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

DINÂMICA DO BANCO DE SEMENTES E GERMINAÇÃO DE GRAMÍNEAS
NATIVAS E INVASORAS DO CERRADO

MARIANA CORREA DAIREL

Dissertação apresentada ao Instituto de Biociências do *campus* de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Mestre em Ciências Biológicas (Biologia Vegetal).

Março
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Orientadora: Prof^a. Dra. Alessandra Fidelis

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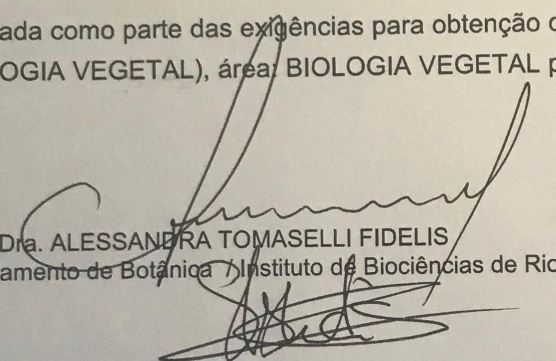
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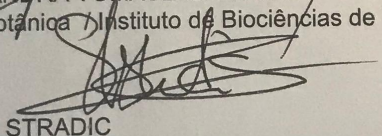
TÍTULO DA DISSERTAÇÃO: DINÂMICA DO BANCO DE SEMENTES E GERMINAÇÃO DE GRAMÍNEAS NATIVAS E INVASORAS DO CERRADO

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Rio Claro, 27 de abril de 2018

É poder apreciar o que se encontra abaixo dos olhos
Descobrir e se encantar com suas variáveis formas
E ainda perceber que dentro do micro existe o macro.

Ao Cerrado, dedico esta dissertação!

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A todos vocês, o meu muito obrigada!

"A natureza é grande nas coisas grandes e grandíssima nas pequeninas."

Saint-Pierre

RESUMO GERAL

Espécies invasoras são aquelas capazes de se estabelecer em áreas além de sua ocorrência natural, ultrapassando barreiras geográficas e constituindo novas populações viáveis. A introdução de gramíneas africanas no Cerrado, como *Melinis minutiflora* P. Beauv e *Urochloa brizantha* (Stapf) R.D. Webster, pode levar a uma baixa heterogeneidade do sistema, deixando-o mais suscetível a distúrbios ambientais, alterando o equilíbrio da comunidade e os serviços ecossistêmicos. Em sistemas frequentemente perturbados, clareiras são criadas na paisagem e novas espécies são recrutadas para colonizar o espaço aberto, processo esse regido pelo nicho da regeneração. Espera-se que estes nichos se sobressaiam nas espécies invasoras em relação às espécies nativas, conferindo-lhes vantagens adaptativas na colonização desses espaços. Dessa maneira, o objetivo do estudo foi avaliar a dinâmica do banco de sementes de gramíneas em áreas com e sem invasão biológica, além de avaliar diferentes fatores que influenciam a germinação de gramíneas nativas. A área de estudo está inserida na Estação Ecológica de Itirapina, onde até 1998 havia uma plantação de *Pinus caribaea*, a qual foi removida e desde então a área está sob regeneração natural, sem interferência, com alto grau de invasão por gramíneas africanas. No primeiro capítulo, as amostras de solo foram coletadas nas parcelas experimentais controle do projeto da Profa. Dra. Alessandra Fidelis (FAPESP 2015/06743-0), delineado então parcelas com três tratamentos: dominada por uma das duas espécies invasoras, *Urochloa brizantha* e *Melinis minutiflora*, e parcelas com domínio de vegetação nativa (4parcelas/tratamento= 12 unidades amostrais). No segundo capítulo, as sementes de gramíneas nativas foram coletadas na Estação Ecológica de Itirapina e na Reserva Natural Serra do Tombador, triadas em laboratório e submetidas a tratamentos de flutuação de temperatura, simulando a oscilação de temperatura diária sobre a superfície do solo, e choques de temperatura simulando a passagem do fogo. O conhecimento mais detalhado sobre o banco de sementes em áreas invadidas é fundamental para elaboração de práticas de controle e manejo dessas espécies. Além disso, a germinação e viabilidade das sementes de gramíneas nativas são fatores importantes tanto para elucidar a regeneração natural via semente, compreendendo processos ecológicos relacionados à quebra da dormência fisiológica, quanto para aplicação de métodos físicos de restauração do Cerrado, como a semeadura direta.

Palavras-chave: banco de sementes, germinação, gramíneas, regeneração natural, Cerrado.

ABSTRACT

Invasive species are those capable of establishing themselves in areas beyond its natural occurrence, surpassing geographical barriers and constituting new populations. The introduction of African grasses in Cerrado, *Melinis minutiflora* P. Beauv and *Urochloa brizantha* (Stapf) R.D. Webster, may low heterogeneity of the system, making it more susceptible to altering the balance of the community and ecosystems. In frequently disturbed systems, clearings are created in the landscape and new species are recruited to colonize the open space, which is governed by the regeneration niche. These niches are expected to be better in invasive species in relation to native species, giving them adaptive advantages in the colonization of these spaces. In this way, the objective of the study was to evaluate the dynamics of grasslands in areas with and without biological invasion, in addition to evaluating different factors that influence the germination of native grasses. The study area is located in the Estação Ecológica de Itirapina, where until 1998 there was a plantation of *Pinus caribaea*, which has been removed and since then the area is under natural regeneration, without interference, with a high invasion of *Urochloa brizantha* and *Melinis minutiflora*. In the first chapter, the soil samples were collected in the control plots of the project of Profa. Dr. Alessandra Fidelis (FAPESP 2015 / 06743-0), outlined then plots with three treatments: dominated by one of the two invasive species, *Urochloa brizantha* and *Melinis minutiflora*, and plots with dominance of native vegetation (4plots / treatment = 12 sample units). In the second chapter, grass seeds were collected at Estação Ecológica de Itirapina and in Reserva Natural Serra do Tombador, triads in the laboratory and submitted to treatments of temperature fluctuation, simulating the daily temperature oscillation on the soil surface, and heat shock simulating the passage of fire. More detailed knowledge about the soil seed bank in invaded areas is fundamental for the elaboration of practices of control and management of these species. In addition, the germination and viability of native grass seeds are important factors both to elucidate the natural regeneration through seed, comprising ecological processes related to break physiological dormancy of seed, and for application of physical methods of restoration of Cerrado, such as direct sowing.

