balance in macronutrient content. Therefore, where the fire recurrence interval is shorter, there is a regular nutrient input into the soil available to the plants (Bond & van Wilgen 1996; Pivello et al. 2010), leading to an accumulation of nutrients that, in turn, could promote enhanced growth and reproductive capacity (Knox & Clarke 2005; Pivello et al. 2010). Additionally, larger vegetative structures appear to support a greater number of reproductive organs, likely because a more extensive structure can support more flowers that, in turn, yield more fruits (Pickering 1994). Moreover, larger plants often exhibit an increased capacity for nutrient storage, allowing them to allocate more resources to reproductive structures—particularly under favorable conditions, such as nutrient-rich soils following fire events (Cruz & Moreno 2001; Knox & Clarke 2005). This pattern is exemplified by *Erica australis*, which showed increases in both reproductive and

vegetative biomass after fire, a response likely linked to its ability to store high amounts of carbon (Cruz & Moreno 2001).

Our initial hypothesis regarding clonality and trade-offs was not supported, as only positive interactions between vegetative and reproductive variables were affected by clonality. Overall, clonal species showed a stronger positive allocation toward non-structural carbohydrates (NCs), whereas non-clonal species exhibited a positive relationship between vegetative biomass and seed/fruit production. When considering fire parameters, clonal plants were more effective at allocating polysaccharides and total NCs under high fire frequency and long intervals since last fire. This suggests that their capacity for storing and mobilizing belowground reserves (Appezzato-da-Glória 2003; Pérez-Harguindeguy et al. 2016; Klimešová et al. 2018) provides a significant advantage in frequently disturbed environments. In contrast, non-clonal species showed a positive correlation between larger plant size (i.e., higher vegetative biomass) and increased seed/fruit output in recently burned areas, potentially due to the elevated nutrient availability typical of post-fire conditions (Bond & van Wilgen 1996; Knox & Clarke 2005) and the ability of larger individuals to more effectively capture and utilize these resources. Ultimately, our results highlight that clonality can enhance a plant's ability to recover from disturbances (Pérez-Harguindeguy et al. 2016), and that shifts in fire regimes may drive changes in resource allocation patterns—particularly between vegetative and reproductive components—in fire-prone ecosystems.

Finally, the absence of additional trade-offs or relationships among the variables may be attributed to the highly efficient resource acquisition and allocation strategies of Cerrado species. These species are capable of storing water and nutrients in their belowground organs (da Silva & Rossatto 2019). Moreover, fire has been shown to increase photosynthetic rates in herbaceous plants, resulting in a rapid accumulation of reserves (de Moraes et al. 2016; Scalon & Rossatto 2021; Antonio 2024). This quick reserve buildup likely reduces the costs associated with resprouting, thereby mitigating significant resource trade-offs. Consequently, understanding how fire regime parameters influence plant processes, resource allocation, and subsequent trade-offs is crucial for accurately predicting plant responses to fire (Bond & van Wilgen 1996; Bond & Midgley 2003; Keeley et al. 2011; Lamont et al. 2011).

Overall, these findings suggest that while trade-offs between reproductive and vegetative traits are not universally observed, they emerge under specific conditions

influenced by fire frequency and history and are highly dependent on species and their ecological strategies. Resource allocation between vegetative and reproductive variables is affected by fire regime parameters and life history strategies, highlighting the importance of fire regime shaping plant responses to disturbance. These findings suggest that future studies should further investigate the interplay between fire regimes, resource allocation strategies, and clonality to better understand the drivers of plant community dynamics in fire-prone ecosystems.

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Supplementary Materials

Figure S1. Generalized linear mixed model (GLMM) outputs showing the relationships between reproductive and vegetative traits under different fire frequencies (panel A) and fire histories (panel B). U.O. = underground organs, Oligo. = oligosaccharides, Poly. = polysaccharides, Total CHOs = total non-structural carbohydrates, N = nitrogen, P = phosphorus, K = potassium, Ca = calcium, Mg = magnesium, and S = sulfur. Regression lines illustrate significant interactions between vegetative and reproductive variables at each level of fire frequency (High = orange, Low = green) and fire history (RB = yellow, LTB = dark cyan). A negative slope suggests a trade-off, whereas a positive slope indicates a positive relationship. R^2m (Marginal R^2): The proportion of variance explained by the fixed effects, indicating how much of the variation in the data is accounted for solely by the fixed predictors, excluding random effects. R^2c (Conditional R^2): The total proportion of variance explained by the model, encompassing both fixed and random effects (species), reflecting the overall fit of the model to the data.

Figure S1 A

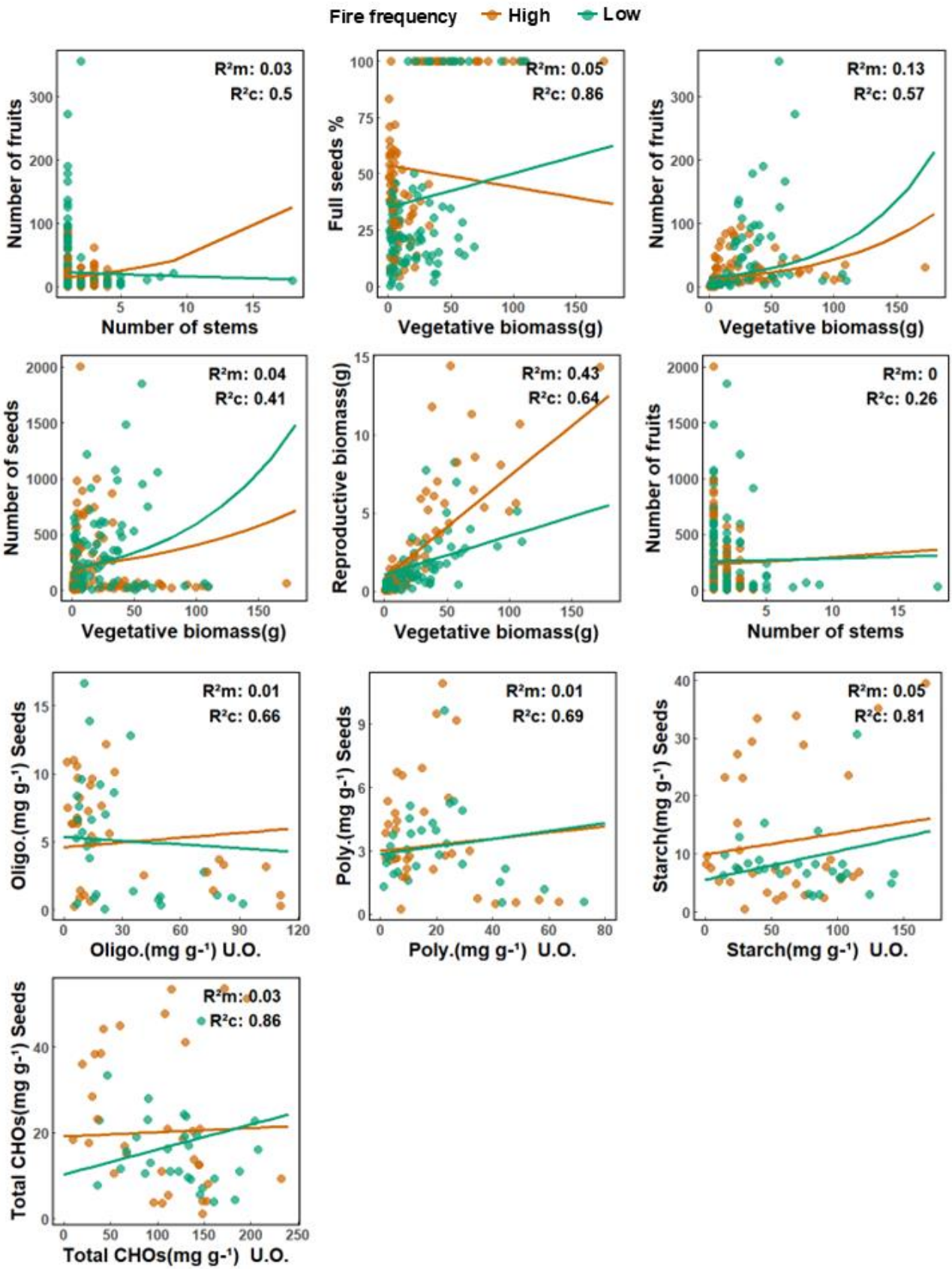

Fire frequency
High
Low
R²m: 0.03
R²c: 0.5
Number of fruits
Number of stems
R²m: 0.05
R²c: 0.86
Full seeds %
Vegetative biomass(g)
R²m: 0.13
R²c: 0.57
Number of fruits
Vegetative biomass(g)
R²m: 0.04
R²c: 0.41
Number of seeds
Vegetative biomass(g)
R²m: 0.43
R²c: 0.64
Reproductive biomass(g)
Vegetative biomass(g)
R²m: 0
R²c: 0.26
Number of fruits
Number of stems
R²m: 0.01
R²c: 0.66
Oligo.(mg g-¹) Seeds
Oligo.(mg g-¹) U.O.
R²m: 0.01
R²c: 0.69
Poly.(mg g-¹) Seeds
Poly.(mg g-¹) U.O.
R²m: 0.05
R²c: 0.81
Starch(mg g-¹) Seeds
Starch(mg g-¹) U.O.
R²m: 0.03
R²c: 0.86
Total CHOs(mg g-¹) Seeds
Total CHOs(mg g-¹) U.O.

Figure S1 A

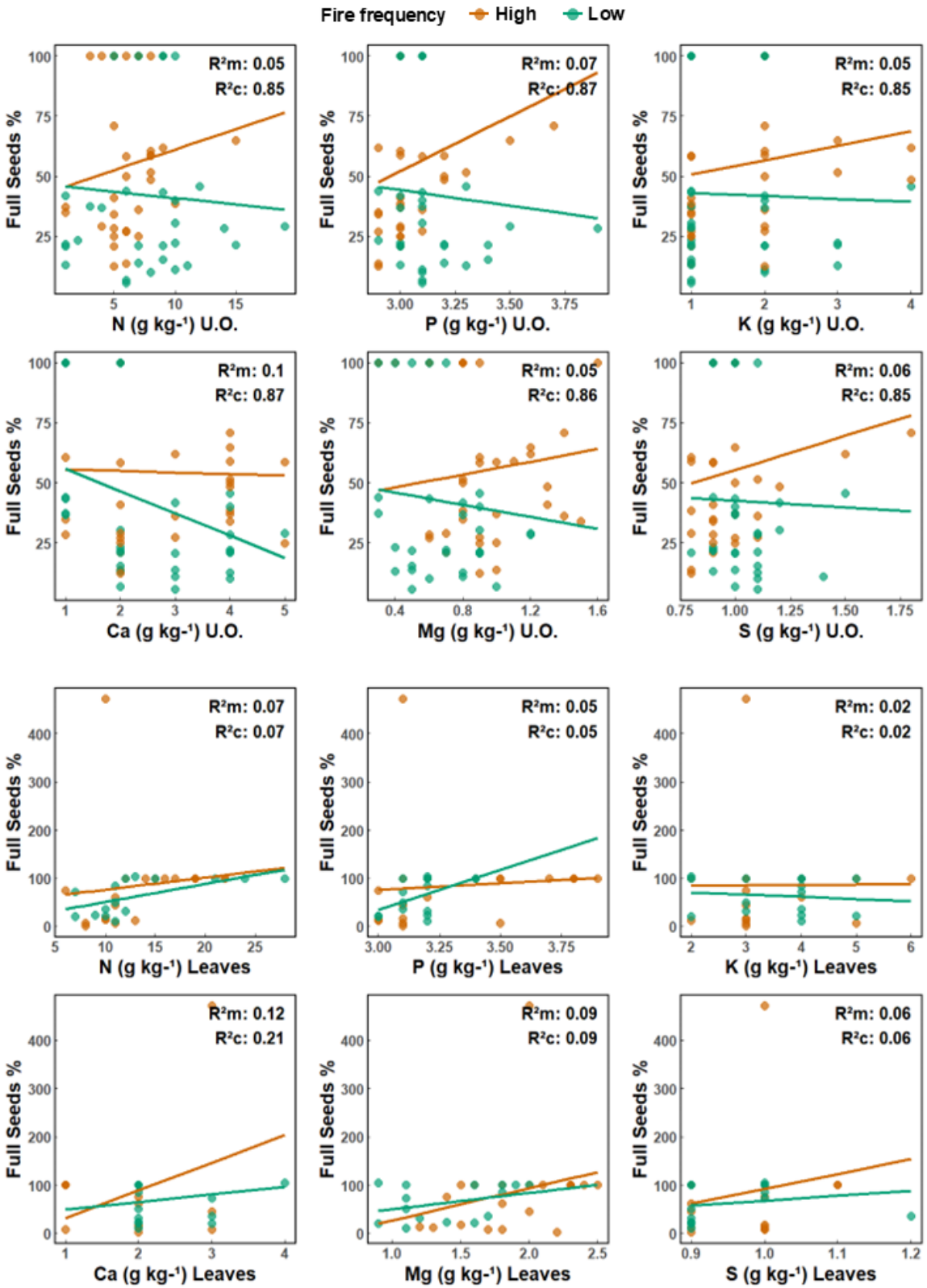

Fire frequency
High
Low
Full Seeds %
R²m: 0.05
R²c: 0.85
N (g kg-¹) U.O.
R²m: 0.07
R²c: 0.87
P (g kg-¹) U.O.
R²m: 0.05
R²c: 0.85
K (g kg-¹) U.O.
R²m: 0.1
R²c: 0.87
Ca (g kg-¹) U.O.
R²m: 0.05
R²c: 0.86
Mg (g kg-¹) U.O.
R²m: 0.06
R²c: 0.85
S (g kg-¹) U.O.
R²m: 0.07
R²c: 0.07
N (g kg-¹) Leaves
R²m: 0.05
R²c: 0.05
P (g kg-¹) Leaves
R²m: 0.02
R²c: 0.02
K (g kg-¹) Leaves
R²m: 0.12
R²c: 0.21
Ca (g kg-¹) Leaves
R²m: 0.09
R²c: 0.09
Mg (g kg-¹) Leaves
R²m: 0.06
R²c: 0.06
S (g kg-¹) Leaves

Figure S1 B

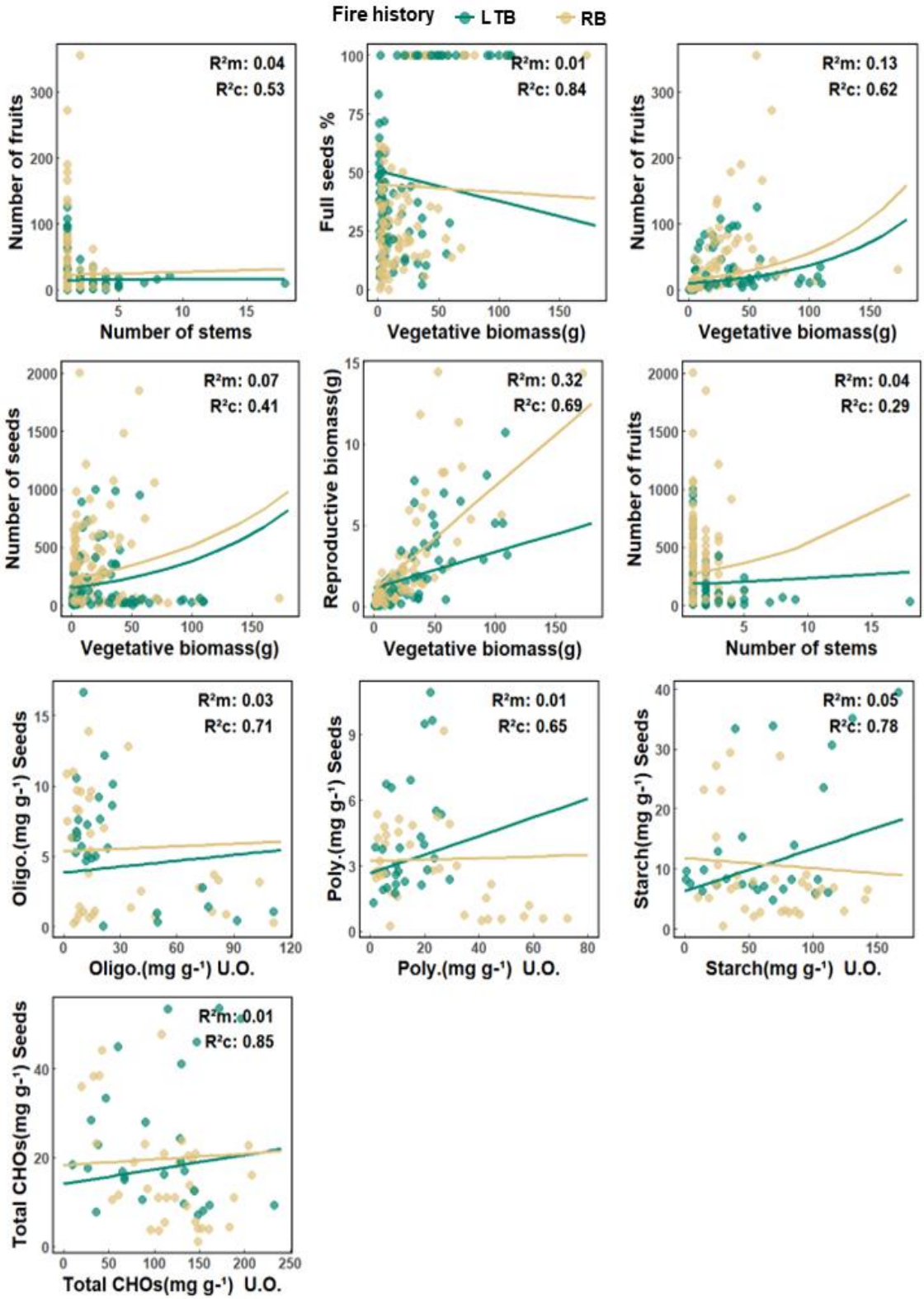
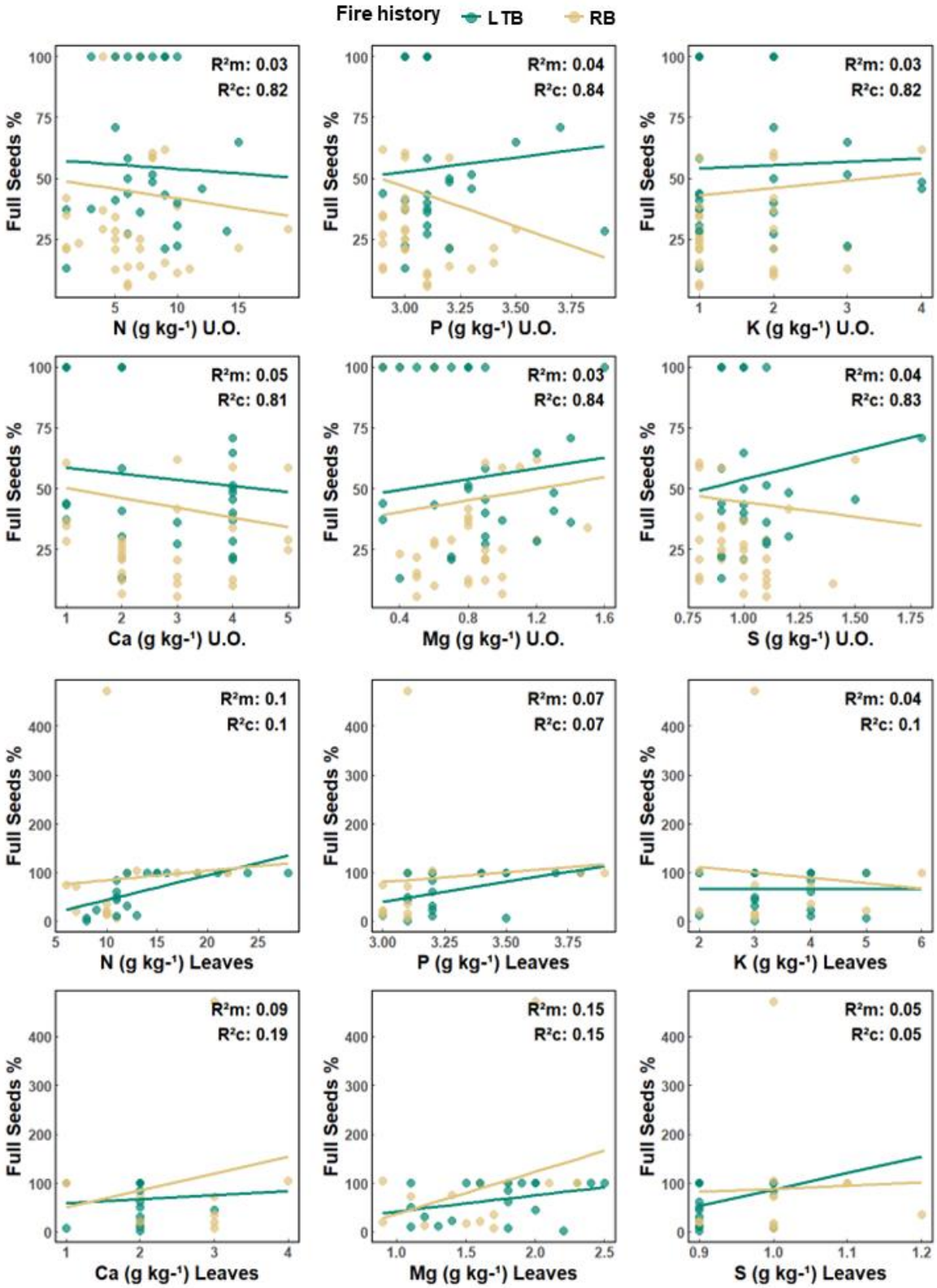

Fire history
LTB
RB
R²m: 0.04
R²c: 0.53
Number of fruits
Number of stems
R²m: 0.01
R²c: 0.84
Full seeds %
Vegetative biomass(g)
R²m: 0.13
R²c: 0.62
Number of fruits
Vegetative biomass(g)
R²m: 0.07
R²c: 0.41
Number of seeds
Vegetative biomass(g)
R²m: 0.32
R²c: 0.69
Reproductive biomass(g)
Vegetative biomass(g)
R²m: 0.04
R²c: 0.29
Number of fruits
Number of stems
R²m: 0.03
R²c: 0.71
Oligo.(mg g-1) Seeds
Oligo.(mg g-1) U.O.
R²m: 0.01
R²c: 0.65
Poly.(mg g-1) Seeds
Poly.(mg g-1) U.O.
R²m: 0.05
R²c: 0.78
Starch(mg g-1) Seeds
Starch(mg g-1) U.O.
R²m: 0.01
R²c: 0.85
Total CHOs(mg g-1) Seeds
Total CHOs(mg g-1) U.O.

Figure S1 B


Fire history
LTB
RB
Full Seeds %
R²m: 0.03
R²c: 0.82
N (g kg-¹) U.O.
R²m: 0.04
R²c: 0.84
P (g kg-¹) U.O.
R²m: 0.03
R²c: 0.82
K (g kg-¹) U.O.
R²m: 0.05
R²c: 0.81
Ca (g kg-¹) U.O.
R²m: 0.03
R²c: 0.84
Mg (g kg-¹) U.O.
R²m: 0.04
R²c: 0.83
S (g kg-¹) U.O.
R²m: 0.1
R²c: 0.1
N (g kg-¹) Leaves
R²m: 0.07
R²c: 0.07
P (g kg-¹) Leaves
R²m: 0.04
R²c: 0.1
K (g kg-¹) Leaves
R²m: 0.09
R²c: 0.19
Ca (g kg-¹) Leaves
R²m: 0.15
R²c: 0.15
Mg (g kg-¹) Leaves
R²m: 0.05
R²c: 0.05
S (g kg-¹) Leaves

Supplementary Table

Table S1. Summary of linear model (LM) outputs analyzing the relationship between reproductive and vegetative traits in plant species. The column "Model" indicates the combination of response and predictor variables used in the analysis, along with interactions with fire-related factors. Factors include the interaction terms used in the LM analysis: Trat (a combination of fire frequency and fire history), Fire Frequency (classified as High or Low), and Fire History, which includes two levels: LTB (areas with a long time since the last fire, "Long Time Since Burned") and RB (areas recently burned, "Recently Burned"). The "Relationship" column describes the associations between variables under different fire regimes, where NS (No Significant Relationship) indicates no significant interaction or association was detected, PR (Positive Relationship) indicates a positive and statistically significant association between variables, and TD (Trade-Off) indicates a trade-off between variables. R^2 (R-squared) represents the proportion of variance in the response variable explained by the predictors in the model, with higher values indicating a better model fit. Adjusted R^2 is a modified version of R^2 that adjusts for the number of predictors in the model, accounting for model complexity and allowing better comparison between models with different numbers of predictors. The P-value indicates whether individual predictors have a statistically significant effect on the response variable, with predictors having P-values < 0.05 being considered significant.