Keywords: soil seed bank, germination, grasses, natural regeneration, Cerrado.

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INTRODUÇÃO GERAL

Espécies invasoras são definidas como espécies exóticas (não naturais daquele local) que apresentam propágulos capazes de ultrapassar barreiras geográficas e ambientais locais, estabelecendo novas populações viáveis. Além disso, também causam danos ecológicos e econômicos ao sistema que estão invadindo (Mayers et al. 2000; Richardson et al. 2006). Portanto, o processo de invasão biológica envolve não apenas a superação da barreira geográfica, mas também da ambiental para o estabelecimento das populações de espécies invasoras em certo local (Richardson et al. 2000).

A dinâmica e estabelecimento dos processos de invasão biológica são produtos da interação da *invasividade* x *invasibilidade* (invasiveness x invasibility, Richardson et al. 2006). A *invasividade* é representada por um conjunto de atributos que a espécie dispõe que a torna capaz de se estabelecer e desenvolver nos sistemas como, por exemplo, o potencial reprodutivo, a propagação vegetativa e dispersão de sementes através do espaço e tempo (Richardson et al. 2006). A *invasibilidade*, por sua vez, descreve características favoráveis do ambiente que o deixam mais susceptíveis à invasão, como a natureza da superfície do solo, influenciando assim o estabelecimento de uma plântula (Richardson et al. 2006). Dessa forma, a invasão biológica é determinada não somente pelas características da própria espécie invasora, como também por um conjunto de atributos do ambiente.

Eventos de distúrbios, sejam eles naturais ou antrópicos, podem facilitar a entrada de espécies exóticas com características invasoras, de rápida colonização e adaptação, no sistema (Grubb, 1977). Contudo, para que as espécies tenham sucesso na colonização da nova área fatores limitantes são expressos no nicho de regeneração, para o estabelecimento dessas espécies (Grubb, 1977). A regeneração pós-distúrbio, além de ser dependente de fatores

abióticos, são dependentes de fatores bióticos como a presença de uma fonte viável de propágulos para restabelecimento da comunidade vegetal aérea (Baskin & Baskin 2014), que podem já estar no sistema (banco de sementes e de gemas), como vir de fora dele, através da dispersão (chuva de sementes) (Fenner & Thompson, 2006; Anderson et al. 2011).

O banco de sementes é uma fonte eficiente para regeneração da comunidade vegetal pós eventos de distúrbio em diversos sistemas (Baskin & Baskin 2014), como já descrito para savanas africanas (Anderson et al. 2011) e australianas (Scott et al. 2010). O banco de sementes é definido como um reservatório que compreende todas as sementes viáveis que se encontram no solo, seja na superfície ou soterradas (Thompson & Grime, 1979) e pode ser classificado como persistente quando as sementes permanecem viáveis no solo por mais de um ano, ou transitório, quando as sementes perdem a viabilidade até um ano (Thompson & Grime, 1979). Além de ser um recurso ligado diretamente à regeneração natural do ambiente, o banco de sementes é um reservatório genético e histórico da comunidade vegetal (Simpson et al. 1989), potencialmente capaz de substituir a vegetação aérea (Baker, 1989). Compreender a dinâmica do banco de sementes, principalmente em ecossistemas onde a sazonalidade é um importante fator, é essencial para a conservação desses ecossistemas (Martins, 2006; Aires, 2013).

As espécies invasoras são caracterizadas por apresentar alta produção de sementes além de apresentarem atributos de recrutamento e estabelecimento que sobressaem quando comparado as espécies nativas (Freitas & Pivello, 2005; Martins, 2006; Aires, 2013). As espécies invasoras são capazes de manter altas densidades de sementes no solo que em conjunto com seus atributos reprodutivos, fazem com que a dinâmica do banco de sementes da área seja totalmente modificada, alterando assim a estrutura e diversidade da comunidade, além de dificultar o estabelecimento de espécies nativas (Belgeri et al. 2014;

Ngruyen et al. 2017). Sendo assim, é imprescindível entender a dinâmica do banco de sementes em áreas invadidas para inferência de novas invasões e até mesmo para elaboração de práticas de manejo eficientes.

As sementes viáveis no banco de sementes precisam germinar e se estabelecer na comunidade vegetal, portanto conhecer os atributos de germinação das espécies da comunidade é de extrema importância para avaliar o estabelecimento de espécies via banco de sementes em um certo local. A falta de conhecimento sobre germinação de sementes (Carmona et al. 1998) e estabelecimento (Klink 1996) das espécies nativas, principalmente do Cerrado, fomenta o uso de espécies exóticas como *Melinis minutiflora* P. Beauv (capim gordura) e *Urochloa brizantha* (Stapf) R.D. Webster (braquiária) para recuperação de áreas degradadas. Sendo assim, o conhecimento mais detalhado sobre o banco de sementes e viabilidade das sementes é fundamental tanto para elucidar a regeneração natural via sementes quanto para inclusão de espécies nativas em programas de restauração.

O Cerrado apresenta cerca de 500 espécies de gramíneas nativas (Mendonça et al. 2008), representando de 60 a 80% do estrato herbáceo de sistemas campestres e savânicos (Munhoz & Felfili, 2007). Porém, os programas de restauração do Cerrado utilizam em sua maioria espécies lenhosas (Durigan et al. 2011) e gramíneas exóticas (Silva et al. 2006), em grande parte, por dificuldades na germinação de espécies de gramíneas nativas (Carmona et al. 1998; Zaidan & Carreira, 2008; Aires, 2013; Kolb et al. 2016; Ramos et al. 2016; Ramos et al. 2017).

Apesar de apresentarem alta produção de sementes, no Cerrado, as gramíneas são caracterizadas pela baixa porcentagem de germinação e estabelecimento (Aires, 2013; Ramos et al. 2016). Esses atributos estão relacionados à alta produção de sementes inviáveis e/ou sem embrião (Carmona et al. 1999; Wright et al. 2014), além de apresentarem espécies com sementes

dormentes (Baskin & Baskin 2004; Erickson et al. 2016), principalmente dormência fisiológica (Baskin & Baskin 2004; Erickson et al. 2016), que pode ser quebrada através da oscilação de temperatura (Adkins et al. 2002, Ramos et al. 2016, Commander et al. 2017).