Species	Regression Model	Factors	Level	R^2	Adjusted R^2	P-values	Relationship
Diplusodon punctatus	**Total reproductive weight ~ Total vegetative aboveground weight**	**Fire_frequency**	**High**	**0.37**	**0.34**	**0.0008**	**PR**
Diplusodon punctatus	**Total reproductive weight ~ Total vegetative aboveground weight**	**Fire_frequency**	**Low**	**0.28**	**0.26**	**0.0026**	**PR**
Diplusodon punctatus	Total reproductive weight ~ Total vegetative aboveground weight	Fire_history	LTB	0.10	0.07	0.1057	NS
Diplusodon punctatus	**Total reproductive weight ~ Total vegetative aboveground weight**	**Fire_history**	**RB**	**0.49**	**0.47**	**0.0000**	**PR**
Diplusodon punctatus	Total number of seeds ~ Total number of stems	Fire_frequency	High	0.00	-0.04	0.9672	NS
Diplusodon punctatus	Total number of seeds ~ Total number of stems	Fire_frequency	Low	0.12	0.08	0.0655	NS
Diplusodon punctatus	Total number of seeds ~ Total number of stems	Fire_history	LTB	0.00	-0.04	0.7584	NS
Diplusodon punctatus	**Total number of seeds ~ Total number of stems**	**Fire_history**	**RB**	**0.37**	**0.35**	**0.0004**	**PR**
Diplusodon punctatus	Number of fruits ~ Total number of stems	Fire_frequency	High	0.01	-0.03	0.5955	NS
Diplusodon punctatus	**Number of fruits ~ Total number of stems**	**Fire_frequency**	**Low**	**0.14**	**0.11**	**0.0404**	**PR**
Diplusodon punctatus	Number of fruits ~ Total number of stems	Fire_history	LTB	0.01	-0.03	0.5975	NS
Diplusodon punctatus	**Number of fruits ~ Total number of stems**	**Fire_history**	**RB**	**0.43**	**0.41**	**0.0001**	**PR**
Diplusodon punctatus	Full seeds percentage ~ Total vegetative aboveground weight	Fire_frequency	High	0.00	-0.04	0.9689	NS

Diplusodon punctatus	Full seeds percentage ~ Total vegetative aboveground weight	Fire_frequency	Low	0.03	-0.01	0.3632	NS
Diplusodon punctatus	Full seeds percentage ~ Total vegetative aboveground weight	Fire_history	LTB	0.05	0.02	0.2438	NS
Diplusodon punctatus	Full seeds percentage ~ Total vegetative aboveground weight	Fire_history	RB	0.12	0.09	0.0637	NS
Diplusodon punctatus	**Number of fruits ~ Total vegetative aboveground weight**	**Fire_frequency**	**High**	**0.25**	**0.22**	**0.0077**	**PR**
Diplusodon punctatus	**Number of fruits ~ Total vegetative aboveground weight**	**Fire_frequency**	**Low**	**0.33**	**0.30**	**0.0010**	**PR**
Diplusodon punctatus	**Number of fruits ~ Total vegetative aboveground weight**	**Fire_history**	**LTB**	**0.20**	**0.17**	**0.0192**	**PR**
Diplusodon punctatus	**Number of fruits ~ Total vegetative aboveground weight**	**Fire_history**	**RB**	**0.57**	**0.56**	**0.0000**	**PR**
Diplusodon punctatus	**Total number of seeds ~ Total vegetative aboveground weight**	**Fire_frequency**	**High**	**0.20**	**0.17**	**0.0190**	**PR**
Diplusodon punctatus	**Total number of seeds ~ Total vegetative aboveground weight**	**Fire_frequency**	**Low**	**0.26**	**0.23**	**0.0039**	**PR**
Diplusodon punctatus	Total number of seeds ~ Total vegetative aboveground weight	Fire_history	LTB	0.03	-0.01	0.4166	NS
Diplusodon punctatus	**Total number of seeds ~ Total vegetative aboveground weight**	**Fire_history**	**RB**	**0.53**	**0.51**	**0.0000**	**PR**
Gymneia chapadensis	**Total reproductive weight ~ Total vegetative aboveground weight**	**Fire_frequency**	**High**	**0.35**	**0.32**	**0.0009**	**PR**
Gymneia chapadensis	**Total reproductive weight ~ Total vegetative aboveground weight**	**Fire_frequency**	**Low**	**0.48**	**0.47**	**0.0000**	**PR**
Gymneia chapadensis	**Total reproductive weight ~ Total vegetative aboveground weight**	**Fire_history**	**RB**	**0.53**	**0.51**	**0.0000**	**PR**
Gymneia chapadensis	**Total reproductive weight ~ Total vegetative aboveground weight**	**Fire_history**	**LTB**	**0.33**	**0.31**	**0.0007**	**PR**
Gymneia chapadensis	Total number of seeds ~ Total number of stems	Fire_frequency	High	0.05	0.01	0.2594	NS
Gymneia chapadensis	Total number of seeds ~ Total number of stems	Fire_frequency	Low	0.05	0.02	0.2089	NS
Gymneia chapadensis	Total number of seeds ~ Total number of stems	Fire_history	RB	0.08	0.05	0.1403	NS
Gymneia chapadensis	Total number of seeds ~ Total number of stems	Fire_history	LTB	0.10	0.07	0.0836	NS
Gymneia chapadensis	Number of fruits ~ Total number of stems	Fire_frequency	High	0.02	-0.01	0.4423	NS
Gymneia chapadensis	Number of fruits ~ Total number of stems	Fire_frequency	Low	0.06	0.02	0.1950	NS
Gymneia chapadensis	Number of fruits ~ Total number of stems	Fire_history	RB	0.02	-0.02	0.5060	NS
Gymneia chapadensis	**Number of fruits ~ Total number of stems**	**Fire_history**	**LTB**	**0.19**	**0.16**	**0.0155**	**PR**
Gymneia chapadensis	Full seeds percentage ~ Total vegetative aboveground weight	Fire_frequency	High	0.05	0.01	0.2564	NS
Gymneia chapadensis	Full seeds percentage ~ Total vegetative aboveground weight	Fire_frequency	Low	0.03	0.00	0.3431	NS
Gymneia chapadensis	**Full seeds percentage ~ Total vegetative aboveground weight**	**Fire_history**	**RB**	**0.32**	**0.29**	**0.0018**	**TD**

Gymneia chapadensis	Full seeds percentage ~ Total vegetative aboveground weight	Fire_history	LTB	0.06	0.03	0.1851	NS
Gymneia chapadensis	**Number of fruits ~ Total vegetative aboveground weight**	**Fire_frequency**	**High**	**0.21**	**0.18**	**0.0147**	**PR**
Gymneia chapadensis	**Number of fruits ~ Total vegetative aboveground weight**	**Fire_frequency**	**Low**	**0.47**	**0.45**	**0.0000**	**PR**
Gymneia chapadensis	**Number of fruits ~ Total vegetative aboveground weight**	**Fire_history**	**RB**	**0.40**	**0.37**	**0.0003**	**PR**
Gymneia chapadensis	**Number of fruits ~ Total vegetative aboveground weight**	**Fire_history**	**LTB**	**0.62**	**0.61**	**0.0000**	**PR**
Gymneia chapadensis	**Total number of seeds ~ Total vegetative aboveground weight**	**Fire_frequency**	**High**	**0.15**	**0.12**	**0.0389**	**PR**
Gymneia chapadensis	**Total number of seeds ~ Total vegetative aboveground weight**	**Fire_frequency**	**Low**	**0.44**	**0.42**	**0.0001**	**PR**
Gymneia chapadensis	**Total number of seeds ~ Total vegetative aboveground weight**	**Fire_history**	**RB**	**0.36**	**0.34**	**0.0007**	**PR**
Gymneia chapadensis	**Total number of seeds ~ Total vegetative aboveground weight**	**Fire_history**	**LTB**	**0.23**	**0.20**	**0.0063**	**PR**
Mimosa leiocephala	**Total reproductive weight ~ Total vegetative aboveground weight**	**Fire_frequency**	**High**	**0.30**	**0.27**	**0.0043**	**PR**
Mimosa leiocephala	Total reproductive weight ~ Total vegetative aboveground weight	Fire_frequency	Low	0.01	-0.08	0.7465	NS
Mimosa leiocephala	Total reproductive weight ~ Total vegetative aboveground weight	Fire_history	LTB	0.15	0.11	0.0637	NS
Mimosa leiocephala	**Total reproductive weight ~ Total vegetative aboveground weight**	**Fire_history**	**RB**	**0.31**	**0.25**	**0.0321**	**PR**
Mimosa leiocephala	**Total number of seeds ~ Total number of stems**	**Fire_frequency**	**High**	**0.27**	**0.24**	**0.0080**	**TD**
Mimosa leiocephala	Total number of seeds ~ Total number of stems	Fire_frequency	Low	0.03	-0.06	0.5947	NS
Mimosa leiocephala	Total number of seeds ~ Total number of stems	Fire_history	LTB	0.01	-0.04	0.6865	NS
Mimosa leiocephala	**Total number of seeds ~ Total number of stems**	**Fire_history**	**RB**	**0.29**	**0.24**	**0.0370**	**TD**
Mimosa leiocephala	Number of fruits ~ Total number of stems	Fire_frequency	High	0.00	-0.04	0.9710	NS
Mimosa leiocephala	Number of fruits ~ Total number of stems	Fire_frequency	Low	0.00	-0.09	0.9234	NS
Mimosa leiocephala	Number of fruits ~ Total number of stems	Fire_history	LTB	0.01	-0.04	0.6775	NS
Mimosa leiocephala	Number of fruits ~ Total number of stems	Fire_history	RB	0.00	-0.07	0.8689	NS
Mimosa leiocephala	Full seeds percentage ~ Total vegetative aboveground weight	Fire_frequency	High				NS
Mimosa leiocephala	Full seeds percentage ~ Total vegetative aboveground weight	Fire_frequency	Low	0.55	0.51	0.8405	NS
Mimosa leiocephala	Full seeds percentage ~ Total vegetative aboveground weight	Fire_history	LTB	0.51	0.49	0.5493	NS
Mimosa leiocephala	Full seeds percentage ~ Total vegetative aboveground weight	Fire_history	RB	0.47	0.43	0.2308	NS
Mimosa leiocephala	Number of fruits ~ Total vegetative aboveground weight	Fire_frequency	High	0.10	0.06	0.1209	NS

Mimosa leiocephala	Number of fruits ~ Total vegetative aboveground weight	Fire_frequency	Low	0.10	0.02	0.2902	NS
Mimosa leiocephala	**Number of fruits ~ Total vegetative aboveground weight**	**Fire_history**	**LTB**	**0.24**	**0.21**	**0.0172**	**PR**
Mimosa leiocephala	Number of fruits ~ Total vegetative aboveground weight	Fire_history	RB	0.09	0.02	0.2800	NS
Mimosa leiocephala	Total number of seeds ~ Total vegetative aboveground weight	Fire_frequency	High	0.06	0.02	0.2545	NS
Mimosa leiocephala	Total number of seeds ~ Total vegetative aboveground weight	Fire_frequency	Low	0.07	-0.01	0.3755	NS
Mimosa leiocephala	Total number of seeds ~ Total vegetative aboveground weight	Fire_history	LTB	0.06	0.01	0.2751	NS
Mimosa leiocephala	Total number of seeds ~ Total vegetative aboveground weight	Fire_history	RB	0.08	0.01	0.3013	NS
Tibouchina melastomoides	Total reproductive weight ~ Total vegetative aboveground weight	Fire_frequency	High	0.14	0.07	0.1771	NS
Tibouchina melastomoides	Total reproductive weight ~ Total vegetative aboveground weight	Fire_frequency	Low	0.03	-0.05	0.5631	NS
Tibouchina melastomoides	Total reproductive weight ~ Total vegetative aboveground weight	Fire_history	RB	0.06	0.03	0.1872	NS
Tibouchina melastomoides	Total number of seeds ~ Total number of stems	Fire_frequency	High	0.02	-0.06	0.6589	NS
Tibouchina melastomoides	Total number of seeds ~ Total number of stems	Fire_frequency	Low	0.01	-0.06	0.6643	NS
Tibouchina melastomoides	Total number of seeds ~ Total number of stems	Fire_history	RB	0.02	-0.02	0.4795	NS
Tibouchina melastomoides	Number of fruits ~ Total number of stems	Fire_frequency	High	0.13	0.06	0.1950	NS
Tibouchina melastomoides	Number of fruits ~ Total number of stems	Fire_frequency	Low	0.00	-0.08	0.9384	NS
Tibouchina melastomoides	Number of fruits ~ Total number of stems	Fire_history	RB	0.05	0.01	0.2426	NS
Tibouchina melastomoides	Full seeds percentage ~ Total vegetative aboveground weight	Fire_frequency	High	0.00	-0.08	0.9214	NS
Tibouchina melastomoides	Full seeds percentage ~ Total vegetative aboveground weight	Fire_frequency	Low	0.13	0.06	0.1928	NS
Tibouchina melastomoides	Full seeds percentage ~ Total vegetative aboveground weight	Fire_history	RB	0.01	-0.03	0.6791	NS
Tibouchina melastomoides	Number of fruits ~ Total vegetative aboveground weight	Fire_frequency	High	0.15	0.09	0.1467	NS
Tibouchina melastomoides	Number of fruits ~ Total vegetative aboveground weight	Fire_frequency	Low	0.05	-0.02	0.4279	NS
Tibouchina melastomoides	**Number of fruits ~ Total vegetative aboveground weight**	**Fire_history**	**RB**	**0.13**	**0.10**	**0.0497**	**PR**
Tibouchina melastomoides	Total number of seeds ~ Total vegetative aboveground weight	Fire_frequency	High	0.17	0.11	0.1279	NS
Tibouchina melastomoides	**Total number of seeds ~ Total vegetative aboveground weight**	**Fire_frequency**	**Low**	**0.27**	**0.22**	**0.0466**	**TD**
Tibouchina melastomoides	Total number of seeds ~ Total vegetative aboveground weight	Fire_history	RB	0.02	-0.01	0.4433	NS
Mimosa leiocephala	Oligosaccharides in Seeds ~ Oligosaccharides in belowground organs	Fire_frequency	High	0.00	-0.12	0.8730	NS

Mimosa leiocephala	Oligosaccharides in Seeds ~ Oligosaccharides in belowground organs	Fire_frequency	Low	0.06	-0.26	0.7015	NS
Mimosa leiocephala	Oligosaccharides in Seeds ~ Oligosaccharides in belowground organs	Fire_history	LTB	0.00	-0.12	0.9479	NS
Mimosa leiocephala	Oligosaccharides in Seeds ~ Oligosaccharides in belowground organs	Fire_history	RB	0.03	-0.30	0.7992	NS
Mimosa leiocephala	**Polysaccharides in Seeds ~ Polysaccharides in belowground organs**	**Fire_frequency**	**High**	**0.53**	**0.47**	**0.0171**	**PR**
Mimosa leiocephala	Polysaccharides in Seeds ~ Polysaccharides in belowground organs	Fire_frequency	Low	0.32	0.10	0.3178	NS
Mimosa leiocephala	Polysaccharides in Seeds ~ Polysaccharides in belowground organs	Fire_history	LTB	0.16	0.06	0.2459	NS
Mimosa leiocephala	**Polysaccharides in Seeds ~ Polysaccharides in belowground organs**	**Fire_history**	**RB**	**0.88**	**0.84**	**0.0192**	**PR**
Mimosa leiocephala	**Starch in Seeds ~ Starch in belowground organs**	**Fire_frequency**	**High**	**0.42**	**0.34**	**0.0443**	**PR**
Mimosa leiocephala	Starch in Seeds ~ Starch in belowground organs	Fire_frequency	Low	0.71	0.61	0.0731	NS
Mimosa leiocephala	**Starch in Seeds ~ Starch in belowground organs**	**Fire_history**	**LTB**	**0.48**	**0.41**	**0.0267**	**PR**
Mimosa leiocephala	Starch in Seeds ~ Starch in belowground organs	Fire_history	RB	0.39	0.18	0.2620	NS
Mimosa leiocephala	**Total CHOs in Seeds ~ Total CHOs in belowground organs**	**Fire_frequency**	**High**	**0.63**	**0.58**	**0.0062**	**PR**
Mimosa leiocephala	Total CHOs in Seeds ~ Total CHOs in belowground organs	Fire_frequency	Low	0.26	0.01	0.3810	NS
Mimosa leiocephala	Total CHOs in Seeds ~ Total CHOs in belowground organs	Fire_history	LTB	0.37	0.29	0.0617	NS
Mimosa leiocephala	**Total CHOs in Seeds ~ Total CHOs in belowground organs**	**Fire_history**	**RB**	**0.78**	**0.70**	**0.0482**	**PR**
Diplusodon punctatus	Oligosaccharides in Seeds ~ Oligosaccharides in belowground organs	Fire_frequency	High	0.11	0.00	0.3465	NS
Diplusodon punctatus	Oligosaccharides in Seeds ~ Oligosaccharides in belowground organs	Fire_frequency	Low	0.25	0.15	0.1426	NS
Diplusodon punctatus	Oligosaccharides in Seeds ~ Oligosaccharides in belowground organs	Fire_history	LTB	0.16	0.05	0.2571	NS
Diplusodon punctatus	Oligosaccharides in Seeds ~ Oligosaccharides in belowground organs	Fire_history	RB	0.16	0.05	0.2597	NS
Diplusodon punctatus	Polysaccharides in Seeds ~ Polysaccharides in belowground organs	Fire_frequency	High	0.16	0.06	0.2475	NS
Diplusodon punctatus	Polysaccharides in Seeds ~ Polysaccharides in belowground organs	Fire_frequency	Low	0.02	-0.10	0.6806	NS
Diplusodon punctatus	Polysaccharides in Seeds ~ Polysaccharides in belowground organs	Fire_history	LTB	0.13	0.02	0.3011	NS
Diplusodon punctatus	Polysaccharides in Seeds ~ Polysaccharides in belowground organs	Fire_history	RB	0.13	0.02	0.3002	NS
Diplusodon punctatus	Starch in Seeds ~ Starch in belowground organs	Fire_frequency	High	0.23	0.13	0.1636	NS
Diplusodon punctatus	**Starch in Seeds ~ Starch in belowground organs**	**Fire_frequency**	**Low**	**0.48**	**0.41**	**0.0382**	**TD**
Diplusodon punctatus	Starch in Seeds ~ Starch in belowground organs	Fire_history	LTB	0.19	0.08	0.2386	NS

Diplusodon punctatus	**Starch in Seeds ~ Starch in belowground organs**	**Fire_history**	**RB**	**0.70**	**0.66**	**0.0025**	**TD**
Diplusodon punctatus	Total CHOs in Seeds ~ Total CHOs in belowground organs	Fire_frequency	High	0.08	-0.04	0.4304	NS
Diplusodon punctatus	Total CHOs in Seeds ~ Total CHOs in belowground organs	Fire_frequency	Low	0.00	-0.12	0.8592	NS
Diplusodon punctatus	Total CHOs in Seeds ~ Total CHOs in belowground organs	Fire_history	LTB	0.29	0.21	0.1058	NS
Diplusodon punctatus	Total CHOs in Seeds ~ Total CHOs in belowground organs	Fire_history	RB	0.02	-0.10	0.6970	NS
Gymneia chapadensis	Oligosaccharides in Seeds ~ Oligosaccharides in belowground organs	Fire_frequency	High	0.19	0.05	0.2838	NS
Gymneia chapadensis	Oligosaccharides in Seeds ~ Oligosaccharides in belowground organs	Fire_frequency	Low	0.07	-0.07	0.5060	NS
Gymneia chapadensis	Oligosaccharides in Seeds ~ Oligosaccharides in belowground organs	Fire_history	RB	0.00	-0.12	0.9089	NS
Gymneia chapadensis	Oligosaccharides in Seeds ~ Oligosaccharides in belowground organs	Fire_history	LTB	0.13	-0.05	0.4332	NS
Gymneia chapadensis	Polysaccharides in Seeds ~ Polysaccharides in belowground organs	Fire_frequency	High	0.13	-0.02	0.3898	NS
Gymneia chapadensis	Polysaccharides in Seeds ~ Polysaccharides in belowground organs	Fire_frequency	Low	0.03	-0.10	0.6374	NS
Gymneia chapadensis	**Polysaccharides in Seeds ~ Polysaccharides in belowground organs**	**Fire_history**	**RB**	**0.49**	**0.43**	**0.0240**	**TD**
Gymneia chapadensis	**Polysaccharides in Seeds ~ Polysaccharides in belowground organs**	**Fire_history**	**LTB**	**0.58**	**0.50**	**0.0467**	**PR**
Gymneia chapadensis	Starch in Seeds ~ Starch in belowground organs	Fire_frequency	High	0.01	-0.16	0.8558	NS
Gymneia chapadensis	Starch in Seeds ~ Starch in belowground organs	Fire_frequency	Low	0.11	-0.02	0.3855	NS
Gymneia chapadensis	Starch in Seeds ~ Starch in belowground organs	Fire_history	RB	0.00	-0.12	0.9715	NS
Gymneia chapadensis	Starch in Seeds ~ Starch in belowground organs	Fire_history	LTB	0.15	-0.02	0.3899	NS
Gymneia chapadensis	Total CHOs in Seeds ~ Total CHOs in belowground organs	Fire_frequency	High	0.07	-0.09	0.5290	NS
Gymneia chapadensis	Total CHOs in Seeds ~ Total CHOs in belowground organs	Fire_frequency	Low	0.02	-0.12	0.7437	NS
Gymneia chapadensis	Total CHOs in Seeds ~ Total CHOs in belowground organs	Fire_history	RB	0.04	-0.07	0.5578	NS
Gymneia chapadensis	Total CHOs in Seeds ~ Total CHOs in belowground organs	Fire_history	LTB	0.03	-0.17	0.7299	NS
Tibouchina melastomoides	Oligosaccharides in Seeds ~ Oligosaccharides in belowground organs	Fire_frequency	High	0.06	-0.26	0.7025	NS
Tibouchina melastomoides	Oligosaccharides in Seeds ~ Oligosaccharides in belowground organs	Fire_frequency	Low	0.22	-0.04	0.4256	NS
Tibouchina melastomoides	Oligosaccharides in Seeds ~ Oligosaccharides in belowground organs	Fire_history	RB	0.03	-0.09	0.6305	NS
Tibouchina melastomoides	Polysaccharides in Seeds ~ Polysaccharides in belowground organs	Fire_frequency	High	0.02	-0.31	0.8384	NS
Tibouchina melastomoides	Polysaccharides in Seeds ~ Polysaccharides in belowground organs	Fire_frequency	Low	0.24	-0.01	0.3989	NS

Tibouchina melastomoides	Polysaccharides in Seeds ~ Polysaccharides in belowground organs	Fire_history	RB	0.05	-0.07	0.5466	NS
Tibouchina melastomoides	Starch in Seeds ~ Starch in belowground organs	Fire_frequency	High	0.06	-0.25	0.6842	NS
Tibouchina melastomoides	Starch in Seeds ~ Starch in belowground organs	Fire_frequency	Low	0.58	0.43	0.1373	NS
Tibouchina melastomoides	**Starch in Seeds ~ Starch in belowground organs**	**Fire_history**	**RB**	**0.47**	**0.40**	**0.0291**	PR
Tibouchina melastomoides	Total CHOs in Seeds ~ Total CHOs in belowground organs	Fire_frequency	High	0.01	-0.32	0.8945	NS
Tibouchina melastomoides	Total CHOs in Seeds ~ Total CHOs in belowground organs	Fire_frequency	Low	0.29	0.05	0.3515	NS
Tibouchina melastomoides	Total CHOs in Seeds ~ Total CHOs in belowground organs	Fire_history	RB	0.35	0.27	0.0718	NS
Mimosa leiocephala	Full seeds percentage ~ Nitrogen in belowground organs	Fire_frequency	High	0.53	0.47	0.9585	NS
Mimosa leiocephala	Full seeds percentage ~ Nitrogen in belowground organs	Fire_frequency	Low	0.52	0.36	0.3273	NS
Mimosa leiocephala	Full seeds percentage ~ Nitrogen in belowground organs	Fire_history	LTB	0.49	0.43	0.5581	NS
Mimosa leiocephala	Full seeds percentage ~ Nitrogen in belowground organs	Fire_history	RB	0.43	0.24	0.3019	NS
Mimosa leiocephala	Full seeds percentage ~ Phosphorus in belowground organs	Fire_frequency	High	0.53	0.47	0.4468	NS
Mimosa leiocephala	Full seeds percentage ~ Phosphorus in belowground organs	Fire_frequency	Low	0.47	0.30	0.4950	NS
Mimosa leiocephala	Full seeds percentage ~ Phosphorus in belowground organs	Fire_history	LTB	0.47	0.41	0.2415	NS
Mimosa leiocephala	Full seeds percentage ~ Phosphorus in belowground organs	Fire_history	RB	0.65	0.53	0.6850	NS
Mimosa leiocephala	Full seeds percentage ~ Potassium in belowground organs	Fire_frequency	High	0.53	0.47	0.4468	NS
Mimosa leiocephala	Full seeds percentage ~ Potassium in belowground organs	Fire_frequency	Low	0.47	0.30	0.4950	NS
Mimosa leiocephala	Full seeds percentage ~ Potassium in belowground organs	Fire_history	LTB	0.53	0.47	0.4468	NS
Mimosa leiocephala	Full seeds percentage ~ Potassium in belowground organs	Fire_history	RB	0.47	0.30	0.4950	NS
Mimosa leiocephala	Full seeds percentage ~ Calcium in belowground organs	Fire_frequency	High	0.48	0.41	0.5447	NS
Mimosa leiocephala	Full seeds percentage ~ Calcium in belowground organs	Fire_frequency	Low	0.47	0.30	0.4950	NS
Mimosa leiocephala	Full seeds percentage ~ Calcium in belowground organs	Fire_history	LTB	0.53	0.47	0.4468	NS
Mimosa leiocephala	Full seeds percentage ~ Calcium in belowground organs	Fire_history	RB	0.65	0.53	0.6850	NS
Mimosa leiocephala	Full seeds percentage ~ Magnesium in belowground organs	Fire_frequency	High	0.49	0.42	0.8199	NS
Mimosa leiocephala	Full seeds percentage ~ Magnesium in belowground organs	Fire_frequency	Low	0.59	0.45	0.5594	NS
Mimosa leiocephala	Full seeds percentage ~ Magnesium in belowground organs	Fire_history	LTB	0.53	0.47	0.8722	NS

Mimosa leiocephala	Full seeds percentage ~ Magnesium in belowground organs	Fire_history	RB	0.51	0.34	0.3734	NS
Mimosa leiocephala	**Full seeds percentage ~ Sulfur in belowground organs**	**Fire_frequency**	**High**	**0.53**	**0.47**	**0.0353**	**NA**
Mimosa leiocephala	Full seeds percentage ~ Sulfur in belowground organs	Fire_frequency	Low	0.56	0.41	0.8304	NS
Mimosa leiocephala	Full seeds percentage ~ Sulfur in belowground organs	Fire_history	LTB	0.54	0.49	0.1981	NS
Mimosa leiocephala	Full seeds percentage ~ Sulfur in belowground organs	Fire_history	RB	0.65	0.53	0.6850	NS
Diplusodon punctatus	Full seeds percentage ~ Nitrogen in belowground organs	Fire_frequency	High	0.01	-0.11	0.7710	NS
Diplusodon punctatus	Full seeds percentage ~ Nitrogen in belowground organs	Fire_frequency	Low	0.04	-0.08	0.5909	NS
Diplusodon punctatus	Full seeds percentage ~ Nitrogen in belowground organs	Fire_history	LTB	0.10	-0.01	0.3635	NS
Diplusodon punctatus	Full seeds percentage ~ Nitrogen in belowground organs	Fire_history	RB	0.34	0.26	0.0746	NS
Diplusodon punctatus	Full seeds percentage ~ Phosphorus in belowground organs	Fire_frequency	High	0.27	0.17	0.1276	NS
Diplusodon punctatus	Full seeds percentage ~ Phosphorus in belowground organs	Fire_frequency	Low	0.13	0.03	0.2964	NS
Diplusodon punctatus	Full seeds percentage ~ Phosphorus in belowground organs	Fire_history	LTB	0.00	-0.12	0.8592	NS
Diplusodon punctatus	Full seeds percentage ~ Phosphorus in belowground organs	Fire_history	RB	0.23	0.13	0.1625	NS
Diplusodon punctatus	Full seeds percentage ~ Potassium in belowground organs	Fire_frequency	High	0.00	-0.12	0.9327	NS
Diplusodon punctatus	Full seeds percentage ~ Potassium in belowground organs	Fire_frequency	Low	0.00	0.00		NS
Diplusodon punctatus	Full seeds percentage ~ Potassium in belowground organs	Fire_history	LTB	0.05	-0.07	0.5310	NS
Diplusodon punctatus	Full seeds percentage ~ Potassium in belowground organs	Fire_history	RB	0.00	0.00		NS
Diplusodon punctatus	Full seeds percentage ~ Calcium in belowground organs	Fire_frequency	High	0.01	-0.11	0.7932	NS
Diplusodon punctatus	**Full seeds percentage ~ Calcium in belowground organs**	**Fire_frequency**	**Low**	**0.81**	**0.79**	**0.0004**	**TD**
Diplusodon punctatus	Full seeds percentage ~ Calcium in belowground organs	Fire_history	LTB	0.05	-0.07	0.5340	NS
Diplusodon punctatus	**Full seeds percentage ~ Calcium in belowground organs**	**Fire_history**	**RB**	**0.72**	**0.69**	**0.0018**	**TD**
Diplusodon punctatus	Full seeds percentage ~ Magnesium in belowground organs	Fire_frequency	High	0.09	-0.03	0.4034	NS
Diplusodon punctatus	Full seeds percentage ~ Magnesium in belowground organs	Fire_frequency	Low	0.00	-0.12	0.9019	NS
Diplusodon punctatus	Full seeds percentage ~ Magnesium in belowground organs	Fire_history	LTB	0.03	-0.10	0.6603	NS
Diplusodon punctatus	Full seeds percentage ~ Magnesium in belowground organs	Fire_history	RB	0.02	-0.10	0.6660	NS
Diplusodon punctatus	Full seeds percentage ~ Sulfur in belowground organs	Fire_frequency	High	0.02	-0.10	0.6958	NS

Diplusodon punctatus	Full seeds percentage ~ Sulfur in belowground organs	Fire_frequency	Low	0.03	-0.10	0.6596	NS
Diplusodon punctatus	Full seeds percentage ~ Sulfur in belowground organs	Fire_history	LTB	0.08	-0.03	0.4230	NS
Diplusodon punctatus	Full seeds percentage ~ Sulfur in belowground organs	Fire_history	RB	0.20	0.10	0.2004	NS
Gymneia chapadensis	Full seeds percentage ~ Nitrogen in belowground organs	Fire_frequency	High	0.08	-0.04	0.4307	NS
Gymneia chapadensis	Full seeds percentage ~ Nitrogen in belowground organs	Fire_frequency	Low	0.10	-0.02	0.3848	NS
Gymneia chapadensis	Full seeds percentage ~ Nitrogen in belowground organs	Fire_history	RB	0.05	-0.07	0.5547	NS
Gymneia chapadensis	Full seeds percentage ~ Nitrogen in belowground organs	Fire_history	LTB	0.05	-0.06	0.5167	NS
Gymneia chapadensis	Full seeds percentage ~ Phosphorus in belowground organs	Fire_frequency	High	0.32	0.23	0.0906	NS
Gymneia chapadensis	Full seeds percentage ~ Phosphorus in belowground organs	Fire_frequency	Low	0.05	-0.07	0.5302	NS
Gymneia chapadensis	Full seeds percentage ~ Phosphorus in belowground organs	Fire_history	RB	0.16	0.05	0.2533	NS
Gymneia chapadensis	Full seeds percentage ~ Phosphorus in belowground organs	Fire_history	LTB	0.11	0.00	0.3482	NS
Gymneia chapadensis	Full seeds percentage ~ Potassium in belowground organs	Fire_frequency	High	0.16	0.05	0.2527	NS
Gymneia chapadensis	Full seeds percentage ~ Potassium in belowground organs	Fire_frequency	Low	0.03	-0.09	0.6322	NS
Gymneia chapadensis	Full seeds percentage ~ Potassium in belowground organs	Fire_history	RB	0.00	-0.12	0.9251	NS
Gymneia chapadensis	Full seeds percentage ~ Potassium in belowground organs	Fire_history	LTB	0.05	-0.07	0.5364	NS
Gymneia chapadensis	Full seeds percentage ~ Calcium in belowground organs	Fire_frequency	High	0.20	0.11	0.1892	NS
Gymneia chapadensis	Full seeds percentage ~ Calcium in belowground organs	Fire_frequency	Low	0.13	0.02	0.3096	NS
Gymneia chapadensis	Full seeds percentage ~ Calcium in belowground organs	Fire_history	RB	0.00	-0.12	0.8879	NS
Gymneia chapadensis	Full seeds percentage ~ Calcium in belowground organs	Fire_history	LTB	0.00	0.00		NS
Gymneia chapadensis	**Full seeds percentage ~ Magnesium in belowground organs**	**Fire_frequency**	**High**	**0.41**	**0.33**	**0.0472**	**PR**
Gymneia chapadensis	Full seeds percentage ~ Magnesium in belowground organs	Fire_frequency	Low	0.24	0.14	0.1544	NS
Gymneia chapadensis	**Full seeds percentage ~ Magnesium in belowground organs**	**Fire_history**	**RB**	**0.51**	**0.45**	**0.0198**	**PR**
Gymneia chapadensis	Full seeds percentage ~ Magnesium in belowground organs	Fire_history	LTB	0.38	0.30	0.0589	NS
Gymneia chapadensis	Full seeds percentage ~ Sulfur in belowground organs	Fire_frequency	High	0.24	0.15	0.1468	NS
Gymneia chapadensis	Full seeds percentage ~ Sulfur in belowground organs	Fire_frequency	Low	0.04	-0.08	0.5905	NS
Gymneia chapadensis	Full seeds percentage ~ Sulfur in belowground organs	Fire_history	RB	0.04	-0.08	0.5662	NS

Gymneia chapadensis	Full seeds percentage ~ Sulfur in belowground organs	Fire_history	LTB	0.33	0.24	0.0836	NS
Tibouchina melastomoides	Full seeds percentage ~ Nitrogen in belowground organs	Fire_frequency	High	0.40	0.20	0.2540	NS
Tibouchina melastomoides	Full seeds percentage ~ Nitrogen in belowground organs	Fire_frequency	Low	0.30	0.07	0.3372	NS
Tibouchina melastomoides	Full seeds percentage ~ Nitrogen in belowground organs	Fire_history	RB	0.00	-0.12	0.8595	NS
Tibouchina melastomoides	Full seeds percentage ~ Phosphorus in belowground organs	Fire_frequency	High	0.21	-0.05	0.4338	NS
Tibouchina melastomoides	Full seeds percentage ~ Phosphorus in belowground organs	Fire_frequency	Low	0.55	0.40	0.1497	NS
Tibouchina melastomoides	Full seeds percentage ~ Phosphorus in belowground organs	Fire_history	RB	0.02	-0.10	0.7010	NS
Tibouchina melastomoides	Full seeds percentage ~ Potassium in belowground organs	Fire_frequency	High	0.02	-0.31	0.8253	NS
Tibouchina melastomoides	Full seeds percentage ~ Potassium in belowground organs	Fire_frequency	Low	0.58	0.44	0.1363	NS
Tibouchina melastomoides	Full seeds percentage ~ Potassium in belowground organs	Fire_history	RB	0.16	0.05	0.2529	NS
Tibouchina melastomoides	Full seeds percentage ~ Calcium in belowground organs	Fire_frequency	High	0.10	-0.20	0.6049	NS
Tibouchina melastomoides	Full seeds percentage ~ Calcium in belowground organs	Fire_frequency	Low	0.00	-0.33	0.9924	NS
Tibouchina melastomoides	Full seeds percentage ~ Calcium in belowground organs	Fire_history	RB	0.04	-0.08	0.6015	NS
Tibouchina melastomoides	Full seeds percentage ~ Magnesium in belowground organs	Fire_frequency	High	0.00	-0.33	0.9385	NS
Tibouchina melastomoides	**Full seeds percentage ~ Magnesium in belowground organs**	**Fire_frequency**	**Low**	**0.98**	**0.97**	**0.0014**	**TD**
Tibouchina melastomoides	Full seeds percentage ~ Magnesium in belowground organs	Fire_history	RB	0.00	-0.12	0.8855	NS
Tibouchina melastomoides	Full seeds percentage ~ Sulfur in belowground organs	Fire_frequency	High	0.02	-0.31	0.8253	NS
Tibouchina melastomoides	Full seeds percentage ~ Sulfur in belowground organs	Fire_frequency	Low	0.27	0.03	0.3652	NS
Tibouchina melastomoides	Full seeds percentage ~ Sulfur in belowground organs	Fire_history	RB	0.00	-0.12	0.8917	NS
Mimosa leiocephala	Full seeds percentage ~ Nitrogen in leaves	Fire_frequency	High	0.49	0.42	0.2902	NS
Mimosa leiocephala	Full seeds percentage ~ Nitrogen in leaves	Fire_frequency	Low	0.56	0.41	0.8910	NS
Mimosa leiocephala	Full seeds percentage ~ Nitrogen in leaves	Fire_history	LTB	0.52	0.46	0.3906	NS
Mimosa leiocephala	Full seeds percentage ~ Nitrogen in leaves	Fire_history	RB	0.49	0.33	0.4089	NS
Mimosa leiocephala	Full seeds percentage ~ Phosphorus in leaves	Fire_frequency	High	0.48	0.41	0.8060	NS
Mimosa leiocephala	Full seeds percentage ~ Phosphorus in leaves	Fire_frequency	Low	0.57	0.43	0.2191	NS
Mimosa leiocephala	Full seeds percentage ~ Phosphorus in leaves	Fire_history	LTB	0.53	0.47	0.8776	NS

Mimosa leiocephala	Full seeds percentage ~ Phosphorus in leaves	Fire_history	RB	0.40	0.20	0.3888	NS
Mimosa leiocephala	Full seeds percentage ~ Potassium in leaves	Fire_frequency	High	0.53	0.47	0.9302	NS
Mimosa leiocephala	Full seeds percentage ~ Potassium in leaves	Fire_frequency	Low	0.61	0.48	0.7519	NS
Mimosa leiocephala	Full seeds percentage ~ Potassium in leaves	Fire_history	LTB	0.49	0.43	0.5447	NS
Mimosa leiocephala	Full seeds percentage ~ Potassium in leaves	Fire_history	RB	0.64	0.51	0.5340	NS
Mimosa leiocephala	Full seeds percentage ~ Calcium in leaves	Fire_frequency	High	0.48	0.41	0.5447	NS
Mimosa leiocephala	Full seeds percentage ~ Calcium in leaves	Fire_frequency	Low	0.00	0.00		NS
Mimosa leiocephala	Full seeds percentage ~ Calcium in leaves	Fire_history	LTB	0.47	0.41	0.7599	NS
Mimosa leiocephala	Full seeds percentage ~ Calcium in leaves	Fire_history	RB	0.44	0.26	0.2722	NS
Mimosa leiocephala	Full seeds percentage ~ Magnesium in leaves	Fire_frequency	High	0.50	0.43	0.5347	NS
Mimosa leiocephala	Full seeds percentage ~ Magnesium in leaves	Fire_frequency	Low	0.56	0.41	0.8406	NS
Mimosa leiocephala	Full seeds percentage ~ Magnesium in leaves	Fire_history	LTB	0.51	0.45	0.3732	NS
Mimosa leiocephala	Full seeds percentage ~ Magnesium in leaves	Fire_history	RB	0.60	0.46	0.5106	NS
Mimosa leiocephala	Full seeds percentage ~ Sulfur in leaves	Fire_frequency	High	0.48	0.41	0.1175	NS
Mimosa leiocephala	Full seeds percentage ~ Sulfur in leaves	Fire_frequency	Low	0.44	0.26	0.2722	NS
Mimosa leiocephala	Full seeds percentage ~ Sulfur in leaves	Fire_history	LTB	0.47	0.41	0.2415	NS
Mimosa leiocephala	Full seeds percentage ~ Sulfur in leaves	Fire_history	RB	0.47	0.30	0.4950	NS
Diplusodon punctatus	Full seeds percentage ~ Nitrogen in leaves	Fire_frequency	High	0.00	-0.12	0.9787	NS
Diplusodon punctatus	Full seeds percentage ~ Nitrogen in leaves	Fire_frequency	Low	0.10	-0.01	0.3789	NS
Diplusodon punctatus	Full seeds percentage ~ Nitrogen in leaves	Fire_history	LTB	0.13	0.02	0.2995	NS
Diplusodon punctatus	Full seeds percentage ~ Nitrogen in leaves	Fire_history	RB	0.01	-0.12	0.8004	NS
Diplusodon punctatus	Full seeds percentage ~ Phosphorus in leaves	Fire_frequency	High	0.01	-0.12	0.8251	NS
Diplusodon punctatus	Full seeds percentage ~ Phosphorus in leaves	Fire_frequency	Low	0.10	-0.01	0.3664	NS
Diplusodon punctatus	Full seeds percentage ~ Phosphorus in leaves	Fire_history	LTB	0.01	-0.11	0.7672	NS
Diplusodon punctatus	Full seeds percentage ~ Phosphorus in leaves	Fire_history	RB	0.07	-0.05	0.4611	NS
Diplusodon punctatus	Full seeds percentage ~ Potassium in leaves	Fire_frequency	High	0.01	-0.12	0.8304	NS

Diplusodon punctatus	Full seeds percentage ~ Potassium in leaves	Fire_frequency	Low	0.09	-0.03	0.4075	NS
Diplusodon punctatus	Full seeds percentage ~ Potassium in leaves	Fire_history	LTB	0.01	-0.12	0.8133	NS
Diplusodon punctatus	Full seeds percentage ~ Potassium in leaves	Fire_history	RB	0.01	-0.11	0.7393	NS
Diplusodon punctatus	Full seeds percentage ~ Calcium in leaves	Fire_frequency	High	0.21	0.11	0.1816	NS
Diplusodon punctatus	Full seeds percentage ~ Calcium in leaves	Fire_frequency	Low	0.30	0.21	0.1042	NS
Diplusodon punctatus	Full seeds percentage ~ Calcium in leaves	Fire_history	LTB	0.11	-0.01	0.3604	NS
Diplusodon punctatus	Full seeds percentage ~ Calcium in leaves	Fire_history	RB	0.07	-0.04	0.4540	NS
Diplusodon punctatus	Full seeds percentage ~ Magnesium in leaves	Fire_frequency	High	0.10	-0.02	0.3836	NS
Diplusodon punctatus	Full seeds percentage ~ Magnesium in leaves	Fire_frequency	Low	0.00	-0.12	0.8835	NS
Diplusodon punctatus	Full seeds percentage ~ Magnesium in leaves	Fire_history	LTB	0.01	-0.12	0.8195	NS
Diplusodon punctatus	Full seeds percentage ~ Magnesium in leaves	Fire_history	RB	0.22	0.12	0.1764	NS
Diplusodon punctatus	Full seeds percentage ~ Sulfur in leaves	Fire_frequency	High	0.06	-0.06	0.4897	NS
Diplusodon punctatus	Full seeds percentage ~ Sulfur in leaves	Fire_frequency	Low	0.12	0.01	0.3269	NS
Diplusodon punctatus	Full seeds percentage ~ Sulfur in leaves	Fire_history	LTB	0.07	-0.05	0.4676	NS
Diplusodon punctatus	Full seeds percentage ~ Sulfur in leaves	Fire_history	RB	0.00	-0.12	0.9398	NS

CAPÍTULO 2

In situ seed storage affects germination responses to fire-related cues in Cerrado species

Abstract

Fire-related cues often influence germination and seedling establishment processes in fire-prone ecosystems through cracks on the seed coat in physically dormant species and chemical compounds modulating germination in species with water-permeable seed coats. However, most studies use fresh seeds and ignore the role of seed storage in soil seed banks in shaping responses to fire-related cues. We aimed to investigate whether *in situ* seed storage (buried in the field) affects seed germination responses to heat shock and smoke. We collected freshly dispersed seeds of 13 Cerrado species at the beginning of the dry and rainy seasons and exposed them to fire-related cues (Fresh T0). We then buried the remaining seeds in the field (1cm depth) in six different plots, where they were exhumed after one (T1), three (T3), six (T6), nine (T9) and twelve (T12) months. Seeds from each storage period were exposed to heat shock (100ºC and 200ºC for 1') and smoke experiments (1:1 smoke solution, 24 hr). Then, seeds were set to germinate in germination chambers at controlled conditions (12h/12h light and dark, 27 °C) for 30 days. Three species with permeable seed coats had an increase in germination when exposed to smoke after three and six months buried *in situ*. Overall, *in situ* seed storage affected germination responses to fire-related cues in >60% of tested species, with two permeable species becoming more sensitive to smoke (increasing germination) and physically dormant (PY) species having a significant alleviation in seed dormancy leading to greater percentages in germination at 200°C at longer storage periods compared to fresh seeds. Therefore, *in situ* seed storage affects germination response to fire-related cues, suggesting that recruitment from seeds in fire-prone tropical savannas is more complex than previously described.

Keywords: Seed storage, seed longevity, fire cues, germination, Cerrado

Introduction

In fire-prone prone ecosystems a variety of species have their germination and seedling establishment affected by fire-related cues, such as heat and smoke (Keeley and Fotheringham, 2000; Keeley et al., 2011; Lamont et al., 2018; Pausas and Lamont 2022). The high temperatures of fire can stimulate germination and break the seed coat of physically dormant species allowing water uptake and subsequent germination (Moreira et al., 2010; Baskin and Baskin, 2014), while chemical compounds present in the smoke can enhance germination in species with water-permeable seed coats (Brown, 1993; Flematti, 2004; Keeley and Pausas, 2016). Therefore, exploring how species and communities respond to the diversity of fire-related cues helps understanding regeneration processes in fire-prone ecosystems worldwide (Bond and van Wilgen, 1996; Keeley and Fotheringham, 2000).

Under favorable conditions, germination of non-dormant seeds occurs following dispersal, but otherwise seeds can be incorporated into soil seed banks (Murdoch and Ellis, 2000; Fenner and Thompson, 2005). While seeds remain in the soil, they are exposed to abiotic (e.g. temperature fluctuations, water and light availability) and biotic (e.g. predation, pathogens, seed aging, etc) factors that affect the germination process and responses to external drivers (Fenner and Thompson, 2005; Baskin and Baskin, 2014). Seed aging is one of these factors that plays an important role in germination response to fire-related cues, since soil storage interacts directly with the major factors driving seed longevity (such as seed moisture content, temperature and period of storage) (Fenner and Thompson, 2005; Baskin and Baskin, 2014). For example, several studies found that seeds in the soil had a gradual decrease in germination ability and viability with increasing storage periods mainly due to seed aging and oxidative processes leading to deterioration (Harrington and Kozlowski, 1972; Walters et al., 2010; Kaeser and Kirkman, 2012; De Vitis et al., 2020). Currently, most studies evaluating the role of fire-related cues on germination use fresh seeds, and largely ignore the potential interactions between fire-related cues and seed storage into soil seed banks. Therefore, such studies provide limited understanding on how fire-related cues affect regeneration dynamics because most seeds are stored in soil seed banks.