Em ambientes savânicos, eventos de fogo são responsáveis por manter a fisionomia, estrutura e diversidade do ecossistema (Bond & Keeley 2005), sendo eventos recorrentes também no Cerrado (Coutinho, 1982; Kauffman et al. 1994). A passagem do fogo cria certas condições ambientais que favorecem a quebra da dormência das sementes que se encontram no sistema, seja sobre o solo ou soterradas (Daibes et al., no prelo). O aumento da temperatura causado pela passagem das chamas (Moreira et al. 2010), ou até mesmo a remoção da vegetação, criando clareiras expondo o solo a alta incidência solar, leva a flutuação térmica diária do solo (Santana et al. 2013; Daibes et al. 2017), podendo levar à quebra de dormência das sementes de espécies com este tipo de estratégia, favorecendo assim, o recrutamento de gramíneas.

Ecossistemas campestres ao redor do mundo são atualmente ameaçados por diversas práticas, entre elas a agropecuária, exclusão de regimes de fogo, invasão biológica e incentivo ao reflorestamento (Veldman et al. 2015). O Cerrado vem sofrendo perdas significativas de biodiversidade devido à pressão antrópica para fins agropecuários (Machado et al. 2004; Sano et al. 2010). As gramíneas africanas, como por exemplo, *Urochloa brizantha* e *Melinis minutiflora*, foram introduzidas para fins forrageiros e acabaram invadindo diversos ambientes, sendo amplamente distribuídas e presentes em praticamente todas as unidades de conservação de Cerrado (Pivello et al. 1999a; Pivello et al. 1999b).

Dentre as fisionomias campestres e savânicas do Cerrado, há o domínio do estrato herbáceo-arbustivo, caracterizada por apresentar elevada riqueza de espécies, conferindo destaque à família Poaceae (Munhoz & Felfili, 2007).

Poaceae é uma das maiores famílias de angiospermas, apresentando alta diversidade entre espécies nativas e exóticas (Watson, 1990). Esse grupo possui certos atributos morfológicos e fisiológicos característicos, que lhe confere vantagem no estabelecimento nestes tipos de vegetação, como a produção em larga escala de sementes, ciclos reprodutivos curtos, grande distribuição espacial, investimento em reprodução assexuada e eficiência fotossintética (Watson, 1990).

As espécies invasoras *Urochloa brizantha* e *Melinis minutiflora*, além de serem espécies perenes com banco de sementes persistente (Simpson et al. 1989), apresentam características intrínsecas à reprodução, como produzir alta densidade de sementes, o que favorece o estabelecimento das mesmas quando comparada às espécies nativas (Pivello et al. 1999; Hoffman et al. 2004; Freitas & Pivello, 2005; Martins, 2006; Gorgone-Barbosa et al., em preparação; Damasceno et al., submetido).

A dinâmica natural das comunidades de plantas no Cerrado vem sendo fortemente modificada pela presença dessas gramíneas (Gorgone-Barbosa et al., em preparação, Damasceno et al., submetido). Devido ao grande poder competitivo, essas espécies são responsáveis pela alteração na composição de espécies e na estruturação da comunidade vegetal (Aires, 2013; Gorgone-Barbosa et al., em preparação; Damasceno et al., submetido).

Dessa forma, o presente trabalho tem como objetivo principal analisar a dinâmica do banco de sementes de *Urochloa brizantha*, *Melinis minutiflora* e gramíneas nativas, em áreas invadidas e não invadidas de campo sujo em regeneração natural após retirada da plantação de *Pinus caribae* há cerca de 20 anos elucidando, assim, o potencial de regeneração de gramíneas do banco de sementes. Finalmente, pretende-se também avaliar os diferentes fatores que influenciam a germinação das gramíneas nativas, elucidando assim, os

mecanismos que influenciam o recrutamento das espécies via banco de sementes.

Delineamento experimental

O trabalho foi desenvolvido na Estação Ecológica de Itirapina, dentro da Zona de Interferência Experimental da Unidade de Conservação (UC), área onde havia cultivo de *Pinus caribaea* (Zanchetta et al. 2006), plantio esse incentivado pelo Estado de São Paulo, na década de 60, pelo programa de reflorestamento do Estado (Fig. 1). O talhão foi retirado entre 1998-2000 e desde então, a área encontra-se em regeneração natural, sem interferência. Atualmente a área apresenta uma fisionomia de campo sujo, com alto grau de invasão por gramíneas exóticas: *Melinis minutiflora* e *Urochloa brizantha*.



Figura 1. Foto aérea da área de estudo: a delimitação maior representa o talhão de *Pinus* removido, as menores representam algumas parcelas experimentais. Foto: Dhemerson Conciani.

Nessa área estão inseridas as parcelas experimentais do projeto da Profa. Dra. Alessandra Fidelis (FAPESP 2015/06743-0), que foram utilizadas para o desenvolvimento deste projeto. O desenho amostral é composto por 48

parcelas de tamanho fixo 15x15 m, divididas em três grupos de acordo com o domínio de espécies: *Urochloa brizantha*, *Melinis minutiflora* e espécies nativas (16 parcelas/espécie).

Banco de sementes

Dentro da área foram delineadas três tratamentos de acordo com a dominância de espécies na comunidade vegetal aérea. Sendo assim foram estabelecidas três tipos de parcelas no tamanho de 15x15m para o levantamento do banco de sementes: com dominância (cobertura >50%) de *Melinis minutiflora* (MM), com dominância de *Urochloa brizantha* (UB) e parcelas com domínio de vegetação nativa (N, Fig. 2). As parcelas com vegetação nativa eram caracterizadas principalmente pela presença de gramíneas como *Axonopus pressus*, *Aristida jubata*, *Loudetiospsis chrysothrix* e *Aristida riparia*. Cada tratamento possui quatro réplicas isoladas e independentes, totalizando assim 12 unidades amostrais (4 réplicas/tratamento).