The few studies testing how fire-related cues affect germination in seeds stored in soil seed banks indicate enhanced germination compared to fresh seeds (Valbuena and Vera, 2002; Turner, 2013; Downes et al., 2015). For example, Turner (2013) showed that germination of *Lepidosperma scabrum* exposed to heat was higher in nutlets stored *in situ* for 29 months. *In situ* storage and environmental conditions can also influence dormancy-breaking processes, particularly when dormancy percentages fluctuate with varying burial periods (Tieu et al., 2001; Turner et al., 2013; Liyanage and Ooi, 2017) or when seed dormancy thresholds are altered by the temperature conditions at the seed's population origin (Zomer et al., 2022). Liyanage and Ooi (2017) showed that seed storage for six and 18 months led to increased germination response to heat treatments and changes in dormancy-breaking thresholds when compared to freshly collected seeds in three physically dormant (PY) Fabaceae species. Given that *in situ* seed storage interact fire-related cues in many species, studies addressing fire-related cues on dormancy and germination should allow the seeds sufficient time to exhibit the complete effects of high temperature treatment to remove the bias of data from fresh seeds (Carreño et al. 2023).

In the Cerrado, most species tested seem to lack a strong response to fire cues (e.g. as dormancy breakage or high increase in germination by smoke) as found in species of other fire-prone ecosystems, with general result being the seed tolerance to high temperatures (Daibes et al 2022, Fichino et al. 2016), indicating that seed responses to fire may have been underestimated (Carreño et al. 2023). Thus, our aim was to determine whether germination responses to fire-related cues of native Cerrado seeds are affected by *in situ* seed storage. Given the seasonality of Cerrado (dry winters and rainy summers) and the presence of fire, seeds would face a great range of changes in the environmental conditions, even more when seed dispersal occurs at the beginning of the dry season. Therefore, we hypothesize that seeds stored in the soil would have responses to fire-related cues modulated by storage time, with seeds becoming more sensitive to heat shock and smoke with increasing storage time, especially for physically dormant seeds. We predict that changes in the environmental conditions (such as temperature and moisture) would directly affect the physiological and physical properties of the seeds (e.g. increased permeability and the activation of metabolic processes), thus, these changes would enhance seeds responsiveness to fire-related cues.

Material and Methods

Study area

The experiments were carried out in two Cerrado areas in Brazil, the Estação Ecológica de Itirapina (EEI, 22° 11'–22° 15' S, 47° 51'–48° 00' W, Southeastern Brazil) and the Reserva Natural Serra do Tombador (RNST, 13 ° 35-38 'S, 47 ° 45'-51' W, Central Brazil). The EEI is a protected area located in Southeastern Brazil with average annual temperatures varying from 18°C in the winter to 22°C in summer and according to Köppen classification the EEI presents a Cwa climate (Zanchetta et al., 2006). The RNST is a private protected area in Central Brazil, with a tropical climate and the average maximum temperatures range from 26 and 36 °C and minimum between 8 and 14° C, and an average annual rainfall between 1300 and 1500 mm (Antonelli-Filho 2011). As usual for Cerrado, both areas have a well-marked seasonality with a dry season from May to September/October and a rainy season from October to April. Although different types of Cerrado vegetation occur at the EEI and the RNST, the experiments were carried out in open savannas areas (*campo sujo)*, which is characterized by the dominance of a continuous, species-rich herbaceous layer, dominated by C4 grasses and forbs, with trees and shrubs scattered along with the vegetation (Coutinho, 1978). Also, Cerrado is a fire-prone ecosystem with surface and frequent fires occurring at the end of dry/beginning of rainy season (caused by lightning) or during the dry season due to fire regimes changes caused by human activities (e.g. land clearing for pastures) (Ramos-Neto and Pivello 2000; Miranda et al. 2009; Pivello 2011).

Seed storage in situ and fire-related cues

To investigate if seed storage and *in situ* burial affect responses to fire-related cues, we collected fresh seeds from 13 native species based on seed availability at their dispersal peak (beginning of the dry season at EEI and beginning of the rainy season at RNST, Table 1). For each species, seeds were sorted and separated into batches. The first batch consisted of fresh seeds exposed to germination experiments immediately after seed collection (Fresh T0). The other batches were buried in the soil (1cm depth) in the areas in six randomly allocated plots and unearthed after one (T1, only for species dispersing in the rainy season), three (T3),

six (T6), nine (T9) and 12 (T12) months, respectively. Seeds were enclosed in two cloth bags to avoid predation. Initially, each treatment comprised six replicates of 50 seeds each. After each seed retrieval, we counted the number of germinated (*in situ*), embryoless, dead, missing and viable seeds and, due to the decreasing number of viable seeds available for the experiments, we mixed seeds of different replicates and rearranged the number of seeds and replicates per germination experiment (at least three replicates of 10 seeds, according to seed availability).

Seeds from T0, T1, T3, T6, T9 and T12 were exposed to heat shock and smoke experiments after the buried period. For the heat shock experiments, seeds were exposed to 100ºC and to 200ºC for one minute, using a pre-heated muffle. For the smoke experiment, we prepared the smoke solution (SW) using 5g of biomass from the study area (mainly grasses and forbs) and according to the protocols of Jäger et al., (1996) and Moreira et al., (2010). Then, seeds were soaked in 1:1 smoke solution for 24h while control seeds (CSW) were soaked in distilled water for the same period. After the experiments, seeds were set to germinate in four chambers under controlled conditions (12h/12h light and dark) and at a constant temperature of 27°C for 30 days (following Zirondi *et al.* 2019). Viability tests were carried out on ungerminated seeds, using 1% tetrazolium solution and final seed viability calculated as the number of viable ungerminated seeds plus germinated seeds during the experiments. We also calculated Mean Germination Time (MGT) and Synchrony (Z) for all species. Germination synchrony (Z) was calculated as shown in Ranal and Santana (2006) with Z values close to 1 meaning higher synchrony and Z values close to 0 indicating lower synchrony.

Table 1. Study species according to their family, growth-form, presence of dormancy (ND: non-dormant; PY: physical dormancy) and dispersal season.

Site	Species	Family	Growth-form	Dormancy	Dispersal season
EEI	*Banisteriopsis stellaris (Griseb.) B.Gates*	Malpighiaceae	Shrub	ND	Dry season
EEI	*Bidens gardneri Baker*	Asteraceae	Forb	ND	Dry season
EEI	*Sida linifolia Cav.*	Malvaceae	Forb	PY	Dry season
EEI	*Solidago chilensis Meyen*	Asteraceae	Forb	ND	Dry season
EEI	*Waltheria communis A.St.-Hil.*	Malvaceae	Shrub	PY	Dry season
RNST	*Bulbostylis paradoxa (Spreng.) Lindm.*	Cyperaceae	Graminoid	ND	Rainy season
RNST	*Diplusodon burchellii Koehne*	Lythraceae	Forb	ND	Rainy season

RNST	*Dyckia brasiliana L.B.Sm.*	Bromeliaceae	Forb	ND	Rainy season
RNST	*Stenodon suberosus Naudin*	Melastomataceae	Shrub	ND	Rainy season
RNST	*Stomatanthes* sp. *R.M.King & H.Rob.*	Asteraceae	Shrub	ND	Rainy season
RNST	*Pseudotrimezia juncifolia (Klatt) Lovo & A.Gil*	Iridaceae	Forb	ND	Rainy season
RNST	*Vellozia squamata Pohl*	Velloziaceae	Vellozia	ND	Rainy season
RNST	*Vellozia swallenii L.B.Sm.*	Velloziaceae	Vellozia	ND	Rainy season

For evaluating the effect of time on fire cues responses we used the retrieved seeds in each period to conduct the experiments. We then adjusted the germination percentage by the number of viable seeds to conduct the statistical analysis (see Table S1 for germination by the total number of seeds for each treatment and time).

Statistical analysis

Differences in germination percentage and viability responses to fire-related cues across burial periods (T0, T1, T3, T6, T9, and T12) were tested using Generalized Linear Mixed Models (GLMM). For each species, we tested several models and selected the one with the best-fitting family for our data distribution. Then, we ran GLMM models for germinability data using a beta distribution with a logit link function and a zero-inflated gamma (ziGamma) distribution with a log link function. Differences in Mean Germination Time (MGT) and Synchrony (Z) were analyzed using GLMM with a Gaussian distribution or a ziGamma distribution when necessary for the Z data. Treatments and burial periods were included as fixed factors. All statistical analyses were conducted using glmmTMB, multcomp, lme4, and lsmeans packages in R (Brooks et al., 2017; Hothorn et al., 2008; Bates et al., 2015; Lenth, 2016; R Core Team, 2016).

Results

For the fire-related cues experiments, we used only the seeds retrieved during each period. However, before conducting these experiments, after each retrieval we quantified the seeds that had germinated (i.e., those still with roots and/or cotyledons attached), as well as

those that were lost, dead, or still apparently viable in the cloth bag (Figures S1, S2). Based on these observations, we found that in situ germination began with the onset of the rainy season for species that disperse during both the dry and rainy seasons (see Figure S2 for details).

Fire cue experiments with fresh seeds (T0)

Results of the experiments in fresh seeds (T0) showed that most species dispersing seeds at the onset of the dry season germinated less than 20% at the Control (C) treatment, except for *Bidens gardneri* (C=81,94%, Figure 1a). Results on the effects of fire-related cues showed that smoke increased germination for two species with permeable seed coats, *Banisteriopsis stellaris* (CSW=4.17±4.17%, SW =12.59±5.93%, Figure 1a) and *Solidago chilensis* (CSW=0, SW =50±22.36%, Figure 1a), while the other species were not affected by the smoke treatment. Heat shocks also affect germination, 100˚C treatment increased germination only for *Bidens gardneri* (C=81,94%, 100˚C=100%, Figure 1a) and decreased for *Solidago chilensis* and *Waltheria communis* from approximately 16% to zero for both species (Figure 1a). When exposed to 200˚C *Sida linifolia* had a partial PY broke at 200˚C (C=0%, 200˚C =65.50±20.16, Figure 1a) while *Banisteriopsis stellaris* and *Bidens gardneri* had a decrease in germination, from 11.43% and 81,94% at control to zero at the highest temperature, respectively (Figure 1a).

For species dispersed at the beginning of the rainy season, the fire-related cues, smoke and heat shocks, did not significantly affect germination of fresh seeds (Figure 1b).

Mean germination time (MGT) and synchrony (Z) were affected only by the smoke water treatment only in two species. *Stenodon suberosus*, that dispersed in the beginning of the rainy season, showed slower germination (an average delay of six days) and more synchronous germination when exposed to smoke water. While *Banisteriopsis stellari*s, that dispersed in the dry season, exhibited an increased synchrony (Z) compared to the smoke water control (Table 2).

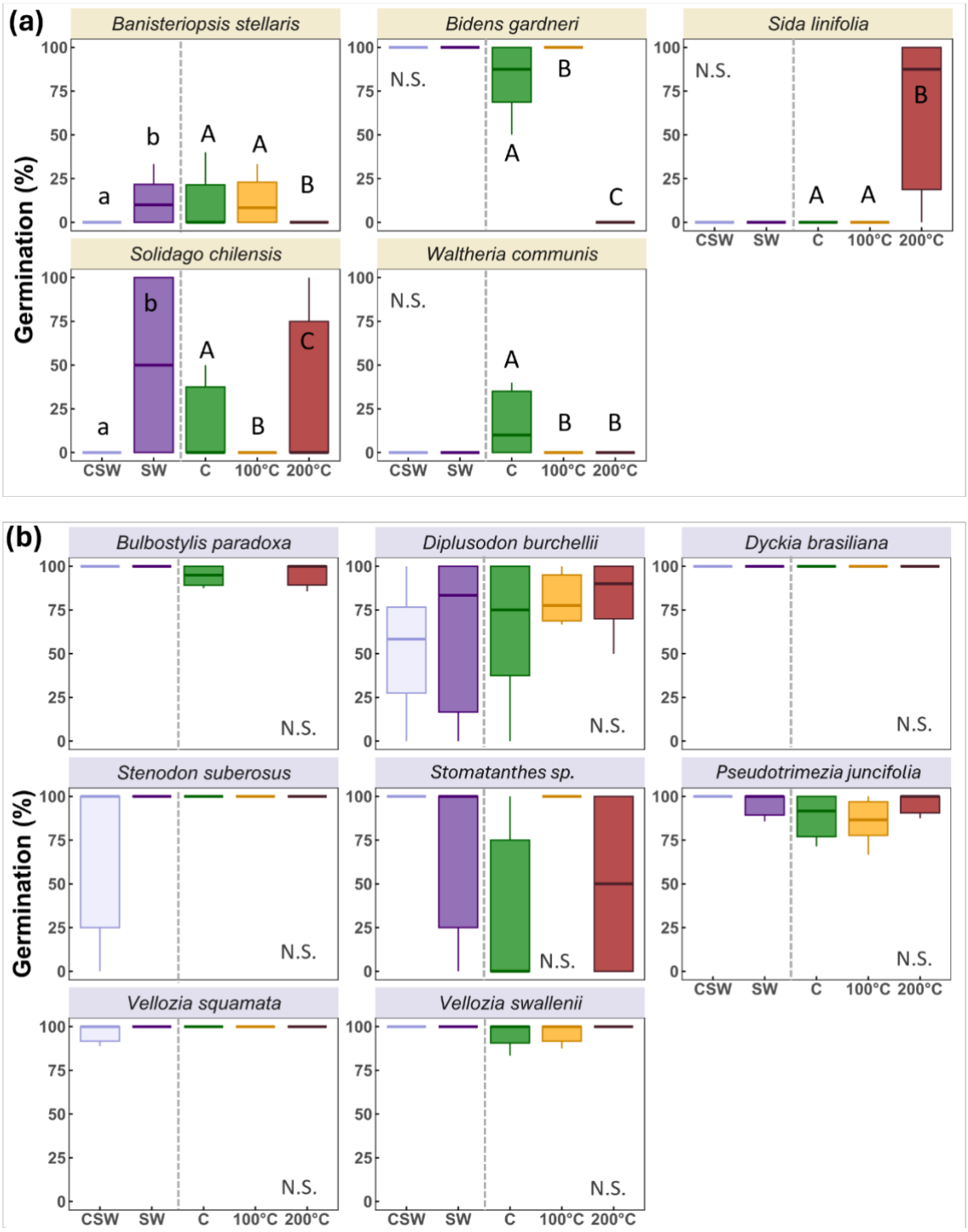


Figure 1. Germination percentage (mean ± SE) for fresh seeds (T0) under fire-related cue treatments: heat shock at 100 °C and 200 °C (for one minute each), a control (C; not exposed to high temperatures), a smoke control (CSW), and smoke water (SW). Lowercase letters indicate significant differences between CSW and SW, while uppercase letters denote significant differences between the heat shock treatments and the control ($p \leq 0.05$). "N.S." indicates no significant difference in germination. Panel (a) shows species dispersing during the dry season, and panel (b) shows species dispersing during the rainy season.

Effects of time on the germination response to fire cue experiments

Our results showed that seed storage time under field conditions affected germination responses to fire-related cues in 61% of the 13 tested species. Two species dispersing in the dry season had the smoke response pattern altered with *Banisteriopsis stellaris* germination not being increased by smoke (SW) after six months and *Solidago chilensis* displaying varying responses to smoke water (positive effects on germination at T0 and T9 and no effects in the other times, see Figure 2). Also, when comparing the germination within the SW along time, we could observe an increase in values after three months buried (Table S2). Response patterns to heat treatment also varied. When exposed to 200˚C at T3 *Banisteriopsis stellaris* and *Bidens gardneri* showed an increased and no effect in germination respectively, opposite responses found in T0 (Figure 2).

The two PY species, *Sida linifolia and Waltheria communis* had response patterns to heat treatment affected (Figure 2). PY in fresh seeds of *Sida linifolia* was overcome only at 200ºC, but seeds stored for 12 months had PY alleviated at 100˚C (Figure 2). In *Waltheria communis* responses went from positive at T6 and T9 to no effect of 200˚C treatment on germination after one year in the field (Figure 2). In addition, when comparing the germination within the heat treatment along time, we observed an increase in germination percentages of at least three-fold at 200˚C compared to the respective controls according to storage time with the highest germination at T9 for both species (Table S2).

Species dispersed in the rainy season: seeds of most species lasted only the first month in the field with only the two Velloziaceae species enduring 12 months in the field. In general, four species had the response pattern to heat shock altered. *Stenodon suberosus* and *Pseudotrimezia juncifolia* germination was negatively affected by the 200˚C when comparing T1 and T0 (Table S2). Meanwhile, *Vellozia squamata* and *Vellozia swallenii* that were not affected by the temperatures at T0, had a significant decrease in germination only at 100˚C in T1 while *Vellozia swallenii* had the germination at 100˚C and 200˚C increased only at T3 (Figure 2).

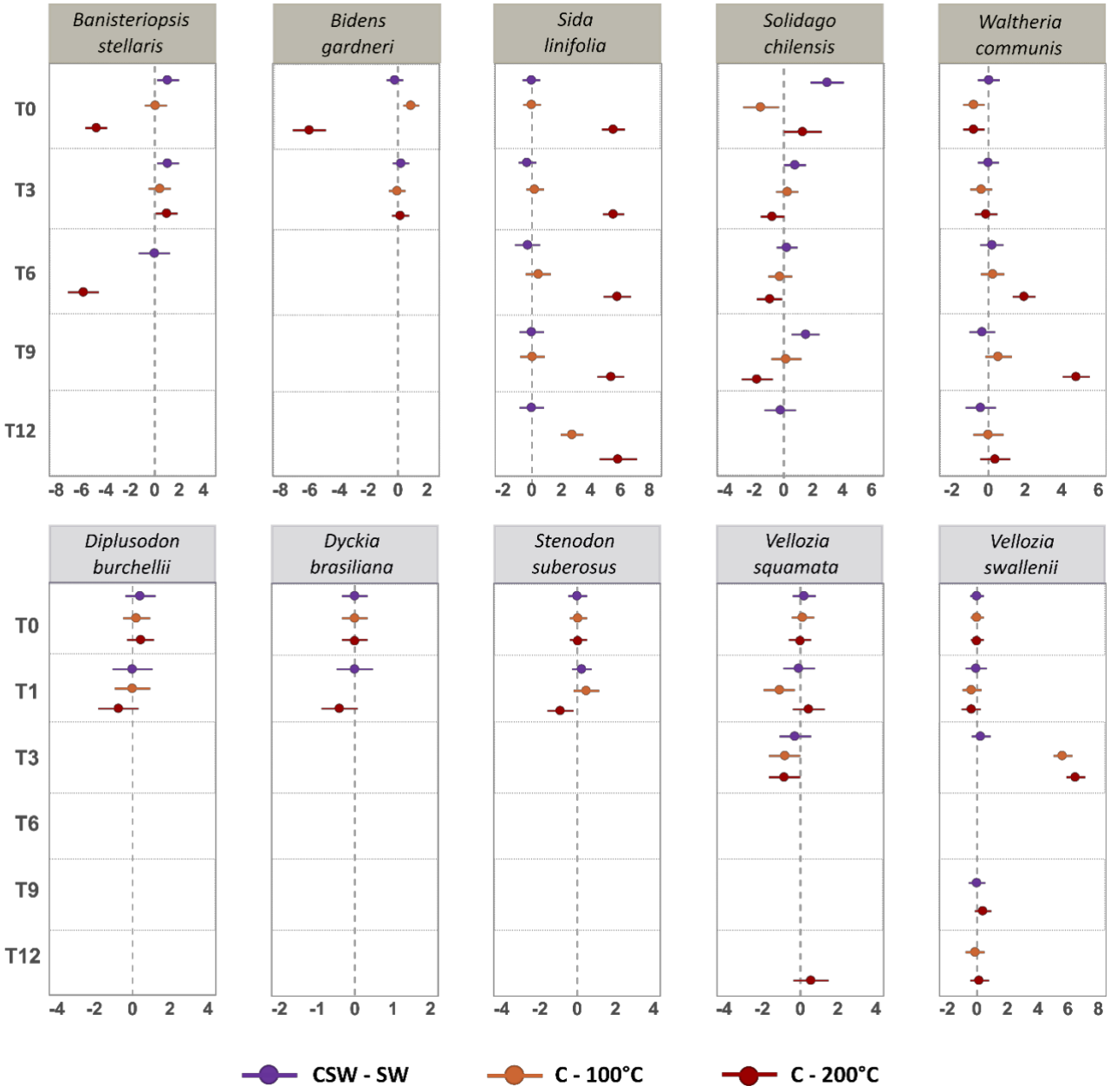


Figure 2. Effect of fire cues on germination for most species according to time buried (estimate ± SE). Values on the X-axis represent the estimated differences (estimate) between treatments. The vertical dashed line at zero indicates no significant difference compared to the control treatment. Positive estimates indicate a higher germination effect compared to the control. Negative estimates indicate a lower germination effect compared to the control. Error bars represent confidence intervals. Results where the confidence intervals do not cross the zero line are statistically significant. Fire cues experiments: 100°C, 200°C for one minute, C=control (not exposed to high temperatures), CSW=smoke control, SW=smoke water. Time buried: T0= fresh seeds not buried; T1= 1 month, T3= 3 months, T6=6 months, T9=9 months and T12= 12 months buried. Blank indicates data not available due to seed unavailability. The first line (yellow) are species that dispersed in the dry season and second line (blue) species that dispersed in the rainy season.

The pattern of mean germination time (MGT) could not be compared for some species since when germination is zero there is no MGT. Therefore, when possible, we compare each treatment along time. For species dispersed in the dry season we found that after three months, *Bidens gardneri* germinated faster than T0 in all treatments (Table 2), while *Solidago chilensis* had slower germination at 100°C after 9 months buried and after 12 months for the controls (C and CSW) (Table 2).

Synchrony values (Z) were variable within species, but we observed a more synchronous germination at the heat shock treatments after three months for *Banisteriopsis stellaris* and *Bidens gardneri* (100°C) and after six and 12 months for *Waltheria communis* (200°C) and *Sida linifolia* (100°C), respectively (Table 2). Also, smoke slowed the germination time for *Banisteriopsis stellaris* at T3 and T6 and for *Solidago chilensis* at T0 and T9 (Table 2).

Some species dispersed in the rainy season showed faster germination after 1 month but not in all treatments, such as *Diplusodon burchellii* and *Dyckia brasiliana* for smoke water control (CSW) and 200°C, *Stenodon suberosus* for 100°C, 200°C and SW and *Pseudotrimezia juncifolia* only for the control (Table 2). On the other hand, *Vellozia swallenii* had slightly slower germination after three months buried and *Bulbostylis paradoxa* showed an increase in germination time for the control along time (from 7 days at C to 15 days at T12) (Table 2). In addition, synchrony was increased after one month buried specially at the controls (C and/or CSW) for three species (*Dyckia brasiliana*, *Stenodon suberosus* and *Vellozia squamata*), while synchrony at SW was lower for *Dyckia brasiliana* at T1 and 200°C for *Vellozia squamata* at T12 (Table 2).

Finally, viability values within the control group for most species dispersed during the dry season did not decrease within *in situ* storage time, except for *Banisteriopsis stellaris* (Table S3). Notably, viability values for *Sida linifolia* and *Solidago chilensis* appeared to increase over time (Table S3), which is unlikely, suggesting that either the tetrazolium test may not be the most accurate method for assessing viability in these species, or that both species may exhibit some degree of physiological or morphophysiological dormancy in fresh seeds. For species dispersed during the rainy season, viability decreased over time for all species, with the most significant reductions occurring within the first month (Table S3). However, species within the Velloziaceae family showed a different pattern. While *Vellozia*

squamata only showed a decrease in viability after 12 months of burial compared to T0, *Vellozia swallenii* experienced a decrease in viability at T1 but maintained values around 30% until the end of the experiment at T12 (Table S3).

Table 2. Mean germination time (MGT in days; mean ± SE) and Synchrony values (Z; mean ± SE) for all species when exposed to the fire cues experiments according to time buried. Z=1 if germination of all seeds occurs at the same time, and if Z=0 at least two seeds could germinate, one at each time. Fire cues experiments: 100°C, 200°C for one minute, C=control (not exposed to high temperatures), CSW=smoke control, SW=smoke water. Time buried: T0= fresh seeds not buried, T1= 1 month, T3= 3 months, T6=6 months, T9=9 months and T12=12 months buried. NA= data not available due not availability of seeds. n.g. = no germination recorded. Asterisks indicate significant differences between the treatment and its respective control at the same time point. Lowercase letters indicate significant differences within the same treatment over time. $P < 0.05$.

Species	Time buried	MGT (days)					Synchrony (Z)				
		C	100°C 1'	200°C 1'	CSW	SW	C	100°C 1'	200°C 1'	CSW	SW
Banisteriopsis stellaris	T0	6.92±4.87	13.17±5.94	n.g.	4.67±4.67	13.58±6.09	0±0	0±0^{a}	n.g.	0±0	0.17±0.17^{a}*
	T3	7.17±2.62	14.03±3.74	17.13±2.03*	8.25±3.20	15.78±0.52	0±0	0.22±0.16^{b}*	0.21±0.10*	0±0	0±0^{b}
	T6	2.67±2.67	NA	n.g.	9.00±9.00	3.67±3.67	0±0	NA	n.g.	0±0	0±0^{b}
	T9	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	T12	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Bidens gardneri	T0	16.61±1.02^{a}	17.89±1.59^{a}	n.g.	13.81±1.92^{a}	11.75±1.41^{a}	0.09±0.04^{a}	0.10±0.05^{a}	n.g.	0.09±0.05^{a}	0.22±0.06
	T3	3.27±0.46^{b}	3.79±0.50^{b}	4.16±1.01	3.53±0.45^{b}	3.63±0.51^{b}	0.47±0.12^{b}	0.47±0.10^{b}	0.55±0.17	0.41±0.13^{b}	0.31±0.06
	T6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	T9	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	T12	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Sida linifolia	T0	n.g.	n.g.	8.08±2.65	2.67±2.67	1.83±1.83	n.g	n.g.	0.27±0.16^{ab}	0±0	0±0
	T3	2.50±2.50	2.42±1.64	6.82±1.85	1.17±1.17	n.g.	0±0	0±0^{a}	0.31±0.15^{ab}*	0±0	n.g.
	T6	n.g.	2.00±2.00	5.67±0.29	2.00±2.00	n.g.	n.g.	0±0^{a}	0.44±0.13^{a}	0±0	n.g.
	T9	2.33±2.33	2.33±2.33	6.84±2.25	n.g.	n.g.	0±0	0±0^{a}	0.22±0.12^{ab}*	n.g.	n.g.
	T12	n.g.	3.73±1.88	2.56±2.56	n.g.	n.g.	n.g.	0.52±0.29^{b}	0.04±0.04^{b}	n.g.	n.g.
Solidago chilensis	T0	2.50±1.59^{a}	n.g.	3.00±1.97	n.g.	4.17±1.96	0±0^{a}	n.g.	0±0	n.g.	0±0^{a}
	T3	3.79±1.35^{a}	3.33±1.05^{a}	6.33±4.01	5.17±1.04^{ab}	5.47±1.31	0.08±0.08^{a}	0.17±0.17	0±0	0.29±0.17	0.57±0.20^{b}
	T6	3.36±1.20^{a}	3.17±1.62^{a}	2.17±1.38	4.94±0.63^{ab}	5.16±0.29	0.47±0.18^{b}	0.19±0.16	0±0*	0.50±0.22	0.67±0.11^{b}
	T9	6.25±2.14^{ab}	15.00±5.21^{b}*	5.25±5.25	3.50±2.02^{a}	9.38±2.12	0±0^{a}	0±0	0±0	0±0	0.05±0.05^{a}

	T12	12.78±0.97[b]	NA	NA	11.86±0.22[b]	9.71±0.77	0.44±0.29[b]	NA	NA	0.14±0.08	0.42±0.14[b]
Waltheria communis	T0	8.08±3.90	n.g.	n.g.	1.17±1.17	1.83±1.83	0±0	n.g.	n.g.	0±0	0±0
	T3	2.00±1.26	n.g.	0.83±0.83	n.g.	n.g.	0±0	0±0	0.17±0.17[a]*	n.g.	n.g.
	T6	n.g.	1.20±1.20	3.60±0.93	n.g.	0.80±0.80	n.g.	0±0	0.57±0.19[b]	n.g.	0±0
	T9	n.g.	7.25±5.99	5.28±0.65	5.75±3.47	2.25±2.25	n.g.	0±0	0.35±0.04[a]	0±0	0±0
	T12	4.33±4.33	2.00±2.00	2.80±2.80	7.67±7.67	n.g.	0±0	0±0	0.13±0.13[a]*	0±0	n.g.
Bulbostylis paradoxa	T0	7.14±0.25[a]	NA	9.26±0.69	8.49±0.26	10.31±0.49	0.50±0.10[a]	NA	0.27±0.07	0.36±0.05	0.19±0.05
	T1	11.33±1.15[ac]	9.74±0.91	11.66±1.58	NA	NA	0.33±0.05[a]	0.20±0.06	0.19±0.05	NA	NA
	T6	20.42±1.89[b]	NA	NA	NA	NA	0.16±0.11[b]	NA	NA	NA	NA
	T12	15.58±3.88[bc]	NA	NA	NA	NA	0.17±0.11[b]	NA	NA	NA	NA
Diplusodon burchellii	T0	16.60±3.45	17.38±1.75	20.17±1.70[a]	18.17±3.90[a]	12.62±4.08	0.11±0.05	0.19±0.09	0.25±0.17	0.03±0.03	0±0
	T1	10.00±2.00	9.22±2.12	4.83±2.74[b]	6.33±0.67[b]	5.33±2.67	0.33±0.33	0.11±0.11	0±0	0.33±0.33	0±0
	T3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	T6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	T9	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	T12	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Dyckia brasiliana	T0	6.38±0.09	6.45±0.18	7.16±0.30[a]	7.49±0.63[a]	6.49±0.14	0.66±0.07[a]	0.70±0.11	0.45±0.06	0.42±0.09	0.63±0.08[a]
	T1	5.00±0.00	NA	3.67±1.86[b]	5.83±0.25[b]	7.00±1.02	1.00±0.00[b]	NA	0.44±0.29*	0.39±0.06	0.22±0.11[b]
	T3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	T6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	T9	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	T12	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Stenodon suberosus	T0	7.11±0.66	6.83±0.40[a]	7.83±0.44[a]	4.67±1.52	10.90±1.09[a]*	0.22±0.16	0.33±0.21	0.34±0.15	0±0[a]	0.31±0.15*
	T1	6.60±1.94	2.80±1.71[b]	1.40±1.40[b]	4.80±1.28	6.33±1.64[b]	0±0	0.20±0.20	0±0	0.60±0.24[b]	0.27±0.19

	T3	3.00±3.00	NA	NA	NA	NA	0±0	NA	NA	NA	NA
	T6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	T9	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	T12	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Stomatanthes sp	T0	3.57±2.56	7.83±1.76	4.53±2.05	7.50±1.96	7.06±2.29	0.03±0.03	0±0	0.06±0.06	0±0	0.22±0.16
	T1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	T12	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Pseudotrimezia juncifolia	T0	15.69±0.87[a]	16.15±0.80	14.60±3.08	15.55±0.72	15.65±0.43	0.71±0.14	0.48±0.11	0.31±0.11	0.45±0.13	0.44±0.13
	T1	10.33±2.73[b]	NA	n.g.	NA	NA	0.33±0.33	NA	n.g.	NA	NA
	T3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	T6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	T9	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	T12	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Vellozia squamata	T0	10.86±0.48	11.42±0.57	11.70±0.44	12.08±0.69	12.38±1.28	0.31±0.04[ac]	0.30±0.05	0.31±0.03[a]	0.30±0.05[a]	0.34±0.04
	T1	8.75±0.25	9.33±1.07	11.21±3.11	12.11±0.11	10.33±3.67	0.76±0.13[b]	0.44±0.08	0.53±0.24[a]	0.78±0.22[b]	0.42±0.05
	T3	13.21±1.09	9.60±1.83	11.93±0.93	13.61±0.84	14.10±2.13	0.13±0.03[a]	0.40±0.31	0.47±0.29[a]	0.07±0.04[a]	0.19±0.12
	T6	13.15±1.47	NA	NA	NA	NA	0.51±0.14[bc]	NA	NA	NA	NA
	T9	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	T12	10.92±1.96	NA	10.92±5.75	NA	NA	0.17±0.17[ac]	NA	0±0[b]*	NA	NA
Vellozia swallenii	T0	11.94±1.42	11.40±1.27	11.50±1.03	11.84±1.84	10.93±1.02[a]	0.41±0.08	0.28±0.07	0.37±0.05	0.47±0.14	0.32±0.04
	T1	9.44±4.12	5.92±3.06	9.28±0.27	7.50±3.77	14.47±2.97[a]	0.33±0.19	0.31±0.16	0.42±0.13	0.50±0.29	0.61±0.06
	T3	n.g.	11.00±6.35	8.33±4.19	20.00±3.61	15.17±4.94[bc]	n.g.	0.33±0.33	0.22±0.11	0.67±0.33	0.11±0.11
	T6	13.68±1.12	NA	NA	NA	NA	0.35±0.08	NA	NA	NA	NA
	T9	8.62±3.05	NA	14.59±1.61	13.33±1.74	13.78±0.92[ca]	0.25±0.25	NA	0.18±0.11	0.42±0.21	0.13±0.08
	T12	10.67±2.17	13.67±1.45	10.17±5.10	NA	NA	0.39±0.31	0.33±0.33	0.06±0.06	NA	NA

Discussion

Although the Cerrado has some species that positively respond to heat shock and smoke (Daibes et al. 2022), germination driven by fire-related cues seems not to be the main regeneration trait for this system as found in other fire-prone ecosystems (e.g. Mediterranean vegetation). Instead, the Cerrado vegetation most common post-fire strategies include resprouting, fire-stimulated flowering and seeds tolerant to high temperatures (Fichino et al., 2016; Oliveira et al., 2018; Zirondi et al., 2019, 2021; Daibes et al. 2019; Fidelis and Zirondi, 2021; Pilon et al. 2021; Zupo et al. 2021). Our results suggest that when investigating seed responses to fire cues, seed aging and *in situ* storage period should be added to the equation to better understand how fire affects the regeneration of tropical savannas. Our main findings were that *in situ* seed storage affected germination responses to fire-related cues in >60% of tested species, with two permeable species becoming more sensitive to smoke (increasing germination) and physically dormant (PY) species having a significant alleviation in seed dormancy leading to greater percentages in germination at 200°C at longer periods of storage when compared to fresh seeds.

After seed dispersal, seeds buried in the soil will have physical and physiological properties influenced by environmental conditions, such as changes in moisture, temperature, light among others (Fenner and Thompson, 2005; Long et al., 2015). In addition, seed aging would negatively affect seed physiology and thus the germination process (e.g. germination time, viability, etc., Dell'aquila, 1994). This holds true for the species dispersed during the rainy season, since their viability decreased over time, with the most significant reductions occurring within the first month. However, for species dispersing in the beginning of the dry season, our results showed otherwise, and the viability seems not to be decreased by the storage period while seeds were still available, probably due to inherent seed longevity (Long et al., 2008) that will allow for seed persistence (Fenner and Thompson, 2005; Long et al., 2015). That is, seed physiological traits (e.g. cellular mobility and ability repair damage, protective compounds, etc) (Long et al., 2015) would

be responsible for ensuring the seed viability of Cerrado plants during this short period that is up to 12 months. Nevertheless, our results showed that to some extent, *in situ* conditions affect germination responses to heat and smoke, thus seed age and storage type might play important roles in fire effects on germination (Franzese and Ghermandi, 2011).

Species from fire-prone ecosystems with water-permeable seed coats are recognized to have increased germination when exposed to smoke compounds (Flematti, 2004; Keeley et al., 2011; Keeley and Pausas, 2018). In the Cerrado, species do not exhibit a consistent response to smoke, showing strong species-specific variations depending on the smoke method and concentration. As observed by Motta et al. (2024), smoke had no effect on germination at the community level. However, at the species level, it influenced germination in 41% of the studied species, either positively or negatively. The smoke stimuli can be observed in fresh seeds of non-dormant species but also in species with physiological dormancy (Roche et al., 1997; Keeley and Fotheringham, 1998; Tieu et al., 2001; Baker et al., 2005a). In addition, seed aging can enhance the smoke effects leading to germination increase in long stored seeds as found by Tieu *et al.* (2001) for Australian species (see also Roche et al., 1997; Baker et al., 2005a; Ooi et al., 2006). Most of our species were not affected by smoke. However, the two species affected by smoke had water-permeable and non-dormant seeds but might follow the same concept of sensing the surrounding environment (e.g. seasonal changes) until the conditions are favorable for germination and establishment (Baker et al., 2005b; Long et al., 2015). Therefore, the increased germination in smoke treatment at long storage treatments could indicate that few Cerrado seeds dispersing in the beginning of the dry season may require an after-ripening period to reach higher germination percentages driven by smoke. On the other hand, *Banisteriopsis stellaris* were no longer positively affected by the smoke after six months buried (beginning of the rainy season), which could indicate the availability of water as a stronger driver of germination.

Interesting enough is the fact that two species *Banisteriopsis stellaris* and *Bidens gardneri*, became resistant to exposure to 200°C after 3 months buried compared to T0, although having water-permeable seed coats. One could speculate that this response might be linked to a decrease in seed moisture content after 3 months in a dry environment, as seed moisture is

known to play a crucial role in a seed's ability to withstand high temperatures (Tangney et al., 2019).

Seed aging in the soil also affects the dormancy-breakage and substantially increased germination percentage and synchrony at 200°C for PY species. It is already known that *in situ* storage can affect seed responses. Orscheg and Enright (2011) found an increase in the percentage of non-dormant seeds of *Acacia* species within 3 years of *in situ* storage. Liyanage and Ooi (2017) observed changes of the dormancy-breaking thresholds of Fabaceae species with seed aging. What we found was partially similar to both studies, an alleviation of seed dormancy with seed aging, where a greater portion of seeds germinate when exposed to 200° C. There was not a change in dormancy-breaking threshold, that is, the only tested temperature capable of breaking the seed dormancy was 200° C, maybe this could change with longer time in storage or with other temperature ranges (e.g. 150° C). Nonetheless, all these studies evidence the importance of seed storage period in dormancy and germination of PY species. A possible explanation for these results can be related to the sensitive cycle to PY break (Jayasuriya et al., 2008, 2009a, 2009b). This cycle is driven by environmental conditions such as soil moisture and temperature and consists of seeds alternating between insensitive and sensitive states to dormancy break, thus dormancy could be broken (e.g. with high temperatures) in sensitive seeds (Jayasuriya et al., 2008, 2009a, 2009b). This mechanism in PY seeds is responsible for increasing the establishment success since it ensures that higher germination would occur only when environmental conditions are suitable for growth (Jayasuriya et al., 2009a) which fits our scenario since our seeds were dispersed at the beginning of the dry season and became more sensitive within the rainy period.

In Cerrado, seeds dispersing in the beginning of the dry season would not have access to water, thus germinating in this period would lead to massive death of the seedlings (Escobar et al. 2018; Long et al. 2015). Thus, the seed capacity to survive through the dry season observed for our studied species can be key in the regeneration processes of those species. In addition, although the Cerrado fire regime has changed due to anthropic actions (most fires occur in the mid and end of the dry season), most natural fires occur at the dry-to-wet transitional period due

to the high lightning incidence that precludes the storms common at this time of the year (Ramos-Neto and Pivello 2000; Miranda et al. 2009; Pivello 2011). This time frame fits with our species becoming more sensitive to fire cues at T6, therefore, if a greater proportion of the seeds can germinate following a fire by the end of the dry/beginning of the rainy season, it would have increased chances of establishing in the post-fire environment as soon as the rainy season starts, since seedlings would have access to water and nutrients required for a successful establishment.

Our results showed that species dispersed during the rainy season experienced high recruitment and significant seed loss in the field within the first month, as expected, given that all these species had non-dormant seeds. Among the seeds that were retrieved, we observed notable alterations in their responses to fire-related cues for half of the species. The most prominent changes included faster germination across different treatments and reduced germination rates at high temperatures after one month, compared to T0. Faster germination enables seeds to access available resources, such as water, providing a competitive edge for early establishment in the ecosystem (Baskin & Baskin, 2014). The observed decline in germination at higher temperatures might be linked to the seeds' increased moisture content, which has been shown to affect their resistance to heat (Tangney et al., 2019). Another point is that although most permeable seeds were recruited during the rainy season, results of *Solidago chilensis, Vellozia squamata* and *Vellozia swallenii* suggest that some species with permeable seeds are also capable of forming soil seed banks. This capacity of having a wide spectrum of seed longevity could be linked to the high frequency of a disturbance (Fenner and Thompson, 2005), as found in the Cerrado (Miranda et al. 2009; Rodrigues et al. 2021) thus leading to a persistent seed bank that will be responsible for future recruitment.

Another aspect to be considered is that PY breakage is generally linked with exposure to fire (high temperatures) or daily temperature fluctuations (Fenner and Thompson, 2005; Baskin and Baskin, 2014). Previous studies on Cerrado species have tested the effects of heat and smoke on germination separately, without identifying any consistent patterns in the responses, being species-specific (Zupo et al., 2016; Daibes et al., 2017; Dairel and Fidelis, 2020; Daibes et al., 2021). However, Zirondi *et al.* (2019) found a positive response when combining heat and smoke

treatments, highlighting that some species exhibit improved germination when exposed to a combination of fire-related cues.

In summary, we found that alterations in germination responses to fire-related cues can be linked to seed aging and *in situ* storage time as well as to the dispersal season (Figure 3), especially when it happens at the onset of the dry season. Together with findings from previous studies, our results suggest that the germination responses of Cerrado species to fire-related cues are highly species-specific and driven by a combination of factors. These include, but are not limited to, the fire regime experienced by the mother plant (Zirondi et al., 2024), seed aging, dormancy type, dispersal season, and storage conditions. This variability in germination responses may reflect bet-hedging strategies in Cerrado species. Bet-hedging strategies increase the likelihood of survival in fluctuating environments, as different strategies may confer advantages under varying competitive conditions (Gianella et al., 2021; Ten Brink & Khan, 2023) what would happen for species in fire-prone ecosystems.

Understanding the interplay of these factors is crucial for deciphering the effects of fire on plant recruitment, regeneration strategies, and germination patterns in Cerrado vegetation. Overall, this study provides novel insights into the role of in situ seed storage in shaping germination responses to fire-related cues, highlighting that seed recruitment in fire-prone tropical savannas is more complex than previously understood.

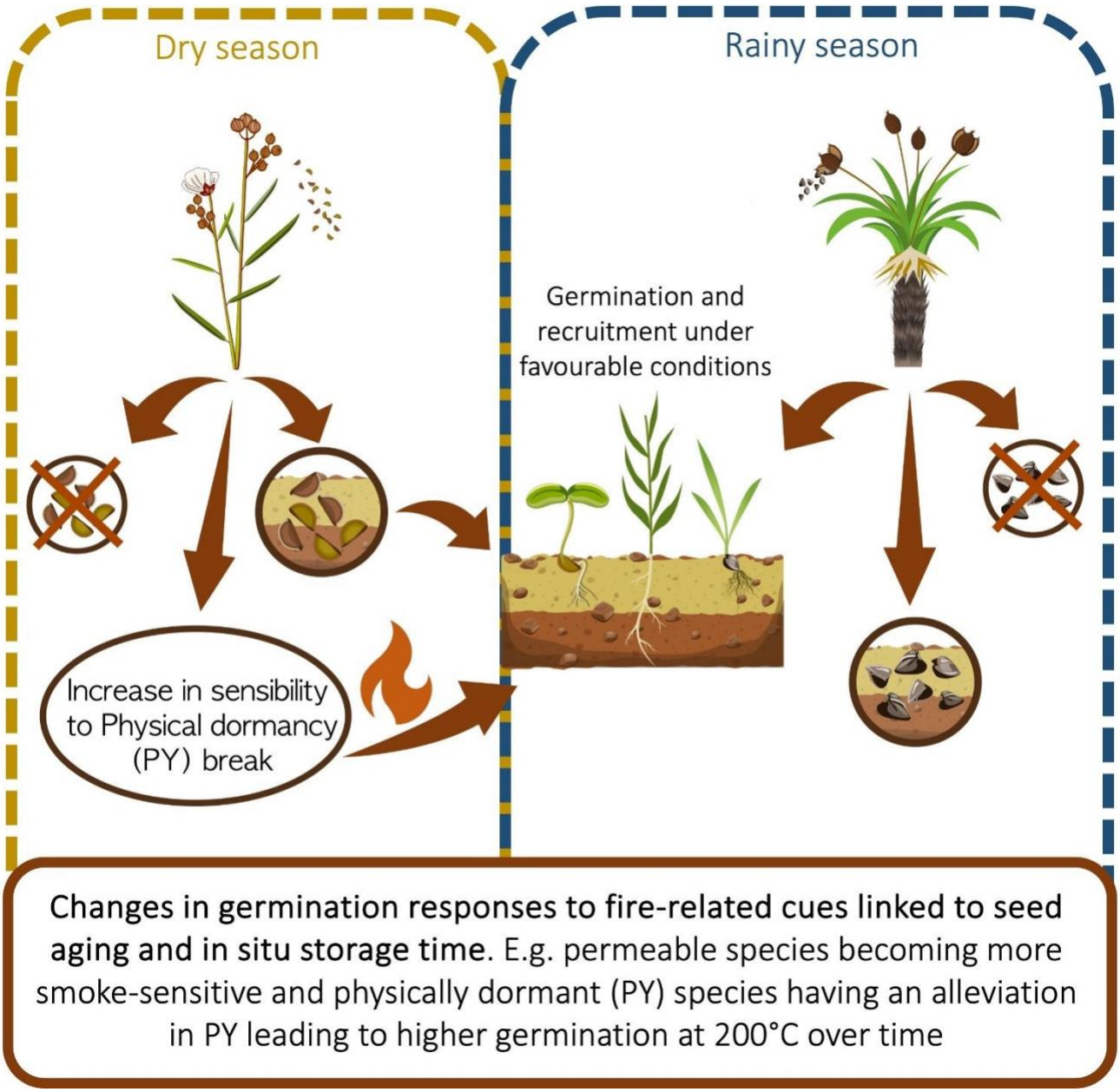


Figure 3. Summary of the findings from this study. Evidencing possible scenarios for seeds following dispersal, highlighting how germination responses to fire-related cues may be influenced by seed aging, in situ storage time, and the dispersal season.

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Supplementary material

Figure S1. Exhumed seeds of *Banisteriopsis stellaris* displaying both germinated seeds (a,b,c) and retrieved seeds (d). We quantified the seeds that had germinated (i.e., those with roots and/or cotyledons still attached) as well as those that were lost, dead, or still apparently viable in the cloth bag.