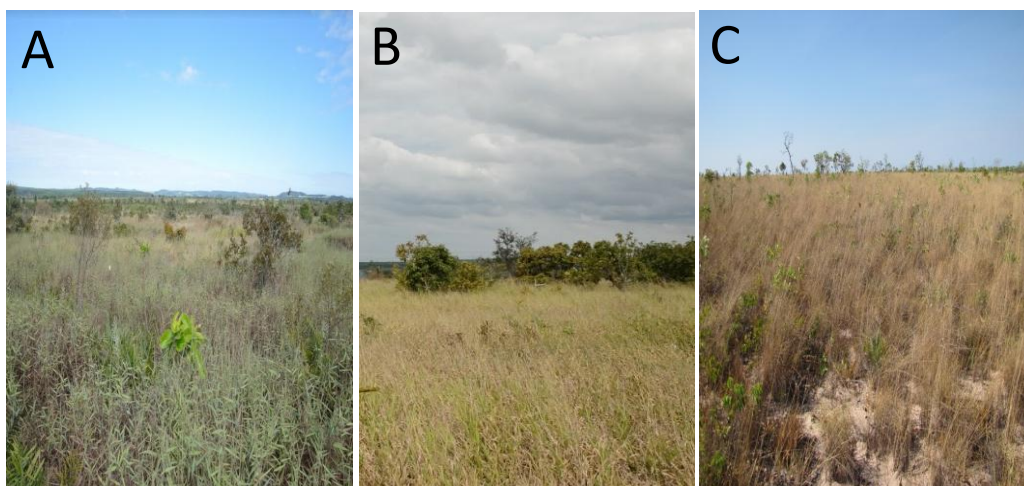


Figura 2. Parcelas experimentais com três tratamentos: (A) dominância de *Melinis minutiflora*- MM. (B) dominância de *Urochloa brizantha* - UB e (C) dominância de gramíneas nativas - N; na foto: *Gymnopogon foliosus*.

A coleta do banco de sementes foi realizada mensalmente, entre o período de Abril de 2016 a Março de 2017. As amostras de solo eram coletadas

com auxílio de uma pá, onde o tamanho era delimitado (20x20cm) e a camada superficial do solo até 2m de profundidade era retirada e levada para laboratório (Fig. 3). Em cada parcela foram coletadas quatro amostras de solo aleatoriamente, totalizando 48 amostras/mês (12 parcelas/4 amostras de solo). Os locais da coleta eram sorteados tendo como ponto referência subparcelas que se encontrava no interior das parcelas de 15x15m para análise da comunidade vegetal do projeto da Prof. Dra. Alessandra Fidelis. Os locais de coleta eram marcados para evitar a coleta no mesmo local nas amostragens seguintes (Fig. 3).



Figura 3. Método de coleta do banco de sementes do solo. Uma amostra (20x20x2cm) era delimitada no solo, sendo coletada com auxílio de uma pá. Fotos: Rafael Consolmagno.

Em laboratório, as amostras passaram por peneiras com distintas malhas (0.2; 0.35 e 0.6mm) a fim de retirar fragmentos maiores como caule, raiz e rochas. Em seguida, foram divididas igualmente em duas amostras de 400cm³ para avaliar o banco de sementes por dois métodos: Contagem Direta e Emergência de Plântulas (Fig. 4).

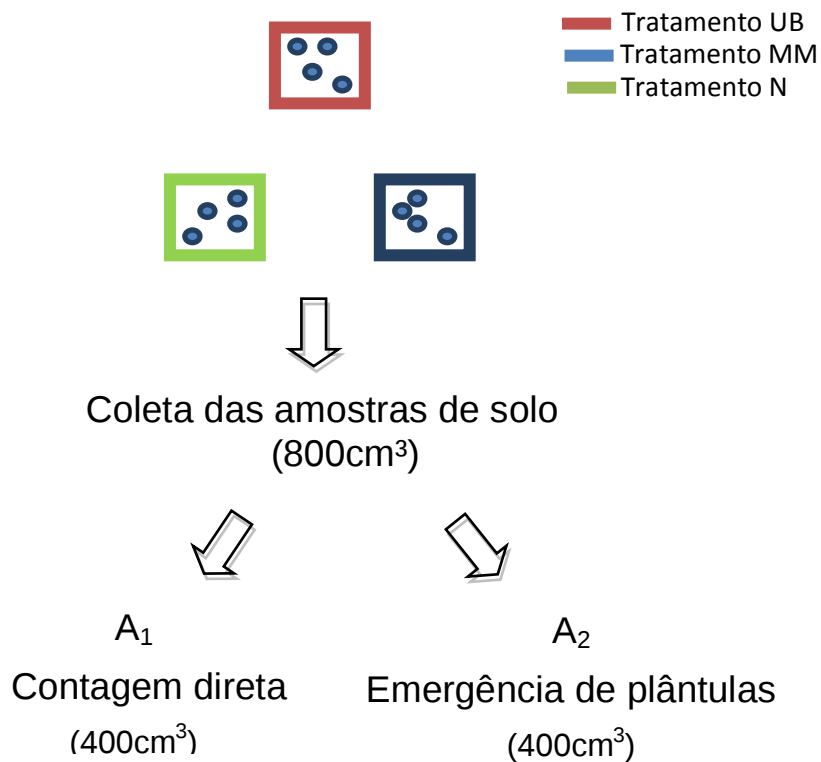


Figura 4. Esquema representando a amostragem do banco de sementes do solo nas parcelas e a separação dessa amostra para análise da dinâmica do banco de sementes com distintas metodologias.

Para o método de Emergência de Plântulas (Fig. 5), as amostras foram espalhadas em bandejas de alumínio (27,5 x 18,5 x 4,5 cm), perfuradas no fundo para evitar acúmulo de água. Após montagem, as bandejas foram acondicionadas em casa de vegetação onde eram irrigadas três vezes ao dia (36mL de água) e ainda eram rotacionadas, semanalmente, para evitar excesso de água nas bandejas. As observações foram realizadas quinzenalmente, durante quatro meses. As plântulas que germinavam foram classificadas em gramíneas nativas e exóticas, separadamente, sendo as espécies exóticas identificadas por espécie.