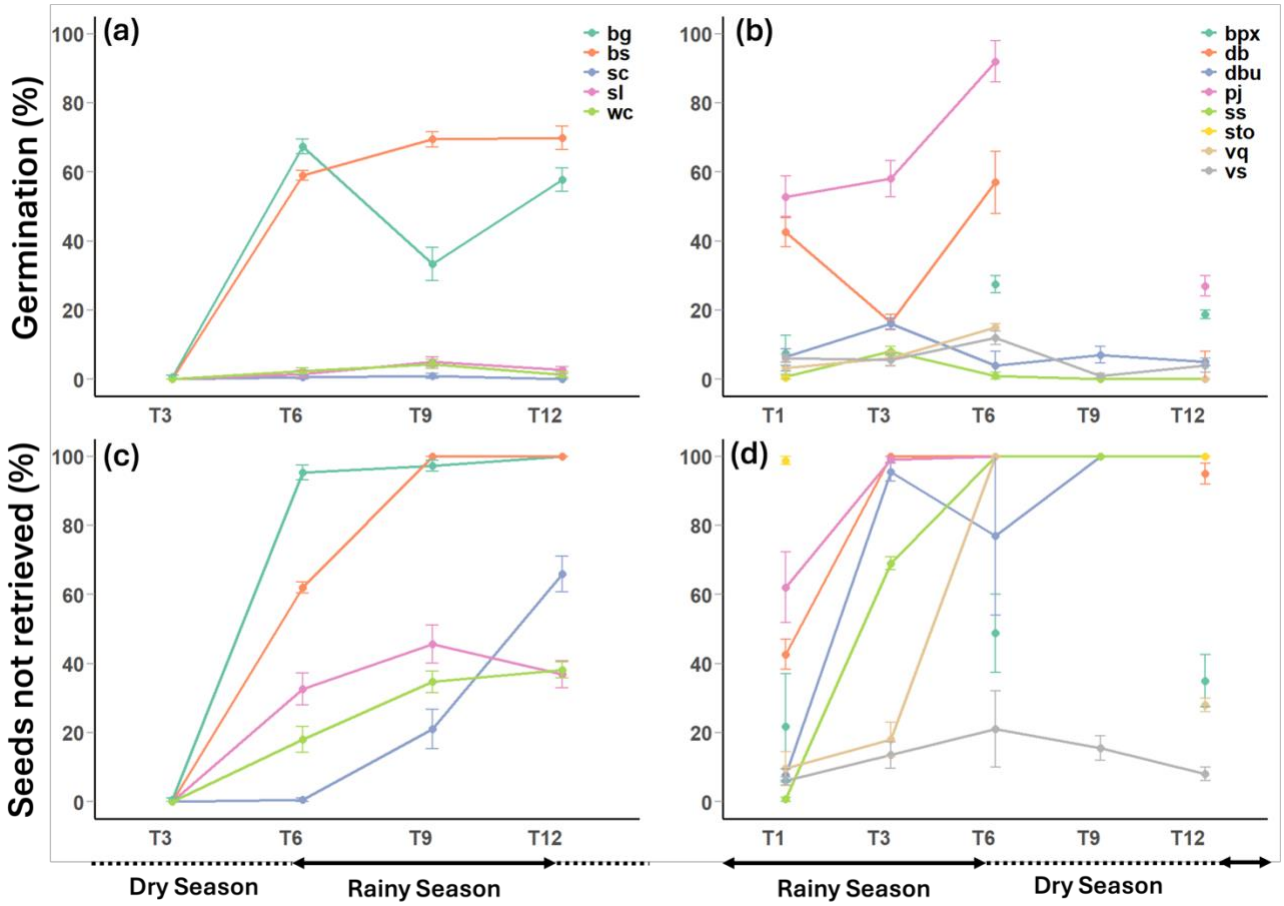

Figure S2. Percentage of in situ germination (mean ± SE) (panels a and b) and non-retrieved seeds (mean ± SE) (panels c and d) for the study species over a 12-month period. Panels (a) and (c) show data for species dispersing at the beginning of the dry season, while panels (b) and (d) show data for species dispersing at the beginning of the rainy season. For species with permeable seeds dispersed at the beginning of the dry season, recruitment commenced with the onset of the rainy season at T6, resulting in few or no remaining seeds after T6 for *Banisteriopsis stellaris* and *Bidens gardneri* (panel a). In contrast, species dispersing at the beginning of the rainy season—particularly photoblastic species such as *Vellozia* spp. and *Bulbostylis paradoxa*—exhibited lower recruitment throughout the study period (panel b). It is important to note that in situ germination may be underestimated for some species, as evidence of germination may have disintegrated in the bag or been lost prior to retrieval. To account for this, we also measured the percentage of non-retrieved seeds, which includes seeds that germinated, were dead, or were lost. The percentage of non-retrieved seeds increased after six months for species dispersed during the dry season and after three months for those dispersed during the rainy season (panels c and d). Species abbreviations: Panels (a) and (c): bg = *Bidens gardneri*; bs = *Banisteriopsis stellaris*; sc = *Solidago chilensis*; sl = *Sida linifolia*; wc = *Waltheria communis*. Panels (b) and (d): bpx = *Bulbostylis paradoxa*; dbu = *Diplusodon burchellii*; db = *Dyckia brasiliana*; ss = *Stenodon suberosus*; sto = *Stomatanthes* sp.; pj = *Pseudotrimezia juncifolia*; vq = *Vellozia squamata*; vs = *Vellozia swallenii*.

Table S1. Percentage of germination (mean ± SE) for all species when exposed to the fire cues experiments according to time buried. Fire cues experiments: 100°C, 200°C for one minute, C=control (not exposed to high temperatures), CSW=smoke control, SW=smoke water. Time buried: T0= fresh seeds not buried, T1= 1 month, T3= 3 months, T6=6 months, T9=9 months and T12=12 months buried. NA= data not available due not availability of seeds. Asterisks indicate significant differences between the treatment and its respective control at each time. *, $P < 0.05$.

Species	Time buried	Fire cues experiments				
		C	100°C 1'	200°C 1'	CSW	SW
Banisteriopsis stellaris	T0	7.03±4.46	5.00±2.24	0.00±0.00*	1.67±1.67	8.33±4.01
	T3	10.00±3.65	16.67±4.22	30.00±6.83*	8.33±3.07	21.67±4.77
	T6	3.33±3.33	NA	0.00±0.00*	3.33±3.33	3.33±3.33
	T9	NA	NA	NA	NA	NA
	T12	NA	NA	NA	NA	NA
Bidens gardneri	T0	41.67±9.80	40.00±3.65	0.00±0.00*	33.33±10.54	77.27±5.24*
	T3	63.33±9.89	55.55±9.65	43.33±9.89	60.73±6.93	80.00±5.77
	T6	NA	NA	NA	NA	NA
	T9	NA	NA	NA	NA	NA
	T12	NA	NA	NA	NA	NA
Sida linifolia	T0	0.00±0.00	0.00±0.00	21.67±8.72*	1.67±1.67	1.67±1.67
	T3	1.67±1.67	5.00±3.42	36.67±11.74*	3.33±3.33	0.00±0.00
	T6	0.00±0.00	2.57±2.57	64.10±12.80*	2.57±2.57	0.00±0.00
	T9	3.33±3.33	3.33±3.33	70.00±20.82*	0.00±0.00	0.00±0.00
	T12	0.00±0.00	31.10±21.88	16.67±16.67	0.00±0.00	0.00±0.00
Solidago chilensis	T0	5.00±3.42	0.00±0.00	3.18±2.02	0.00±0.00	5.00±2.24
	T3	11.67±6.01	8.33±3.07	3.33±2.11	21.67±7.92	35.00±9.92
	T6	18.33±6.54	15.00±5.63	5.00±3.42	25.00±5.63	46.67±6.67
	T9	7.50±2.50	7.50±2.50	2.50±2.50	5.00±2.89	30.00±10.00*
	T12	27.43±1.44	NA	NA	37.70±12.52	45.73±2.40
Waltheria communis	T0	8.33±4.01	0.00±0.00	0.00±0.00	1.67±1.67	1.67±1.67
	T3	3.33±2.11	0.00±0.00	3.33±3.33	0.00±0.00	0.00±0.00
	T6	0.00±0.00	4.00±4.00	26.88±7.93*	0.00±0.00	2.22±2.22
	T9	0.00±0.00	5.00±2.89	71.67±6.45*	5.28±3.05	2.50±2.50
	T12	2.77±2.77	2.77±2.77	12.83±12.83	5.57±5.57	0.00±0.00
Bulbostylis paradoxa	T0	88.33±4.77	NA	76.67±6.67	85.00±6.71	66.67±7.15
	T1	78.17±2.78	90.00±0.00	70.00±5.77	NA	NA
	T6	52.50±6.29	NA	NA	NA	NA
	T12	24.00±5.10	NA	NA	NA	NA
Diplusodon burchellii	T0	26.67±8.03	31.67±4.01	16.67±4.94	15.00±5.63	15.00±6.19
	T1	16.67±3.33	23.33±6.67	10.00±5.77	13.33±3.33	16.67±8.82
	T3	NA	NA	NA	NA	NA
	T6	NA	NA	NA	NA	NA
	T9	NA	NA	NA	NA	NA

	T12	NA	NA	NA	NA	NA
Dyckia brasiliana	T0	68.33±5.43	85.00±5.00	81.67±7.03	68.33±8.72	85.00±6.71
	T1	26.67±6.67	NA	20.00±11.55	33.33±3.33	23.33±6.67
	T3	NA	NA	NA	NA	NA
	T6	NA	NA	NA	NA	NA
	T9	NA	NA	NA	NA	NA
	T12	NA	NA	NA	NA	NA
Stenodon suberosus	T0	16.67±4.22	15.00±2.24	26.67±7.60	8.33±3.07	23.33±4.94*
	T1	12.00±3.74	6.00±4.00	2.00±2.00*	20.00±8.37	14.00±5.10
	T3	1.67±1.67	NA	NA	NA	NA
	T6	NA	NA	NA	NA	NA
	T9	NA	NA	NA	NA	NA
	T12	NA	NA	NA	NA	NA
Stomatanthes sp.	T0	10.00±8.16	8.33±1.67	10.00±5.16	8.33±1.67	11.67±4.77
	T1	NA	NA	NA	NA	NA
	T12	NA	NA	NA	NA	NA
Pseudotrimezia juncifolia	T0	53.33±8.03	51.67±8.72	56.67±12.02	53.33±4.94	58.33±7.03
	T1	13.33±3.33	NA	0.00±0.00*	NA	NA
	T3	NA	NA	NA	NA	NA
	T6	NA	NA	NA	NA	NA
	T9	NA	NA	NA	NA	NA
	T12	NA	NA	NA	NA	NA
Vellozia squamata	T0	91.67±4.77	86.67±4.94	91.67±4.77	76.67±6.15	95.00±3.42*
	T1	63.60±5.25	57.60±16.86	48.47±10.90	45.47±10.48	63.03±17.87
	T3	53.33±13.33	50.00±5.77	40.00±15.28	60.00±11.55	60.00±23.09
	T6	56.67±8.82	NA	NA	NA	NA
	T9	NA	NA	NA	NA	NA
	T12	22.20±7.35	NA	20.00±11.55	NA	NA
Vellozia swallenii	T0	75.00±7.19	75.00±2.24	85.00±6.71	80.00±6.83	73.33±5.58
	T1	30.30±13.19	36.37±18.92	40.63±2.65	18.20±10.51	48.47±6.03*
	T3	0.00±0.00	13.33±8.82*	20.00±10.00*	16.67±3.33	20.00±10.00
	T6	21.67±3.07	NA	NA	NA	NA
	T9	15.00±6.45	NA	27.50±8.54	22.50±4.79	27.50±10.31
	T12	26.67±6.67	13.33±3.33	16.67±12.02	NA	NA

Table S2. Percentages of germination adjusted per viability (mean ± SE) for all species when exposed to the fire cues experiments according to time buried. Fire cues experiments: 100°C, 200°C for one minute, C=control (not exposed to high temperatures), CSW=smoke control, SW=smoke water. Time buried: T0= fresh seeds not buried, T1= 1 month, T3= 3 months, T6=6 months, T9=9 months and T12=12 months buried. NA= data not available due not availability of seeds. Asterisks indicate significant differences between the treatment and its respective control at the same time point. Lowercase letters indicate significant differences within the same treatment over time. No symbols (asterisks or letters) indicate no significant differences in the comparisons. $P < 0.05$.

Species	Time buried	Fire cues experiments				
		C	100°C 1'	200°C 1'	CSW	SW
Banisteriopsis stellaris	T0	11.43±7.38	12.50±5.99	0±0[a]*	4.17±4.17	12.59±5.93*
	T3	25.00±8.33	38.61±9.53	67.46±9.02[b]*	20.83±7.68	61.11±13.02*
	T6	33.33±33.33	NA	0.00±0.00*	33.33±33.33	33.33±33.33
	T9	NA	NA	NA	NA	NA
	T12	NA	NA	NA	NA	NA
Bidens gardneri	T0	81.94±8.72	100.00±0.00*	0±0[a]*	100.00±0.00	97.92±2.08
	T3	98.15±1.85	95.24±4.76	83.33±16.67[b]	96.67±3.33	100.00±0.00
	T6	NA	NA	NA	NA	NA
	T9	NA	NA	NA	NA	NA
	T12	NA	NA	NA	NA	NA
Sida linifolia	T0	0.00±0.00	0.00±0.00[a]	62.50±20.16*	2.38±2.38	4.17±4.17
	T3	5.56±5.56	8.10±5.24[a]	83.33±16.67*	8.33±8.33	0.00±0.00
	T6	0.00±0.00	4.17±4.17[a]	100.00±0.00*	3.03±3.03	0.00±0.00
	T9	3.70±3.70	5.56±5.56[a]	100.00±0.00*	0.00±0.00	0.00±0.00
	T12	0.00±0.00	43.33±29.63[b]*	33.33±33.33*	0.00±0.00	0.00±0.00
Solidago chilensis	T0	16.67±10.54	0.00±0.00[a]*	33.33±21.08*	0.00±0.00[a]	50.00±22.36*
	T3	50.00±18.26	55.56±20.49[b]	25.00±17.08*	58.33±15.96[b]	83.33±16.67
	T6	66.67±21.08	61.11±15.32[b]	33.33±21.08	84.72±11.06[b]	97.22±2.78
	T9	50.00±20.41	75.00±25.00[b]	12.50±12.50*	37.50±23.94[b]	100.00±0.00*
	T12	67.62±16.93	NA	NA	65.71±17.84[b]	77.18±7.37
Waltheria communis	T0	16.67±8.03	0.00±0.00*	0.00±0.00[a]*	2.78±2.78	5.56±5.56
	T3	5.16±3.28	0.00±0.00	6.67±6.67[a]	0.00±0.00	0.00±0.00
	T6	0.00±0.00	5.71±5.71	37.78±11.84[b]*	0.00±0.00	3.33±3.33
	T9	0.00±0.00	5.56±3.21	80.62±8.56[c]*	5.28±3.06	2.50±2.50
	T12	3.03±3.03	3.70±3.70	27.78±27.78[a]	6.67±6.67	0.00±0.00
Bulbostylis paradoxa	T0	94.40±2.53[a]	NA	95.24±3.01	98.33±1.67	98.15±1.85
	T1	89.26±0.37[a]	100.00±0.00	95.24±4.76	NA	NA
	T6	100.00±0.00[a]	NA	NA	NA	NA
	T12	68.33±10.67[b]	NA	NA	NA	NA
Diplusodon burchellii	T0	63.89±17.44	81.39±6.24	82.78±8.62	52.78±15.31	61.11±20.03
	T1	100.00±0.00	100.00±0.00	44.44±29.40	100.00±0.00	66.67±33.33
	T3	NA	NA	NA	NA	NA
	T6	NA	NA	NA	NA	NA

	T9	NA	NA	NA	NA	NA
	T12	NA	NA	NA	NA	NA
Dyckia brasiliana	T0	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
	T1	100.00±0.00	NA	66.67±33.33	100.00±0.00	100.00±0.00
	T3	NA	NA	NA	NA	NA
	T6	NA	NA	NA	NA	NA
	T9	NA	NA	NA	NA	NA
	T12	NA	NA	NA	NA	NA
Stenodon suberosus	T0	95.83±4.17	100.00±0.00	100.00±0.00[a]	66.67±21.08	100.00±0.00
	T1	50.00±16.67	40.00±24.49	10.00±10.00[b]*	80.00±20.00	80.00±20.00
	T3	16.67±16.67	NA	NA	NA	NA
	T6	NA	NA	NA	NA	NA
	T9	NA	NA	NA	NA	NA
	T12	NA	NA	NA	NA	NA
Stomatanthes sp.	T0	33.33±21.08	83.33±16.67	50.00±22.36	83.33±16.67	66.67±21.08
	T1	NA	NA	NA	NA	NA
	T12	NA	NA	NA	NA	NA
Pseudotrimezia juncifolia	T0	88.29±5.47	85.81±5.44	81.25±16.38[a]	95.24±4.76	95.24±3.01
	T1	73.33±26.67	NA	0±0[b]*	NA	NA
	T3	NA	NA	NA	NA	NA
	T6	NA	NA	NA	NA	NA
	T9	NA	NA	NA	NA	NA
	T12	NA	NA	NA	NA	NA
Vellozia squamata	T0	98.33±1.67	100.00±0.00[a]	98.33±1.67	93.39±4.75	98.15±1.85
	T1	92.13±3.96	77.13±10.23[b]*	95.24±4.76	90.28±5.01	87.96±7.23
	T3	93.33±6.67	76.39±11.87[b]	73.02±20.27	84.13±9.66	71.67±17.40
	T6	89.81±5.45	NA	NA	NA	NA
	T9	NA	NA	NA	NA	NA
	T12	77.78±22.22	NA	66.67±33.33	NA	NA
Vellozia swallenii	T0	95.14±3.12[a]	96.06±2.50[a]	95.83±4.17	97.62±2.38	95.24±4.76
	T1	95.24±4.76[a]	66.67±33.33[ab]	65.28±1.39	66.67±33.33	95.24±4.76
	T3	0.00±0.00[b]	28.33±17.40[b]*	66.67±33.33*	44.44±11.11	56.67±23.33
	T6	86.90±8.73[a]	NA	NA	NA	NA
	T9	54.17±20.83[a]	NA	79.17±12.50	75.00±14.43	75.00±14.43
	T12	56.67±12.02[a]	50.00±9.62[b]	66.67±33.33	NA	NA

Table S3. Viability percentages (mean ± SE) for all species when exposed to the fire cues experiments according to time buried. Fire cues experiments: 100°C, 200°C for one minute, C=control (not exposed to high temperatures), CSW=smoke control, SW=smoke water. Time buried: T0= fresh seeds not buried, T1= 1 month, T3= 3 months, T6=6 months, T9=9 months and T12=12 months buried. NA= data not available due not availability of seeds. Lowercase letters indicate significant differences within the same control treatment over time. $P < 0.05$.

Species	Time buried	Fire cues experiments				
		C	100°C 1'	200°C 1'	CSW	SW
Banisteriopsis stellaris	T0	47.59±7.74^{a}	46.67±6.67	35.00±5.63	53.33±6.67	45.00±11.76
	T3	36.67±4.94^{a}	45.00±4.28	45.00±8.47	43.33±2.11	38.33±4.77
	T6	3.33±3.33^{b}	NA	0.00±0.00	3.33±3.33	3.33±3.33
	T9	NA	NA	NA	NA	NA
	T12	NA	NA	NA	NA	NA
Bidens gardneri	T0	48.33±7.49	40.00±3.65	1.67±1.67	33.33±10.54	78.79±4.63
	T3	65.00±10.57	58.89±9.84	43.33±9.89	62.41±6.08	80.00±5.77
	T6	NA	NA	NA	NA	NA
	T9	NA	NA	NA	NA	NA
	T12	NA	NA	NA	NA	NA
Sida linifolia	T0	31.67±4.01^{a}	26.67±5.58	23.33±9.19	36.67±7.15	25.00±5.00
	T3	36.67±4.94^{a}	51.67±8.72	36.67±11.74	45.00±8.06	45.00±8.47
	T6	79.49±9.25^{b}	87.18±12.82	64.10±12.82	87.18±2.56	82.05±2.56
	T9	76.67±6.67^{b}	73.33±8.82	70.00±20.82	63.33±3.33	70.00±5.77
	T12	63.89±7.35^{ab}	71.11±2.22	16.67±16.67	65.17±6.15	61.11±2.78
Solidago chilensis	T0	10.00±6.83^{a}	5.00±2.24	3.18±2.02	5.00±3.42	5.00±2.24
	T3	18.33±4.77^{bc}	15.00±3.42	13.33±4.22	31.67±4.77	40.00±7.30
	T6	20.00±5.77^{a}	20.00±5.77	8.33±3.07	30.00±5.16	48.33±7.03
	T9	12.50±4.79^{a}	10.00±0.00	10.00±4.08	10.00±4.08	30.00±10.00
	T12	45.10±9.86^{c}	NA	NA	55.10±6.80	59.85±3.58
Waltheria communis	T0	50.00±6.83^{a}	38.89±5.88	23.03±3.41	55.00±4.28	55.00±7.64
	T3	66.67±6.67^{ab}	60.00±4.47	45.00±10.57	68.33±6.01	56.67±7.15
	T6	81.86±5.09^{b}	85.78±6.75	75.78±6.67	81.78±4.81	73.33±6.91
	T9	92.22±4.84^{b}	89.44±4.55	89.72±4.09	95.00±5.00	97.50±2.50
	T12	75.00±8.33^{ab}	80.56±5.56	23.72±11.47	83.33±9.62	86.11±5.56
Bulbostylis paradoxa	T0	93.33±3.33^{a}	NA	80.00±5.16	86.67±7.15	68.33±7.92
	T1	87.58±2.89^{a}	90.00±0.00	73.33±3.33	NA	NA
	T6	52.50±6.29^{b}	NA	NA	NA	NA
	T12	34.00±2.45^{c}	NA	NA	NA	NA
Diplusodon burchellii	T0	38.33±3.07^{a}	38.33±3.07	21.67±6.54	26.67±8.43	20.00±5.77
	T1	16.67±3.33^{b}	23.33±6.67	16.67±8.82	13.33±3.33	16.67±8.82
	T3	NA	NA	NA	NA	NA
	T6	NA	NA	NA	NA	NA
	T9	NA	NA	NA	NA	NA
	T12	NA	NA	NA	NA	NA

Dyckia brasiliana	T0	68.33±5.43[a]	85.00±5.00	81.67±7.03	68.33±8.72	85.00±6.71
	T1	26.67±6.67[b]	NA	20.00±11.55	33.33±3.33	23.33±6.67
	T3	NA	NA	NA	NA	NA
	T6	NA	NA	NA	NA	NA
	T9	NA	NA	NA	NA	NA
	T12	NA	NA	NA	NA	NA
Stenodon suberosus	T0	18.33±5.43[a]	15.00±2.24	26.67±7.60	8.33±3.07	23.33±4.94
	T1	20.00±5.48[a]	6.00±4.00	6.00±4.00	22.00±7.35	14.00±5.10
	T3	3.33±2.11[b]	NA	NA	NA	NA
	T6	NA	NA	NA	NA	NA
	T9	NA	NA	NA	NA	NA
	T12	NA	NA	NA	NA	NA
Stomatanthes sp.	T0	10.00±8.16	8.33±1.67	10.00±5.16	8.33±1.67	11.67±4.77
	T1	NA	NA	NA	NA	NA
	T12	NA	NA	NA	NA	NA
Pseudotrimezia juncifolia	T0	60.00±7.30[a]	58.33±7.92	61.67±9.46	56.67±5.58	61.67±7.49
	T1	26.67±12.02[b]	NA	3.33±3.33	NA	NA
	T3	NA	NA	NA	NA	NA
	T6	NA	NA	NA	NA	NA
	T9	NA	NA	NA	NA	NA
	T12	NA	NA	NA	NA	NA
Vellozia squamata	T0	93.33±4.94[a]	86.67±4.94	93.33±4.94	81.67±4.01	96.67±2.11
	T1	69.70±8.02[a]	72.73±13.89	51.52±12.12	51.52±13.21	69.39±16.68
	T3	56.67±12.02[ab]	66.67±6.67	50.00±11.55	70.00±5.77	76.67±14.53
	T6	61.67±8.33[a]	NA	NA	NA	NA
	T9	NA	NA	NA	NA	NA
	T12	27.78±2.78[b]	NA	20.00±11.55	NA	NA
Vellozia swallenii	T0	78.33±6.01[a]	78.33±3.07	88.33±4.77	81.67±6.01	76.67±3.33
	T1	33.33±16.03[b]	42.42±13.21	62.42±5.39	18.18±10.50	51.52±8.02
	T3	23.33±3.33[b]	40.00±5.77	30.00±0.00	40.00±10.00	36.67±8.82
	T6	25.83±3.52[b]	NA	NA	NA	NA
	T9	25.00±2.89[b]	NA	32.50±6.29	30.00±4.08	32.50±7.50
	T12	46.67±3.33[b]	26.67±3.33	20.00±10.00	NA	NA

CAPÍTULO 3

Seeds of Fire: Unraveling the link between heat tolerance and seed traits in Cerrado species

Abstract

In fire-prone ecosystems, plants possess adaptive traits that enable survival and persistence, including seed and germination traits that influence their response to fire-related cues. However, Cerrado species do not appear to exhibit a consistent pattern of response to fire (e.g., increased germination after smoke exposure or dormancy break). Instead, studies indicate that most seeds from this tropical savanna are fire tolerant. In this study, we investigated how seed traits (seed mass and shape), vegetation type, and growth form influence seed tolerance to high temperatures. We compiled viability data for 158 Cerrado species from various sources, including published studies and experimental databases on heat shock experiments. Heat shock experiments included temperatures ranging from 80°C to 200°C with exposure times of 1 to 5 minutes. To account for variations in temperature and exposure time across experiments, we calculated a heat index (H) to represent the cumulative heat dose experienced by seeds. Our findings demonstrate that the heat index is a reliable proxy for assessing the cumulative heat dose experienced by seeds. While increased heat index and temperature resulted in reduced seed viability, 63% of the species retained over 50% viability even at the highest heat levels. Seed traits significantly influenced fire tolerance, with dormant, smaller, and more elongated seeds showing greater heat resistance. Vegetation type and growth form also played critical roles: forest species exhibited higher resistance to the heat index, whereas savanna species showed better resilience to elevated temperatures. These findings highlight the importance of seed traits and habitat-specific factors in shaping seed tolerance to heat. They offer valuable insights into the persistence and regeneration strategies of plants in fire-prone savanna ecosystems.