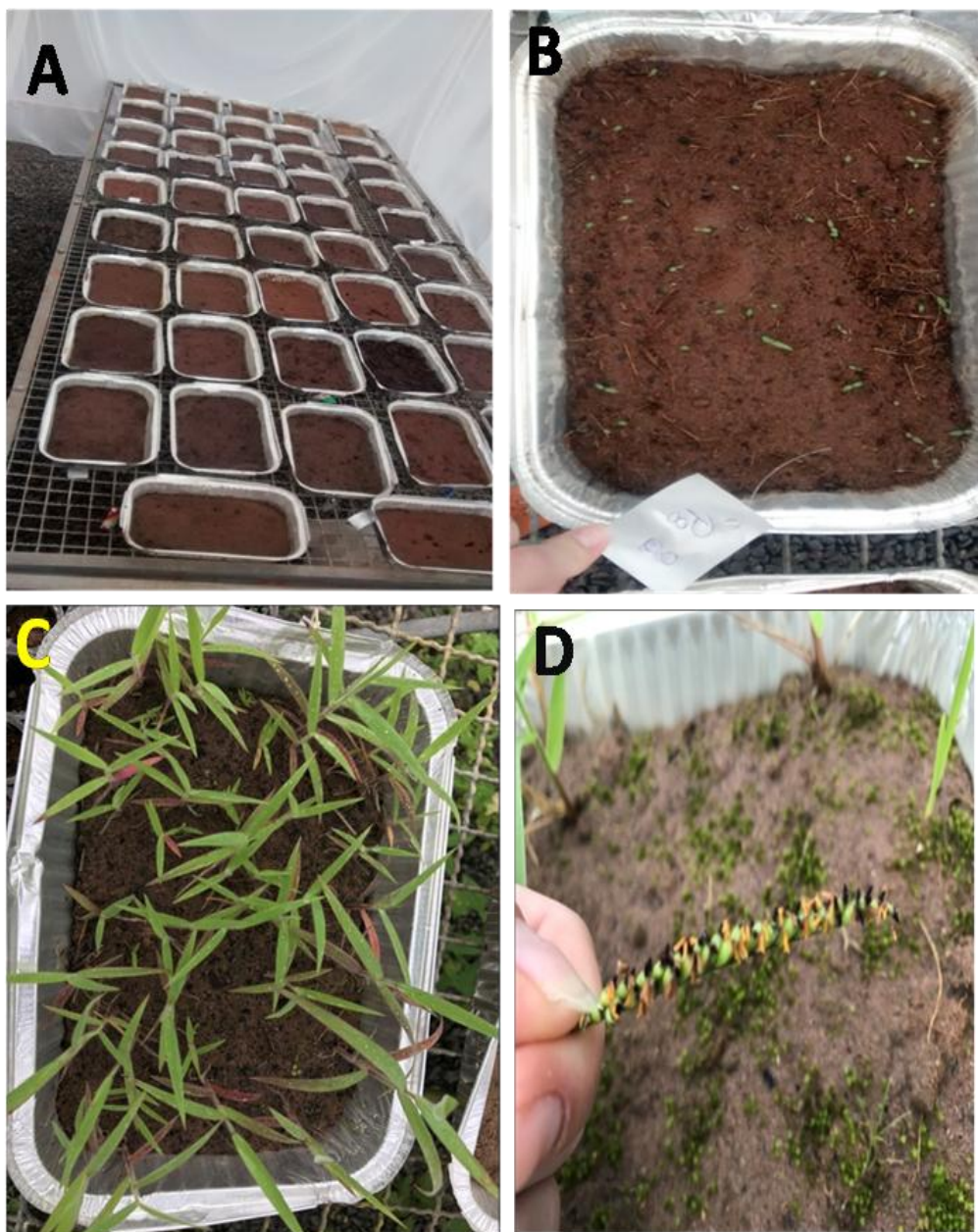


Figura 5. Método de emergência de plântulas. (A) início da observação do mês de coleta. (B) Germinação das sementes presentes no banco de sementes do solo após 15 dias. (C) Desenvolvimento de *Melinis minutiflora* após 30 dias. (D) *Urochloa brizantha* com flor após 90 dias. Fotos: Mariana Dairel.

Já no método de Contagem Direta (Fig. 6), as amostras de solo foram triadas com auxílio de lupa e as sementes encontradas no solo foram contabilizadas e classificadas em gramíneas nativas e exóticas, separadamente, sendo as espécies exóticas identificadas por espécie.



Figura 6. Método de contagem de sementes: com auxílio de uma lupa, as sementes encontradas no solo foram contabilizadas. Fotos: Mariana Dairel.

Germinação

Para os experimentos de germinação foram coletadas, durante todo ano de 2016, sementes de gramíneas nativas (Fig. 7) em duas áreas: Estação Ecológica de Itirapina (EEI) e na Reserva Natural Serra do Tombador (RNST), ao longo da época de dispersão de cada espécie (Tabela 1).

Tabela 1. Espécies de gramíneas nativas coletadas, durante o ano de 2016, na Estação Ecológica de Itirapina (EEI) e na Reserva Natural da Serra do Tombador (RNST), com a época de dispersão e ciclo de vida de cada espécie.

Espécies	Local de coleta	Época de dispersão	Ciclo de vida
<i>Andropogon lateralis</i> L.	EEI	Janeiro - Abril	Perene
<i>Andropogon bicornis</i> L.	EEI	Janeiro - Abril	Perene
<i>Aristida megapotamica</i> Spreng.	EEI	Agosto - Maio	Perene
<i>Aristida riparia</i> Trin.	EEI/RNST	Agosto - Maio	Perene
<i>Aristida jubata</i> (Arechav.) Herter	EEI	Setembro - Junho	Perene
<i>Axonopus aureus</i> P.Beauv	RNST	Agosto - Abril	Perene
<i>Gymnopogon foliosus</i> (Willd.) Nee	EEI	Maio - Junho	Anual
<i>Loudetiopsis chrysothrix</i> (Nees) Conert	EEI	Janeiro - Abril	Perene
<i>Sporobolus aeneus</i> R.Br.	EEI	Janeiro - Abril	Perene
<i>Sporobolus cubensis</i> Hitchc.	RNST	Agosto - Abril	Perene



Figura 7. Coleta de sementes de gramíneas nativas dominantes nas áreas. (A) *Andropogon bicornis*. (B) *Gymnopogon foliosus*. (C) *Aristida jubata*. (D) *Loudetiopsis chrysothrix*. Fotos: (A); (B); (D) Heloiza Zironi/ Mariana Dairel, (C) Alessandra Fidelis/ Mariana Dairel.

As sementes foram coletadas de diferentes indivíduos e populações para garantir a variabilidade genética, coletando apenas sementes de indivíduos aleatórios na comunidade vegetal e que não se encontravam dentro das parcelas experimentais. Foram acondicionadas em sacos de papel e posteriormente, sacos ziplock em campo. Em laboratório, as sementes foram triadas, separando-se em lupa os frutos vazios daqueles com sementes, a fim de

serem submetidas a diferentes experimentos de germinação: Flutuação térmica, Choque de temperatura e Longevidade.

Para os experimentos de flutuação térmica foram utilizadas quatro réplicas com 25 sementes por réplica, colocadas em diferentes câmaras de germinação (1 réplica/câmara – 4 câmaras) que simularam a flutuação térmica diária (variação de temperatura de 19°C a 55°C) por 45 dias. O tratamento controle permaneceu em temperatura ambiente

Já para os experimentos de choques de temperaturas foram utilizadas cinco réplicas com 20 sementes por réplica, submetidas a quatro tratamentos: 100°C por 1 minuto, 100°C por 3 minutos, 200°C por 1 minuto e controle. Os choques foram realizados com auxílio de uma mufla eletrônica sendo cada réplica colocada separadamente na mufla. As sementes do tratamento controle permaneceram em temperatura ambiente.

Nos experimentos de longevidade, as sementes foram estocadas por 6 e 12 meses após a coleta, armazenadas em sacos ziplock e em local com temperatura ambiente, e posteriormente colocadas para germinar. Foram utilizadas quatro réplicas com 25 sementes por réplica.

Após os tratamentos (flutuação térmica, choques de temperaturas e longevidade), as sementes foram colocadas para germinar (Fig. 8A) a 27°C e regime de luz 12/12 horas (Fichino et al. 2016). Observações semanais foram realizadas (3x na semana), ao longo de quatro semanas e todas as sementes germinadas foram contadas e retiradas das placas. Ao fim dos experimentos, aquelas que não germinaram foram submetidas ao teste de tetrazólio (1%) para avaliar a viabilidade das sementes (Lakon, 1949) (Fig. 8B).

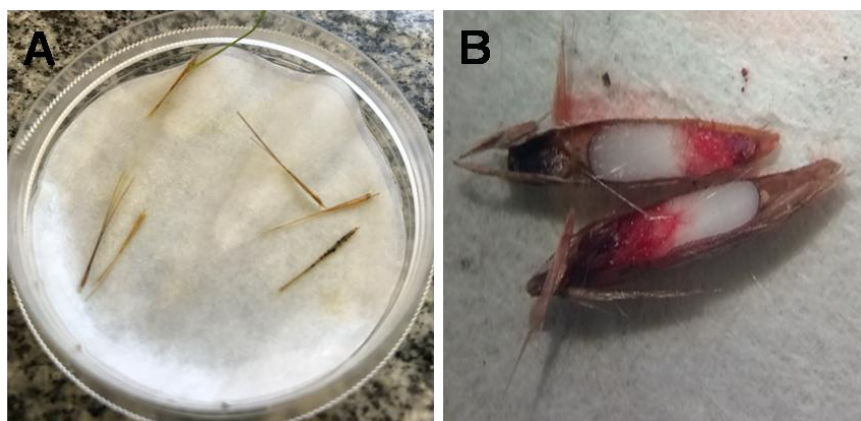


Figura 8. Teste de germinação. (A) Semente de *Aristida megapotamica* germinando. (B) Teste de tetrazólio para viabilidade em semente de *Loudetiopsis chrysothrix*.

Estrutura da dissertação

O conhecimento mais detalhado sobre o banco de sementes e viabilidade das sementes de graminóides nativos é fundamental tanto para elucidar a regeneração natural via semente quanto para inclusão desse grupo em práticas de restauração do Cerrado. Sendo assim, a dissertação foi estruturada em dois capítulos visando trabalhar os objetivos específico em cada um deles. Os capítulos foram elaborado sem forma de manuscrito, redigidos em inglês e na formatação da revista científica de interesse, da seguinte forma:

Capítulo I: ***“Soil seed bank dynamics in invaded and non-invaded areas of open savannas of Cerrado”***. Avaliação da dinâmica do banco de sementes de gramíneas invasoras e nativas, em áreas com e sem invasão biológica, de uma área de campo sujo em regeneração ao longo de um ano. Tem-se como hipótese de que o banco de sementes do capim-gordura (*Melinis minutiflora*) e da braquiária (*Urochloa brizantha*) possuam uma maior densidade de sementes do que das espécies de gramíneas nativas nas áreas invadidas. Além disso, haverá uma variação sazonal de sementes encontradas no banco de

sementes, acompanhando o período de dispersão destas espécies na comunidade vegetal. Este capítulo será submetido para *Biological Invasions*.

Capítulo II: ***“What influences grass germination in Cerrado?”***.

Avaliação da germinação de gramíneas nativas, usando os tratamentos de flutuação térmica e choques de temperatura, tendo em vista que a maioria das gramíneas podem apresentar dormência fisiológica. Tem-se como hipótese que a maioria das espécies apresentará dormência e sua germinação será estimulada pelos tratamentos de flutuação térmica e choques de temperatura. Ainda foi analisada a longevidade dessas espécies no sistema. Além disso, espera-se encontrar uma grande quantidade de sementes inviáveis e sem embrião na população. Este capítulo será submetido para *Plant Ecology*.

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Soil seed bank dynamics in invaded and non-invaded areas of open savannas of Cerrado

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Abstract

One of the major threats of Cerrado is the invasion of African grasses. Areas under natural regeneration can be more easily invaded and after disturbance events, new individuals can be recruited from the seed bank. Thus, this study aims to analyze the dynamics of the soil seed bank in invaded and non-invaded areas elucidating the potential of grasses regeneration from the soil seed bank. Soil samples (0.2x0.2x0.02m, 4 samples/area) were monthly collected (for one year) in areas with different invasive species dominance: *Urochloa brizantha* (UB), *Melinis minutiflora* (M) and in plots with native vegetation (N). Soil seed bank was assessed using two techniques, in emergence technique the soil sample was put to germinate in greenhouse and monitored for four months; in counted technique the sample was sorted in the lab and the seeds were counted. The highest densities of seeds were found in UB areas, where invasive grasses (IG) contributed with 95%, composed mostly by *U. brizantha* (91%, $670 \pm 382 \text{ seeds.m}^{-2}$). The lowest density was of native species (5%, 102 seeds.m^{-2}), represented by *Axonopus pressus* and *Gymnopogon foliosus*. In MM areas, IG dominated with 85% of total seeds, represented mostly by *M. minutiflora* (949 seeds.m^{-2}), and a low density of native species seeds could be found (15%, 58 seeds.m^{-2}). In N areas, native species presented the highest density with 65% (180 seeds.m^{-2}). IG represented 35% of total seeds, from which was dominated

by *U.brizantha*. The seed bank had the highest densities from June to September. The seed bank was influenced by the species dominance in each area and it was dependent on vegetation phenology. Finally, N areas have a higher potential of regeneration by native grasses seeds, while invaded areas soil seed bank are dominated by invasive species and thus, management efforts should be carried out in order to control and avoid their establishment.