Keywords: seed trait, Cerrado, fire tolerance, heat shock, resistance, tropical savanna

Introduction

Fire is a major disturbance in many ecosystems worldwide, significantly influencing organisms and populations by altering plant community composition and dynamics (Whelan

1995; Bond & van Wilgen 1996; Bowman *et al.* 2009; Keeley *et al.* 2011). It can impact nutrient cycling, plant interactions, and responses, as well as vegetation distribution and community structure (Bond & van Wilgen 1996; Keeley *et al.* 2011). Plants in fire-prone ecosystems have physical, morphological, and physiological traits that enable them to survive, reproduce, and persist under different fire regimes (Bond & van Wilgen 1996; Keeley *et al.* 2011; Simon & Pennington 2012). Traits related to post-fire plant persistence include thick bark (Gignoux *et al.* 1997, Chiminazzo et al. 2023), protected buds, and meristems, which confer resprouting capacity to individuals (Clarke *et al.* 2013; Pausas *et al.* 2018, Chiminazzo et al. 2021).

Additionally, plants in flammable ecosystems display adapted seed and germination traits, affecting regeneration processes. These traits include enhanced germination triggered by fire cues (Auld & Ooi 2009; Moreira *et al.* 2010; Soós *et al.* 2019; Zirondi, Silveira, *et al.* 2019), the breaking of physical dormancy through heat shocks (Moreira & Pausas 2012; Ooi *et al.* 2014), and seed tolerance to high temperatures (Ribeiro *et al.* 2013; Fichino *et al.* 2016; Tangney *et al.* 2019; Zirondi, de Pinho José, *et al.* 2019). Seed tolerance to heat is critical for plant regeneration in fire-prone ecosystems, as seeds are often directly exposed to high temperatures during fires. Among others, traits such as seed size (Hanley *et al.* 2003; Ribeiro *et al.* 2015), shape (Gomez-Gonzalez *et al.* 2016), seed coat thickness (Bell & Williams 1998), dormancy type (Ramos *et al.* 2016), and water content (Tangney *et al.* 2019) may influence a seed's ability to survive and germinate after exposure to heat. For example, seed shape will affect seed heat tolerance as rounded seeds might experience less heat transfer at their surface (Gomez-Gonzalez *et al.* 2016). Similarly, seeds with lower water content are less likely to experience lethal damage from heat-induced water vaporization (Ruprecht *et al.* 2016; Tangney *et al.* 2019). Dormancy will also play a bigger part in fire tolerance since dormant seeds demonstrate higher survival rates in extreme heat conditions compared to non-dormant seeds, suggesting that dormancy is a key trait for fire tolerance (Ramos *et al.* 2016; Daibes *et al.* 2019).

Finally, seed size has been widely discussed as a factor influencing heat tolerance, with smaller seeds often considered less heat-tolerant (Escudero *et al.* 2000; Ribeiro *et al.* 2015; Calvo *et al.* 2016; Daibes *et al.* 2019). Larger seeds are often associated with better survival in disturbed

ecosystems due to their ability to produce vigorous seedlings (Moles *et al.* 2004; Moles & Westoby 2006; Lahoreau *et al.* 2006). Daibes et al. (2019) further observed that tree species seed mortality decreased with larger seed mass, especially at 200°C. However, this relationship is not universal with studies demonstrating that small-sized seeds can also exhibit heat tolerance (Hanley et al., 2003; Overbeck et al., 2006; Oliveira et al., 2018; Zirondi et al., 2019). These conflicting findings underscore that heat tolerance is influenced by additional factors, such as provenance, phylogenetic lineage, and microhabitat conditions (Bradstock *et al.* 1994; Delgado *et al.* 2008; Calvo *et al.* 2016; Zirondi, Silveira, *et al.* 2019) and that the interplay between these traits and fire conditions may affect plant community assembly in fire-prone ecosystems. Thus, understanding how these traits relate to seed tolerance to high temperatures is essential for explaining species' fire adaptations and their ability to persist in fire-prone ecosystems like the Cerrado.

While some seed traits may enhance fire tolerance, it is essential to consider that not all species exhibit the same adaptive responses, leading to varied community dynamics and potential vulnerabilities in changing fire regimes. Especially, in present day scenario, where fire regime is being altered by climate change and human activities (Pausas *et al.* 2004; Pivello 2011; Archibald *et al.* 2013), being able to understand how fire affects plant responses and to understand reproductive traits variability facing these changes may be a great tool in many areas of vegetation science. The understanding about seed and germination traits will not only give information about plant interactions, community functions and ecosystems processes in response to fire but also on genetic changes and adaptations, responses to climate change, and could also be used in restoration ecology (Jiménez-Alfaro *et al.* 2016; Saatkamp *et al.* 2019), which is the main challenges when we focus on herbaceous species from savanna ecosystems.

In the Cerrado, a recognized biodiversity hotspot (Myers *et al.* 2000), fire is present for at least 4 mya. (Simon *et al.* 2009). This tropical savanna is characterized by having fast, surface-level, and frequent fires (Coutinho 1990; Rodrigues *et al.* 2021). Unlike other fire-prone ecosystems, Cerrado plants do not exhibit a consistent pattern of seed and germination responses to fire-related cues (Daibes *et al.* 2022). Instead, the most prominent response observed is a high

tolerance of seeds to heat exposure, however, most studies investigating the factors driving this heat tolerance have focused on woody species or taxa within a single family like Fabaceae (Ribeiro *et al.* 2015, 2021; Fichino *et al.* 2016; Ramos *et al.* 2016; Daibes *et al.* 2019; Zirondi, Silveira, *et al.* 2019; Zupo *et al.* 2021). Broader research is needed to evaluate heat tolerance at the community level, particularly incorporating species from the herbaceous layer, to gain a more comprehensive understanding of these traits and their ecological drivers.

Therefore, in this study, we aimed to determine whether there is a relationship between seed tolerance to high temperatures and seed traits in Cerrado species. Our hypothesis is that seeds with higher tolerance to high temperatures will exhibit smaller, more rounded shapes and lower water content. Additionally, we expect stronger relationship between seed tolerance to high temperatures and seed traits in species from open ecosystems of the Cerrado, where fire occurs more frequently. By addressing these questions, our study provides insights into the functional ecology of Cerrado plants and contributes to a broader understanding of fire adaptations in tropical savannas.

Material and Methods

Study design and data collection

To investigate the seed tolerance to high temperatures in Cerrado species, we analyzed seed and viability data of Cerrado species available on the *Cerrado Seed Trait Database* (including published data) from the Lab of Vegetation Ecology (LEVeg), plus data from collaborators and published studies in the literature. The final database comprised data from 158 Cerrado species sampled from different regions of Brazil, including Southeastern, Central, and Northern regions (Fig. 1). To assess the seed tolerance to high temperatures we used seed viability in the controls and the heat shock treatments which ranged from 80°C to 200°C with varying exposure times (from 1 to 5 minutes). Due to the variation in temperatures and exposure time in all experiments we calculated the heat index (H) to estimate heat dose received by the seeds in each treatment

(see Paula & Pausas 2008). The heat index is calculated by multiplying the temperature (in degrees Celsius) by the natural logarithm of the exposure time plus one (in minutes). The heat index (H) ranged from 17.3 to 358.4 units. We examined the relationship between seed tolerance and key traits, including seed mass (mg), seed shape (width:length ratio), and dormancy type. Water content was excluded from the analysis due to the low amount of data available for this trait. Additionally, we analyzed the relationship between seed tolerance and vegetation type and species growth form always accounting for phylogeny.

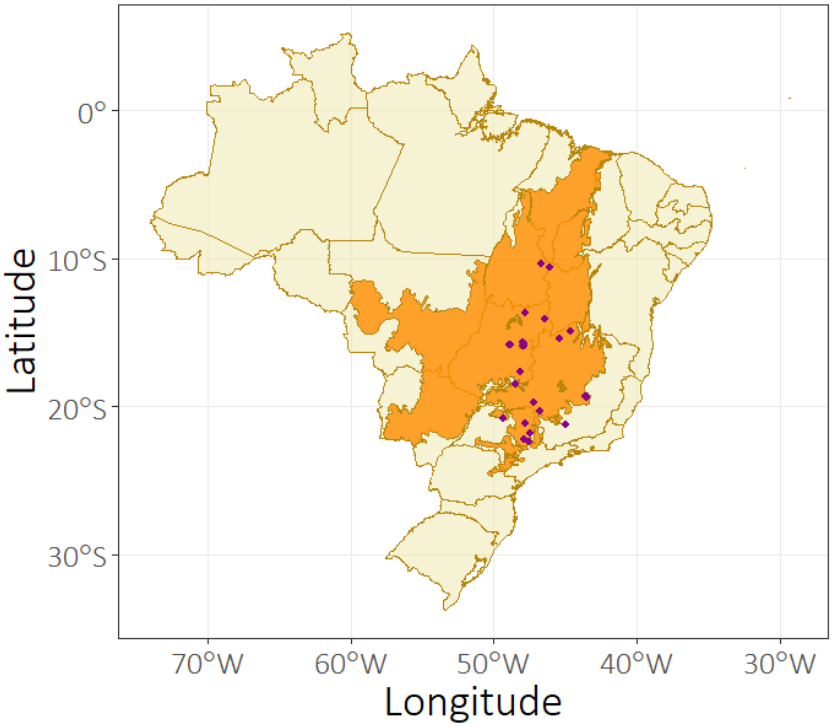

Figure 1. Map displaying the boundaries of the Cerrado biome (dark orange) and the locations of seed collection sites (purple dots).

Data treatment

As the data originated from multiple sources, adjustments were necessary before statistical analyses. When the number of viable seeds was not explicitly reported, we used the number of germinated seeds as the viable seed's values. Furthermore, if the viability proportion

of the Control (C) treatment was lower than 0.75, we standardized the total number of seeds based on the control viability. In addition, Control temperature was set as 25°C for the comparison purpose.

Phylogeny tree construction

To account for phylogenetic relationships among the 158 studied species, we constructed a phylogenetic tree using the *U.PhyloMaker* package in R (Jin & Qian 2022). The tree was based on the updated GBOTB phylogeny for seed plants (Smith & Brown 2018), as implemented in the package using *megatree*. First it binds together our species to the *megatree*, with non-matching species excluded. When a species was not present it was attached to the closest relative or genus node. The resulting tree was used in subsequent statistical models.

Statistical analysis

The relationship between seed tolerance (viability) at high temperatures and seed traits (seed mass, seed shape, dormancy type), growth form (woody and non-woody) and vegetation type (forest, rocky outcrops, savanna a wet grasslands) were analyzed using MCMCglmm Generalized Linear Mixed Models (GLMMs) with Markov Chain Monte Carlo (MCMC) techniques, as implemented in the *MCMCglmm* package (Hadfield 2010). Random factors included phylogeny, species, and site. All statistical analyses were conducted in the R software (R Core Team 2016).

Results

Heat index, temperature and exposition time effects on seed viability

A significant decline in seed viability as the heat index increases could be observed, with a pronounced drop occurring only after 200 units ($p<0.001$, Fig. 2a). We found that 63% of the species still had viability higher than 50% in the *Heat index* values from 132 units (equivalent to

120°C for 2') up to the highest values. A similar pattern was observed with heat shock temperatures, even when differences in exposure time were not considered, with a pronounced decrease in viability occurring from ~110°C (Fig. 2b). Although less pronounced, exposure time also negatively affected seed viability (Fig. 2c).

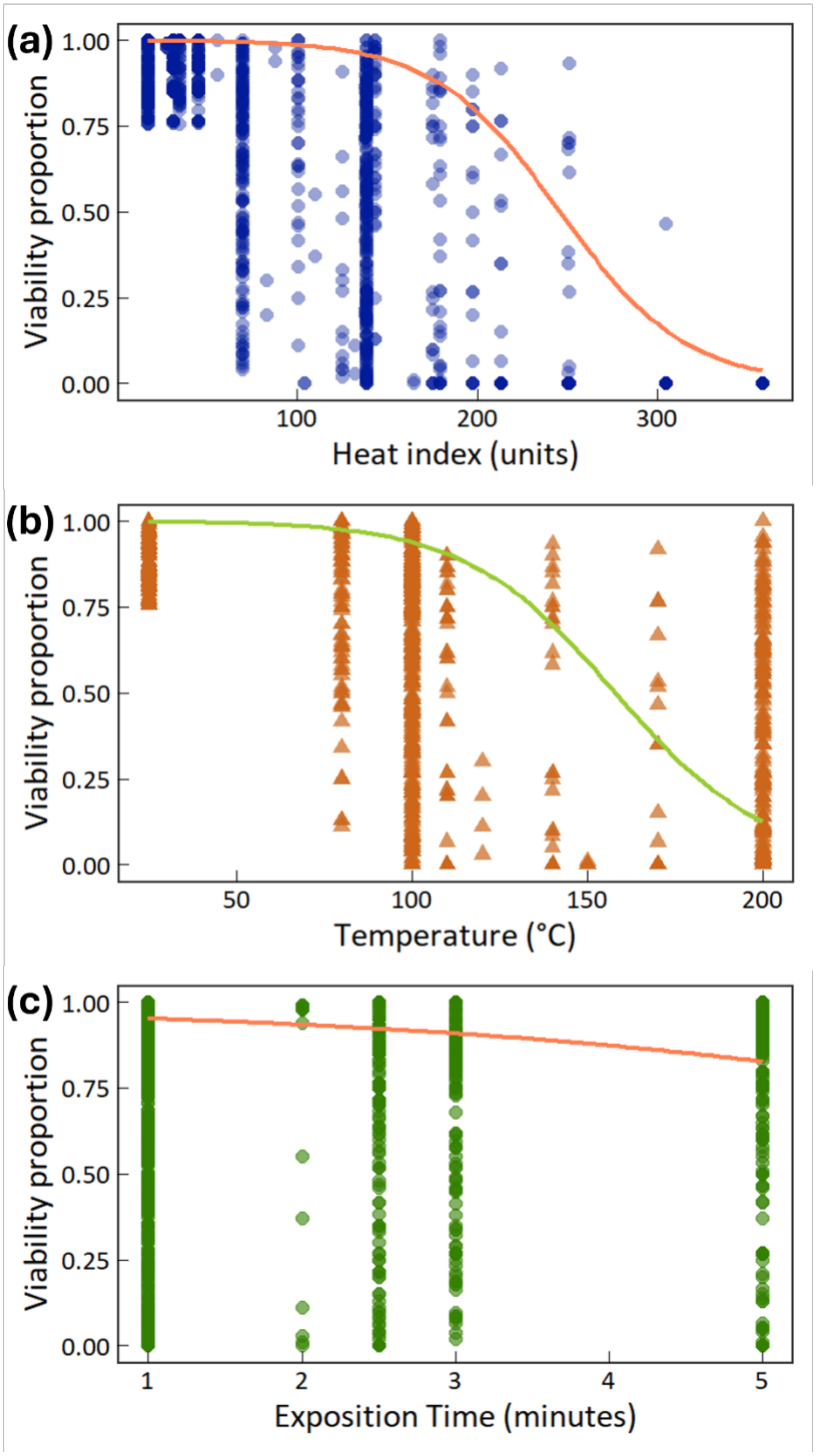

Figure 2. Seed viability proportion according to (a) the heat index (units), (b) the treatments temperatures and (c) exposition time in minutes. Curve showing the decrease in viability of Cerrado seeds of 158 species after exposure to increasing heat index and temperatures that represent the high temperatures during fire. The line represents the predicted values based on a MCMC Generalized Linear Mixed Model (MCMCglmm).

To further investigate the influence of exposure time we checked the interaction between temperature and time and then we analyzed the impact of different exposure durations at various temperatures. Results showed significant interaction between the two fixed factors, temperature and time (p=0.0006) and demonstrated that prolonged exposure led to a gradual decrease in viability at higher temperatures, starting at 110°C. In contrast, at lower temperatures (e.g., 80°C and 100°C), exposure time did not exhibit a clear pattern of influence on viability, with greater variability in the data (p<0.05, Fig. 3).

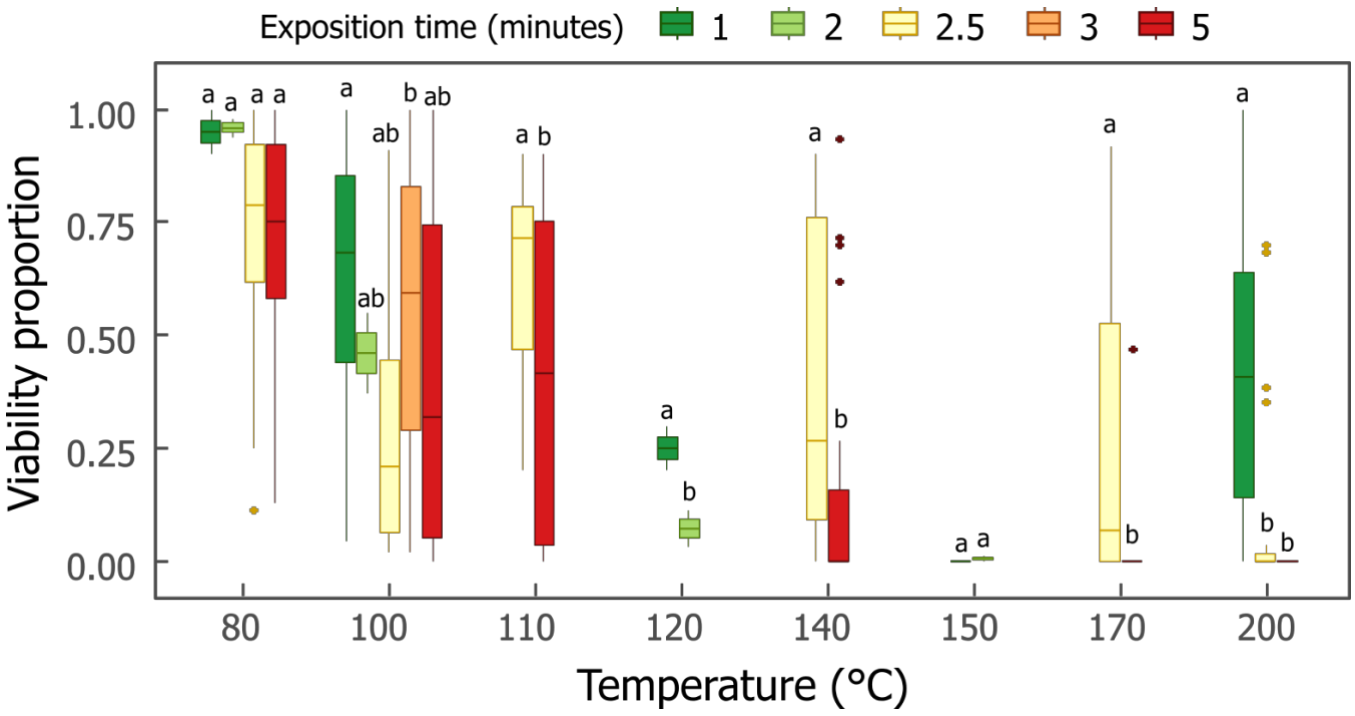

Figure 3. Proportion of seed viability of 158 Cerrado species at different exposure times (in minutes) for each temperature. Significant statistical differences between exposure times within each heat treatment are indicated by lowercase letters; different letters denote significant differences (p < 0.05).

Factors affecting seed tolerance to fire

Our analysis using the heat index (H) as a measure of seed tolerance to high temperatures revealed that smaller seeds with a more elongated shape, as well as seeds from woody species,

exhibited greater resistance to elevated heat index levels (Table 1). On a broader scale, both dormant (D) and non-dormant (ND) seeds were negatively affected by higher heat index values, however dormant seeds were more resistant to higher values of H (Table 1). When looking at the subcategories of dormant seeds we observed that seeds with physical dormancy (PY) were the ones with higher heat tolerance, followed by seeds with physiological dormancy (PD) (Table 1). Moreover, when considering vegetation type, species from forests displayed the highest resistance, followed by those from savanna, rocky outcrops and wet grasslands (Table 1). In addition, regarding the random effects, the low value of posterior mean indicates that phylogeny has little effect explaining data variation (Table S2).

Regarding only the temperature effect on viability, the results mirrored the patterns found for the heat index, where woody species, and species with smaller, dormant and more elongated seeds demonstrating greater resistance to high temperatures (Table 1). Interestingly, savanna species showed better resistance to elevated temperatures, contrasting with the findings for the heat index, where forest ones had a higher tolerance to heat (Table 1).

Table 1. Summary of phylogenetic mixed models with Bayesian estimation (MCMCglmms) to identify Seed traits and factors related to seed tolerance (viability) to high fire temperatures. Model coefficients (posterior mean), lower and upper 95% credible intervals (95% CI) of parameters, with significant pMCMC values. The results indicate a decline in seed viability as heat index and temperatures increase. For Dormancy categories: D=dormant, ND=nondormant; IN=intermediary, MPD= morphophysiological, PD= Physiological and PY= Physical dormancy. Random effects used (phylogeny, species and site) explained equally the variation in the data. Asterisks indicate significant effects.

Model	Posterior mean	95% CI	pMCMC
Heat Index	-2.947	(-3.312;-2.565)	0.001*
Heat Index:Seed mass	-0.447	(-0.612;-0.289)	0.001*
Heat Index:Dormancy simplified D	-2.35	(-2.916;-1.747)	0.001*
Heat Index:Dormancy simplified ND	-3.324	(-3.774;-2.824)	0.001*
Heat Index:Dormancy IN	-3.544	(-5.246;-1.718)	0.001*
Heat Index:Dormancy MPD	-0.801	(-5.146;3.54)	0.732
Heat Index:Dormancy ND	-3.319	(-3.777;-2.815)	0.001*
Heat Index:Dormancy PD	-2.313	(-3.558;-1.128)	0.001*
Heat Index:Dormancy PY	-2.21	(-2.954;-1.491)	0.001*
Heat Index:Seed shape	-3.488	(-3.971;-3.054)	0.001*
Heat Index:Growth form group Non_woody	-3.027	(-3.569;-2.494)	0.001*
Heat Index:Growth form group Woody	-2.873	(-3.37;-2.32)	0.001*
Heat Index:Vegetation type forest	-2.517	(-3.551;-1.556)	0.001*

Heat Index:Vegetation type rocky outcrops	-3.454	(-4.656;-2.405)	0.001*
Heat Index:Vegetation type savanna	-2.9	(-3.338;-2.47)	0.001*
Heat Index:Vegetation type wet grassland	-4.039	(-5.559;-2.215)	0.001*
Temperature	-2.432	(-2.781;-2.066)	0.001*
Temperature:Seed mass	-0.353	(-0.504;-0.208)	0.001*
Temperature:Dormancy simplified D	-1.957	(-2.459;-1.327)	0.001*
Temperature:Dormancy simplified ND	-2.724	(-3.182;-2.321)	0.001*
Temperature:Dormancy IN	-2.146	(-3.733;-0.59)	0.008*
Temperature:Dormancy MPD	-1.123	(-5.011;3.172)	0.588
Temperature:Dormancy ND	-2.729	(-3.156;-2.277)	0.001*
Temperature:Dormancy PD	-3.362	(-4.601;-2.146)	0.001*
Temperature:Dormancy PY	-1.557	(-2.21;-0.852)	0.001*
Temperature:Seed shape	-2.87	(-3.304;-2.431)	0.001*
Temperature:Growth form group Non_woody	-2.596	(-3.088;-2.044)	0.001*
Temperature:Growth form group Woody	-2.284	(-2.747;-1.756)	0.001*
Temperature:Vegetation type forest	-2.559	(-3.511;-1.669)	0.001*
Temperature:Vegetation type rocky outcrops	-3.666	(-4.652;-2.678)	0.001*
Temperature:Vegetation type savanna	-2.143	(-2.537;-1.749)	0.001*
Temperature:Vegetation type wet grassland	-5.655	(-7.617;-3.49)	0.001*

Discussion

Overall, our results showed that heat index is a good proxy to evaluate the heat dose seeds are exposed to and captures the combined effects of temperature and exposure time on seed viability. Nonetheless, our findings indicate that temperature is a dominant factor influencing seed viability, outweighing the importance of exposure time at lower temperatures but interacting at higher temperatures. These findings highlight the role of both temperature and exposure time in determining seed viability under heat, with significant effects observed at higher temperatures and prolonged exposure durations. In addition, we evidenced that not only the seed traits (shape, mass and dormancy) but also habitat and growth form are drivers of seed tolerance to heat for Cerrado species at a broader level. However, we did not investigate this within groups (families) and this result can differ on a minor scale.