Keywords: savannas; grasses; germination; natural regeneration

Introduction

Biological invasion is one of the greatest threats to biodiversity and it has become a major concern in ecosystem conservation (Callaway and Maron 2006; Bajwa et al. 2016). The natural dynamics of plant communities have been strongly modified by the presence of invasive species (Rejmanek et al. 1989; D'Antonio & Vitousek 1992; Pivello et al. 1999). These species not only alter community equilibrium and species composition (Gorgone-Barbosa et al. unpublished data; Damasceno et al. submitted; Nguyen et al. 2017), but also the ecosystem services, preventing system productivity and efficiency (D'Antonio & Vitousek 1992; Rossiter et al. 2014; Bajwa et al. 2016). In addition, invasive species can reduce ecosystem resilience by altering biotic and/or abiotic factors, triggering feedback between limiting factors and invasive species. Because of that, invasive species are favored and thus, they dominate these areas (Gaertner et al. 2012, 2014). As a consequence, they modify the dynamics of disturbance in the ecosystem (D'Antonio & Vitousek 1992; Hughes & Vitousek 1993).

The process of biological invasion is influenced by the invasiveness of the system (set of environment characteristics that favor invasion, Richardson et al. 2006) and the species traits that allows them to colonize the environment

(invasibility, Richardson & Pyšek 2006). Thus, the invasive species can influence invasiveness by altering the soil composition, nutrient cycling, water and light availability, productivity and the disturbance regime (D'Antonio & Vitousek 1992; Hughes & Vitousek 1993; D'Antonio 2000; Levine et al. 2003). The invasibility is usually related to traits linked to reproductive strategies, germination and establishment (Facon et al. 2006), which favors the use of resources with greater effectiveness when compared to the native ones (Hoffman et al. 2004; Freitas & Pivello, 2005; Reed et al. 2005; Martins 2006).

Disturbance events are known to enhance invasion processes (Hobbs & Huenneke 1992). They create gaps in the vegetation, where species can be recruited, a process governed by the regeneration niche (Grubb 1977). Since invasive species produce more seeds and can grow faster than native ones (Pyšek & Richardson 2007), they occupy these gaps fast, outcompeting native species. These establishment can occur from the soil seed bank and/or bud bank (propagules are already in the system), as well as from propagules coming from neighboring areas through the seed rain (Fenner & Thompson 2006; Anderson et al. 2011). Therefore, maintaining a local pool of propagules might be an advantage for species to rapid colonize after disturbance (Uhl & Clark 1983), being the presence of a persistent and viable soil seed bank an advantage (Williams et al. 2005).

In many ecosystems, the main source of propagules is the soil seed bank (Fenner & Thompson 2006). The seed bank is a reservoir comprising all viable seeds that are on or below the soil surface (Thompson & Grime 1979), capable of producing physiologically independent individuals, replacing the aerial vegetation (Baker 1989). It can be classified as transient or persistent, being distinguished by the longevity of the seeds: the transient seed bank is the one that remain

viable in the soil up to one year after dispersion, while the persistent seed bank has viable seeds in the soil for more than one year (Thompson & Grime 1979).

Invasive species are characterized to invest in high seed production, being able to form a persistent soil seed bank (Simpson et al. 1989; Freitas & Pivello 2005; Martins et al. 2006). The deposition of seeds in the soil seed bank coupled with the reproductive traits, high germination percentage and establishment of invasive species causes the natural dynamics of the soil seed bank to be totally altered, influencing negatively the native species diversity (see Belgeri et al. 2014; Ngruyen et al. 2017; Galloway et al. 2017). Additionally, some invasive species have their germination enhanced in gaps, due to the higher temperature fluctuation (Gorgone-Barbosa et al. 2016), being an advantage for their rapid establishment over the native species.

In Cerrado, *Melinis minutiflora* and *Urochloa* spp are the major invasive species. Although they do not change the physiognomy of herbaceous layer, they affect plant community, having a negative impact by decreasing species diversity and altering biomass composition (Aires 2013; Gorgone-Barbosa, unpublished data; Damasceno et al. submitted). Considered as a hotspot of biodiversity (Myers et al. 2000), Cerrado has a high number of endemic species and, in the last decades, it has suffered significant losses of biodiversity (Machado et al. 2004; Sano et al. 2010), being biological invasion one of the greatest threats to the conservation of this species-rich system (Pivello et al. 1999b; Machado et al. 2004; Durigan et al. 2007; Abreu et al. 2017).

Although its known that the presence of invasive species leads to significant changes in the ecosystem (Pivello et al. 1999; D'Antonio 2000; Levine et al. 2003; Bajwa et al. 2016), because of their efficiency in using local resources (Hughes & Vitousek 1993; Levine et al. 2003), little is known about the soil seed

bank dynamics of invaded areas and how it would affect aboveground plant community.

Therefore, this study aims to analyze the soil seed bank composition and dynamics of the most common invasive grasses of Cerrado: *Melinis minutiflora* and *Urochloa brizantha*, as well as of native graminoids in areas both invaded and non-invaded throughout the year. It is hypothesized that the seed bank of areas invaded by *Melinis minutiflora* and *Urochloa brizantha* will have a higher seed density than the native graminoids species. In addition, there will be a seasonal variation of seeds found in the soil seed bank, following the dispersal period of the invasive and native grasses: *Melinis minutiflora*: March to July and *Urochloa brizantha*: throughout the year (Oliveira et al. 2016b).

Material and methods

Study area

This study was carried out at the Ecological Station of Itirapina (EEI, Southeastern Brazil, 22° 11 'to 22° 15' S, 47° 51 'and 48° 00' W), which has a total area of 2,300 ha. The altitude ranges from 710 to 830m (Zanchetta et al. 2006). The climate is hot with and rainy summers and dry winters, with an average annual temperature of 21.9°C and an average annual precipitation of 1459 mm (Zanchetta et al. 2006).