Results showed that many Cerrado species have heat tolerant seeds and although a decline in seed viability is observed as the heat index/ temperature increases, 63% of the species

still had more than 50% viability at the highest values of heat. This aligns with previous studies emphasizing the impact of high temperatures on seed survival in fire-prone ecosystems, and heat tolerance as a major fire-related feature in Cerrado seeds (Ribeiro *et al.* 2015, 2021; Fichino *et al.* 2016; Daibes *et al.* 2019; Zirondi, Silveira, *et al.* 2019; Zupo *et al.* 2021). Such heat tolerance is essential for species persistence in fire-prone ecosystems, ensuring that a portion of the population survives adverse environmental conditions, thereby enhancing opportunities for future establishment and regeneration.

As for what factors influence seed tolerance to heat, seed traits emerged as significant drivers of heat tolerance. Contrary to the general hypothesis that smaller seeds are less heat-tolerant (Escudero *et al.* 2000; Calvo *et al.* 2016), and to previous findings for woody species (Ribeiro *et al.* 2015; Daibes *et al.* 2019), our results showed that smaller seeds exhibited greater resistance to high temperatures. Notably, earlier analyses focused exclusively on Fabaceae species found that larger seeds were more heat-tolerant – a pattern potentially explained by the high prevalence of physical dormancy (PY) in those taxa (Ribeiro et al. 2015; Daibes et al. 2019). However, our expanded analysis including species from various families indicates that the relationship between seed size and heat tolerance is more complex and influenced by ecological differences among taxa. Our findings align with those of Hanley et al. (2003) and Overbeck et al. (2006), who reported similar patterns in small-seeded species. Some authors have proposed that the fire tolerance of smaller seeds is more closely linked to their vertical positioning in the soil (Bond *et al.* 1999; Hanley *et al.* 2003; Overbeck *et al.* 2006). Larger seeds, which are often buried deeper, are naturally insulated from extreme temperatures and can still germinate after a fire event. In contrast, smaller seeds typically remain near the soil surface, where they are exposed to higher temperatures and must exhibit greater tolerance to survive and germinate under such conditions (Hanley *et al.* 2003; Overbeck *et al.* 2006).

In terms of shape, more elongated seeds demonstrated higher heat tolerance, contrary to our hypothesis and other studies where rounder seeds were associated with greater resistance to fire (Ruprecht *et al.* 2015; Gomez-Gonzalez *et al.* 2016). Gomez-Gonzalez *et al.* (2011) found the same pattern within-population, with less rounded seeds being selected by fire, opposing the

regional pattern. This suggests that seed shape may interact with other traits, such as seed coat thickness or dormancy, as well as with abiotic factors (e.g. soil texture and fire intensity) (Gomez-Gonzalez *et al.* 2011) to influence heat tolerance. Further research is needed to clarify these interactions and to test whether these patterns persist across different plant families or within specific ecological contexts.

Dormancy also played a critical role in seed resistance. Dormant seeds were generally more heat-tolerant than non-dormant seeds corroborating our hypothesis. Seeds exhibiting physical dormancy (PY) showed the highest tolerance followed by species with physiological dormancy (PD). Physical dormancy provides a mechanical barrier against heat damage while PD seeds may have a high amount of heat shock protein to provide protection against heat shock (Kaur *et al.* 2015; Ramos *et al.* 2016; Daibes *et al.* 2017, 2018, 2019). This trait is particularly advantageous in fire-prone ecosystems, where seeds must withstand high temperatures to ensure post-fire recruitment.

Finally, vegetation type and growth form further influenced seed tolerance to heat. Interestingly, species from forest habitats, particularly woody species, displayed the highest resistance to heat index values, while savanna species performed better under high-temperature treatments. This pattern could suggest that seeds from forest species may be adapted to endure less frequent but more intense heat events, whereas savanna species are better adapted to cope with the frequent, lower-severity fires characteristic of open ecosystems. This response in heat tolerance strategies could be explained by the evolutionary history of Cerrado species, where woody species are thought to have evolved from fire-free forest lineages in adjacent regions (Simon et al. 2009). During this transition, forest species may have played a significant role in facilitating the establishment of savanna-adapted species by diversifying into contrasting habitats (Hoffmann 2000; Daibes et al. 2019).

However, one potential bias in our study is that all the forest species included are woody, and a high proportion of them exhibit physical dormancy (PY). This limited representation may skew our interpretation of seed heat tolerance across vegetation types, as woody species with PY are inherently more adapted to withstand high temperatures due to their thick seed coats and

protective mechanisms (Moreira & Pausas 2012; Ramos et al. 2016; Daibes et al. 2019). Consequently, the higher heat tolerance observed in forest species may not reflect a general pattern among all forest taxa, but rather the specific characteristics of the sampled species. Future research should aim to include a broader range of forest species, such as herbaceous taxa and those with other dormancy strategies (e.g., physiological or morphological dormancy), to more accurately assess the influence of vegetation type on seed heat tolerance. This would provide a more comprehensive understanding of how different life forms and dormancy strategies contribute to fire resilience in forest ecosystems.

Nonetheless, although there is a potential bias toward woody species, it is important to note that these species occur within the Cerrado domain as part of savanna forests. Notably, even though adult individuals in these savanna forests tend to be more sensitive to fire (Hoffmann 2000), their seeds exhibit high resistance to fire. This finding indicates that for this forest species the bottleneck for the establishment is not at the germination stage—they are capable of germinating successfully in savanna environments. Instead, the transition from seed to mature individual might depend on other factors during later life stages, such as post-germination growth, and tolerance to additional environmental stresses (Hoffmann 2000).

Overall, these findings underscore the multifactorial nature of seed heat tolerance, with seed traits (mass, shape, and dormancy) interacting with habitat-specific factors to shape species' responses to high temperatures. Understanding the drivers of seed heat tolerance has important implications for fire ecology and restoration in the Cerrado. The variability in seed responses highlights the need for habitat-specific management strategies that account for both seed traits and environmental conditions. Furthermore, these findings can inform seed selection for restoration projects, particularly in areas subject to altered fire regimes due to climate change and human activities. By addressing the relationships between seed traits, habitat, and fire, our study contributes to a broader understanding of plant responses to fire in tropical savannas and emphasizes the importance of further research within taxonomic groups and diverse environmental contexts to better predict community dynamics and ecosystem resilience.

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Suplementary material

Table S1. Summary of phylogenetic mixed models with Bayesian estimation (MCMCglmms) to identify Seed traits and factors related to seed tolerance (viability) to heat index (H) and high fire temperatures. Model coefficients (posterior mean), lower and upper 95% credible intervals (95% CI) of parameters, with significant pMCMC values. The results indicate a decline in seed viability as heat index and temperatures increase. For Dormancy categories: D=dormant, ND=nondormant; IN=intermediary, MPD= morphophysiological, PD= Physiological and PY= Physical dormancy. Random effects used (phylogeny, species and site) explained equally the variation in the data.

	Fixed effects			Random effects		
Models	**Posterior mean**	**95% CI**	**pMCMC**	**Phylogeny**	**Species**	**Site**
Heat Index (H)						
(Intercept)	2.135	(1.51;2.72)	0.0006	0.381(0;1.094)	0.542(0;1.046)	0.375(0.001;0.935)
scale(heat_index)	-2.947	(-3.31;-2.56)	0.0006	0.381(0;1.094)	0.542(0;1.046)	0.375(0.001;0.935)
(Intercept)	2.308	(1.71;2.88)	0.0006	0.386(0;1.136)	0.473(0;1.045)	0.308(0;0.812)
scale(heat_index):log(mass_mean)	-0.447	(-0.61;-0.29)	0.0006	0.386(0;1.136)	0.473(0;1.045)	0.308(0;0.812)
(Intercept)	2.122	(1.57;2.74)	0.0006	0.386(0;1.098)	0.514(0;1.055)	0.362(0;0.894)
scale(heat_index):dormancy_simpD	-2.350	(-2.92;-1.75)	0.0006	0.386(0;1.098)	0.514(0;1.055)	0.362(0;0.894)
scale(heat_index):dormancy_simpND	-3.324	(-3.77;-2.82)	0.0006	0.386(0;1.098)	0.514(0;1.055)	0.362(0;0.894)
(Intercept)	2.123	(1.57;2.78)	0.0006	0.391(0;1.098)	0.506(0;1.052)	0.363(0;0.872)
scale(heat_index):dormancy_typeIN	-3.544	(-5.25;-1.72)	0.0006	0.391(0;1.098)	0.506(0;1.052)	0.363(0;0.872)
scale(heat_index):dormancy_typeMPD	-0.801	(-5.15;3.54)	0.7322	0.391(0;1.098)	0.506(0;1.052)	0.363(0;0.872)
scale(heat_index):dormancy_typeND	-3.319	(-3.78;-2.81)	0.0006	0.391(0;1.098)	0.506(0;1.052)	0.363(0;0.872)
scale(heat_index):dormancy_typePD	-2.313	(-3.56;-1.13)	0.0006	0.391(0;1.098)	0.506(0;1.052)	0.363(0;0.872)
scale(heat_index):dormancy_typePY	-2.210	(-2.95;-1.49)	0.0006	0.391(0;1.098)	0.506(0;1.052)	0.363(0;0.872)
(Intercept)	2.137	(1.55;2.72)	0.0006	0.361(0;1.057)	0.506(0;1.017)	0.369(0;0.939)
scale(heat_index):sqrt(shape_mean)	-3.488	(-3.97;-3.05)	0.0006	0.361(0;1.057)	0.506(0;1.017)	0.369(0;0.939)
(Intercept)	2.125	(1.56;2.69)	0.0006	0.368(0;1.079)	0.53(0;1.033)	0.365(0;0.908)
scale(heat_index):form_binNon_woody	-3.027	(-3.57;-2.49)	0.0006	0.368(0;1.079)	0.53(0;1.033)	0.365(0;0.908)
scale(heat_index):form_binWoody	-2.873	(-3.37;-2.32)	0.0006	0.368(0;1.079)	0.53(0;1.033)	0.365(0;0.908)
(Intercept)	2.141	(1.59;2.81)	0.0006	0.374(0;1.087)	0.541(0;1.059)	0.399(0.001;0.957)

scale(heat_index):veg_typeforest	-2.517	(-3.55;-1.56)	0.0006	0.374(0;1.087)	0.541(0;1.059)	0.399(0.001;0.957)
scale(heat_index):veg_typerocky outcrops	-3.454	(-4.66;-2.4)	0.0006	0.374(0;1.087)	0.541(0;1.059)	0.399(0.001;0.957)
scale(heat_index):veg_typesavanna	-2.900	(-3.34;-2.47)	0.0006	0.374(0;1.087)	0.541(0;1.059)	0.399(0.001;0.957)
scale(heat_index):veg_typewet grassland	-4.039	(-5.56;-2.21)	0.0006	0.374(0;1.087)	0.541(0;1.059)	0.399(0.001;0.957)
Temperature						
(Intercept)	2.166	(1.72;2.69)	0.0006	0.22(0;0.725)	0.358(0;0.824)	0.217(0;0.572)
scale(treat_temp)	-2.432	(-2.78;-2.07)	0.0006	0.22(0;0.725)	0.358(0;0.824)	0.217(0;0.572)
(Intercept)	2.379	(1.87;2.87)	0.0006	0.186(0;0.652)	0.348(0;0.823)	0.175(0;0.498)
scale(treat_temp):log(mass_mean)	-0.353	(-0.5;-0.21)	0.0006	0.186(0;0.652)	0.348(0;0.823)	0.175(0;0.498)
(Intercept)	2.157	(1.71;2.63)	0.0006	0.192(0;0.648)	0.376(0;0.804)	0.221(0;0.562)
scale(treat_temp):dormancy_simpD	-1.957	(-2.46;-1.33)	0.0006	0.192(0;0.648)	0.376(0;0.804)	0.221(0;0.562)
scale(treat_temp):dormancy_simpND	-2.724	(-3.18;-2.32)	0.0006	0.192(0;0.648)	0.376(0;0.804)	0.221(0;0.562)
(Intercept)	2.159	(1.65;2.63)	0.0006	0.232(0;0.753)	0.366(0;0.797)	0.235(0;0.611)
scale(treat_temp):dormancy_typeIN	-2.146	(-3.73;-0.59)	0.0078	0.232(0;0.753)	0.366(0;0.797)	0.235(0;0.611)
scale(treat_temp):dormancy_typeMPD	-1.123	(-5.01;3.17)	0.5878	0.232(0;0.753)	0.366(0;0.797)	0.235(0;0.611)
scale(treat_temp):dormancy_typeND	-2.729	(-3.16;-2.28)	0.0006	0.232(0;0.753)	0.366(0;0.797)	0.235(0;0.611)
scale(treat_temp):dormancy_typePD	-3.362	(-4.6;-2.15)	0.0006	0.232(0;0.753)	0.366(0;0.797)	0.235(0;0.611)
scale(treat_temp):dormancy_typePY	-1.557	(-2.21;-0.85)	0.0006	0.232(0;0.753)	0.366(0;0.797)	0.235(0;0.611)
(Intercept)	2.166	(1.75;2.64)	0.0006	0.193(0;0.627)	0.362(0;0.806)	0.208(0;0.564)
scale(treat_temp):sqrt(shape_mean)	-2.870	(-3.3;-2.43)	0.0006	0.193(0;0.627)	0.362(0;0.806)	0.208(0;0.564)
(Intercept)	2.159	(1.65;2.58)	0.0006	0.209(0;0.728)	0.379(0;0.801)	0.219(0;0.592)
scale(treat_temp):form_binNon_woody	-2.596	(-3.09;-2.04)	0.0006	0.209(0;0.728)	0.379(0;0.801)	0.219(0;0.592)
scale(treat_temp):form_binWoody	-2.284	(-2.75;-1.76)	0.0006	0.209(0;0.728)	0.379(0;0.801)	0.219(0;0.592)
(Intercept)	2.128	(1.61;2.62)	0.0006	0.246(0;0.785)	0.396(0;0.838)	0.272(0.001;0.678)
scale(treat_temp):veg_typeforest	-2.559	(-3.51;-1.67)	0.0006	0.246(0;0.785)	0.396(0;0.838)	0.272(0.001;0.678)
scale(treat_temp):veg_typerocky outcrops	-3.666	(-4.65;-2.68)	0.0006	0.246(0;0.785)	0.396(0;0.838)	0.272(0.001;0.678)
scale(treat_temp):veg_typesavanna	-2.143	(-2.54;-1.75)	0.0006	0.246(0;0.785)	0.396(0;0.838)	0.272(0.001;0.678)
scale(treat_temp):veg_typewet grassland	-5.655	(-7.62;-3.49)	0.0006	0.246(0;0.785)	0.396(0;0.838)	0.272(0.001;0.678)

CONSIDERAÇÕES FINAIS

No geral, o estudo forneceu evidências da importância dos atributos para se avaliar as respostas das plantas ao fogo no Cerrado. Os resultados, que podem ajudar a preencher as lacunas de conhecimento ainda existentes nessa savana tropical, incluem: i) a eficácia das estratégias de sobrevivência e regeneração das plantas do Cerrado, que apresentam poucos trade-offs na alocação de recursos, e como essas relações são influenciadas pelo regime de fogo; ii) a influência do ambiente, do tempo no solo e da época de dispersão como fatores cruciais que afetam os atributos germinativos e as respostas das sementes à exposição a altas temperaturas e à fumaça e iii) a identificação de atributos de sementes, em combinação com outros fatores, como determinantes da tolerância das sementes a altas temperaturas, destacando a relação espécie-específica e, além disso, condição-específica na adaptação dos atributos reprodutivos ao fogo.

Sabe-se que a maioria das espécies do Cerrado são rebrotadoras, como parte da estratégia de persistir nesse ambiente de constante distúrbio (Zupo *et al.* 2021; Chiminazzo *et al.* 2023). Portanto as espécies precisam sempre alocar recursos para a formação dos novos ramos (Bazzaz & Grace 1997) após o distúrbio. No entanto, esses recursos também são necessários na formação de frutos e sementes (Reekie & Bazzaz 2005) os quais continuam a ser produzidos por essas espécies. Apesar da germinação não ser a principal estratégia, ainda é de grande importância para eventualmente garantir a colonização de novas áreas (Zupo *et al.* 2021) e variabilidade genética da população. Ademais, estudos recentes demonstraram que a exclusão do fogo, mesmo que por curtos períodos, afeta os atributos de sementes e a germinação quando comparados a áreas recém-queimadas (Zirondi *et al.* 2024). Essas evidências levantam questões importantes: o uso contínuo de recursos para o rebrote pode levar a trade-offs entre atributos vegetativos e reprodutivos? E, em áreas de alta frequência de fogo, onde os distúrbios são mais recorrentes, a relação entre esses atributos seria afetada de forma diferente?

Ao contrário do que se esperava, os resultados do Capítulo 1 indicaram que, que primeiramente há uma baixa ocorrência de trade-offs entre atributos vegetativos e reprodutivos

nas espécies avaliadas. Além disso, essa relação entre atributos reprodutivos e vegetativos pode ser positiva ou na maioria dos casos inexistente. Isso sugere que as espécies do Cerrado possuem acesso a recursos o suficiente eliminando a necessidade de trade-offs entre a maioria de seus atributos, possivelmente devido à capacidade de armazenamento de reservas e às altas taxas fotossintéticas (Scalon & Rossatto 2021; Cozin *et al.* 2024). As estratégias das plantas só foram alteradas em nível de comunidade nas áreas de baixa frequência de fogo, onde o tempo sem distúrbios pode ter sido suficiente para esgotar macronutrientes específicos, impactando negativamente as estruturas reprodutivas. Já em nível de espécie, a relação entre atributos vegetativos e reprodutivos variou, sem um padrão definido, podendo estar associada a estratégias de vida específicas e aos parâmetros do regime de fogo. Portanto, os atributos das plantas e as interações entre eles são fortemente influenciados pelas condições ambientais vivenciadas por cada indivíduo, evidenciando uma elevada plasticidade das respostas das espécies frente ao distúrbio.

Isso nos leva ao segundo capítulo. Se a relação entre atributos vegetativos e reprodutivos é influenciada por uma combinação de condições e fatores, não seria razoável supor que a resposta das sementes aos fatores de fogo (choque de temperatura e fumaça) também fosse alterada? Os resultados confirmaram essa hipótese, demonstrando que o efeito dos fatores de fogo na germinação varia de acordo com o período de dispersão das sementes (sazonalidade) e o tempo que permanecem no solo. No entanto, diferentemente de outros ambientes pirofíticos, não foi identificado um padrão claro nessas respostas (Daibes *et al.* 2022). Dessa forma, para compreender completamente as respostas das espécies a esses fatores, é essencial conduzir experimentos com uma gama mais ampla de sementes, considerando diferentes idades (tempo após a dispersão) e condições de armazenamento. Isso permitirá eliminar o viés associado ao uso exclusivo de dados de sementes frescas ou de um único lote/coleta, proporcionando uma visão mais precisa dos processos de recrutamento em ecossistemas inflamáveis.

Até o presente momento, a maioria dos estudos realizados para o Cerrado com este enfoque de avaliar as respostas germinativas a choques de temperatura não encontraram resultados padrões na quebra de dormência ou estímulo de germinação, porém observaram a

tolerância das sementes às altas temperaturas (Ribeiro *et al.* 2015; Ramos *et al.* 2016; Zupo *et al.* 2021; Daibes *et al.* 2022). O que levou ao terceiro capítulo, que demonstrou a importância de atributos como dormência, tamanho e forma das sementes, forma de crescimento, e fatores como o habitat na definição da tolerância das sementes às altas temperaturas.

Portanto, os resultados deste estudo têm importantes implicações tanto ecológicas quanto práticas para a conservação do Cerrado. Este trabalho foi o primeiro a mostrar que a relação entre atributos vegetativos e reprodutivos em plantas rebrotadoras é espécie-específico e influenciada pelos parâmetros do fogo e clonalidade. E esclarecer que as condições e tempos de armazenamento vão alterar as respostas aos fatores de fogo, portanto sendo enviesado afirmações sobre as sementes do Cerrado não terem adaptações ao fogo, sendo que não foi avaliado isso em sementes de diversos lotes e idade. As respostas das plantas demonstraram uma notável plasticidade, influenciada por fatores como tempo, sazonalidade e atributos. Os atributos vegetativos e reprodutivos avaliados se mostraram fundamentais para compreender os padrões e estratégias adaptativas em relação ao fogo no Cerrado. No entanto, sua análise isolada é limitada; a inclusão de outros fatores ambientais é essencial para uma compreensão mais abrangente.

Ao revelar a complexidade das respostas das plantas e a influência de atributos específicos na persistência e regeneração em ecossistemas inflamáveis, este trabalho fornece subsídios essenciais para o desenvolvimento de estratégias de restauração mais eficientes. O conhecimento sobre os efeitos do tempo de armazenamento das sementes e suas respostas a estímulos relacionados ao fogo pode ser aplicado na restauração de áreas degradadas, especialmente em fisionomias abertas do Cerrado. Atualmente, a restauração por semeadura direta tem ganhado espaço em diversos ecossistemas devido ao seu menor custo em comparação com outras técnicas (Raupp *et al.* 2020). No entanto, sua implementação ainda enfrenta desafios, como a aplicabilidade em larga escala, a disponibilidade de sementes nativas viáveis (de Souza & Engel 2023), a influência do solo e a presença de espécies invasoras (Silva & Vieira 2017). Em fisionomias abertas, resultados promissores começaram a ser encontrados (ver Sampaio et al. 2019; Wiederhecker et al. 2024). No entanto ainda falta conhecimento prévio sobre como o fogo,

distúrbio presente no Cerrado, interage na germinação e consequentemente o estabelecimento das espécies herbáceas e arbustivas no processo de restauração. Ao se ter conhecimento de como as respostas germinativas aos fatores de fogo são moduladas pelo tempo de armazenamento, essa informação poderia guiar na escolha de espécies para a restauração nesse ambiente inflamável, levando a um maior sucesso no estabelecimento, garantindo diversidade de espécies e serviços ecossistêmicos.

Por fim, este estudo reforça a necessidade de pesquisas que abordem atributos funcionais em um espectro mais amplo de espécies, permitindo conclusões mais robustas. Os desafios se encontram muitas vezes na logística da pesquisa, uma vez que alguns dos atributos são consideram *hard traits*, que demandam grande investimentos financeiros e logísticos.

Esses achados também destacam a importância de práticas de conservação que considerem a variabilidade das respostas das espécies ao fogo, contribuindo para a manutenção da biodiversidade e dos serviços ecossistêmicos dessa savana tropical.

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