Soils at the EEI are Gleissol and Neosol Quartizenic soils and one can find different physiognomies of Cerrado, from open areas (wet grasslands, open savannas called *campo sujo*) to woodlands (Zanchetta et al. 2006). The area where the soil was sampled was planted in the past with *Pinus caribaea* was cultivated until 1998 (Zanchetta et al. 2006). The pine trees were removed and

the area is under natural regeneration, with a high degree of invasion by the African grasses *Melinis minutiflora* and *Urochloa brizantha*.

Seed bank sampling

To collect the soil seed bank in invaded and non-invaded areas, we established plots (15x15m) in patches of at least 50% of dominance of *Melinis minutiflora* (MM), *Urochloa brizantha* (UB), and >90% native species (N, mainly composed by grasses, such as *Axonopus pressus*, *Aristida jubata*, *Loudetiospsis chrysothrix* and *Aristida riparia*. We established one plot in each patch, with a total of four patches/treatment (12 sample units).

In each plot, we sorted four subplots of 1x1m in a grid, as reference for the soil seed bank sampling. The soil seed bank was sampled monthly (April/2016 to March/2017, 12 months), and we used the subplot as reference, with 12 soil samples being collected surrounding each subplot. Each soil sample point was marked to avoid sampling at the same site in the following month (1 sample/subplot, 4 subplots/plot, 16 samples/treatment, total 48 samples/month). Considering that 90% of the seeds are found in the first five centimeters of depth (Andrade et al. 2002), we sampled 20x20x2 cm (including litter, totaling 800 cm³/sample).

Soil samples were sieved to remove larger fragments such as stems, roots and rocks. They were then divided equally into two samples of 400cm³, which went through different processes: one was used for the direct counting method and the other for the seedling emergence method, since the use of only one method may underestimate the species richness (Price et al. 2010).

The samples submitted to the direct counting process were sieved by different meshes: 0.2, 0.35 and 0.6mm. Afterwards, they were sorted in the lab,

with the help of magnifying glasses, and seeds found were counted and classified into native and exotic grasses. For the seedling emergence method, samples were transferred into trays (27.5 x 18.5 x 4.5 cm), perforated at the bottom to prevent water accumulation. The collected soil was distributed in the trays, forming layers of approximately 1cm high. Trays were conditioned in a greenhouse, where they were irrigated three times a day, totaling 36mL of water/day. Observations were carried out every 15days for four months.

Both seeds and seedlings were classified into two major groups: Invasive Grasses and Native Species. Invasive grasses were represented by *Urochloa brizantha* and *Melinis minutiflora*. The native species included only the graminoids (Cyperaceae and Poaceae).

Statistical analysis

To analyze the dynamics of the seed bank of invasive grasses and native graminoids and its variation in seed density over time, we used additive mixed modeling (GAMM). We proceeded to model selection based on the restricted maximum likelihood criteria (REML). We used treatment and time as fixed factors and plot as a random effect. All statistical analyzes were performed using R program (R Core Team 2016) with the packages *vegan* (Oksanen et al. 2016), *lme* (Bates et al. 2015), *mgcv* (Wood 2011) and *ggplot2* (Wickham 2009).

Results

The seed bank in the study area is mainly dominated by invasive species (Fig.1). *Urochloa brizantha* showed to have 4-fold the density of the native species (Fig.1), while *Melinis minutiflora* seed bank had the double density than the seed bank of native species (Fig.1).

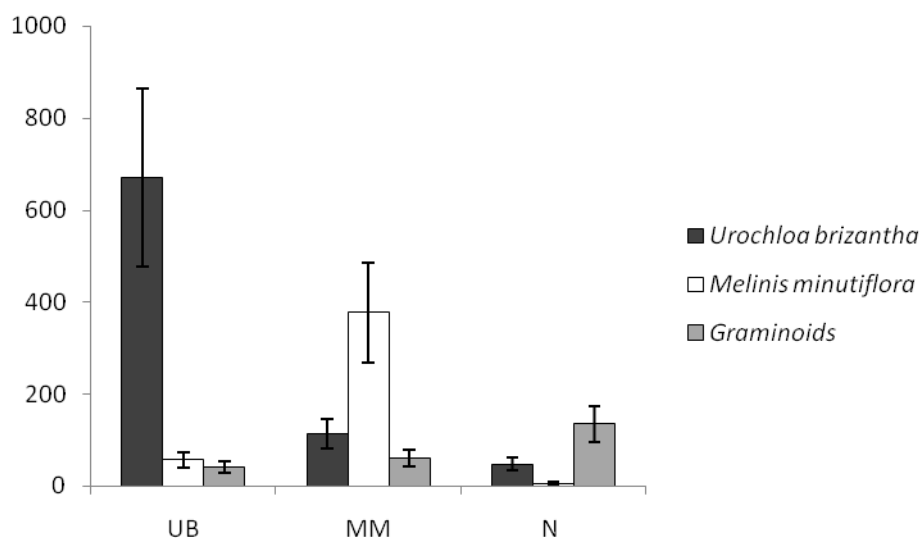


Figure 1. Density of the soil seed bank (mean $\pm$ SE) of *Urochloa brizantha*, *Melinis minutiflora* and native graminoids (Poaceae and Cyperace) in areas invaded by *Urochloa brizantha* (UB), *Melinis minutiflora* (MN) and dominated by native species (N) in open savannas of Cerrado using the seed counting.

In general, seeds of *Urochloa brizantha* corresponded to 55% (830 ± 239 seeds.m⁻², Fig.1) of seeds found in the soils and *Melinis minutiflora* represented nearly 30% (440 ± 127 seeds.m⁻², Fig.1) of the total soil seed bank. The native species dominated 15% (Fig.1), being composed mostly by Poaceae (157 ± 51 seeds.m⁻²) and Cyperaceae (78 ± 22 seeds.m⁻²).

In areas with the dominance of *U. brizantha* (UB), 94% of the seed bank was dominated by *U.brizantha* seeds (649 ± 371 seeds.m⁻²), when we used the the seed counting method. The density of this species oscillated throughout the year ($p<0.001$), peaking from June to September (1194 seeds.m⁻², Fig.2A). When we compared to the seedling emergence method, we found the same pattern, with 46% (23 ± 21 seeds.m⁻²) of *U. brizantha* seeds deposited in the soil seed bank being able to germinate (Fig.2B) and varying along the year ($p<0.001$). The density of *M. minutiflora* and native graminoid species seeds (Poaceae and Cyperaceae), comprised 14% (56 ± 27 seeds.m⁻²) and 17% (28 ± 14 seeds.m⁻²), respectively of the total seed bank in the UB area.

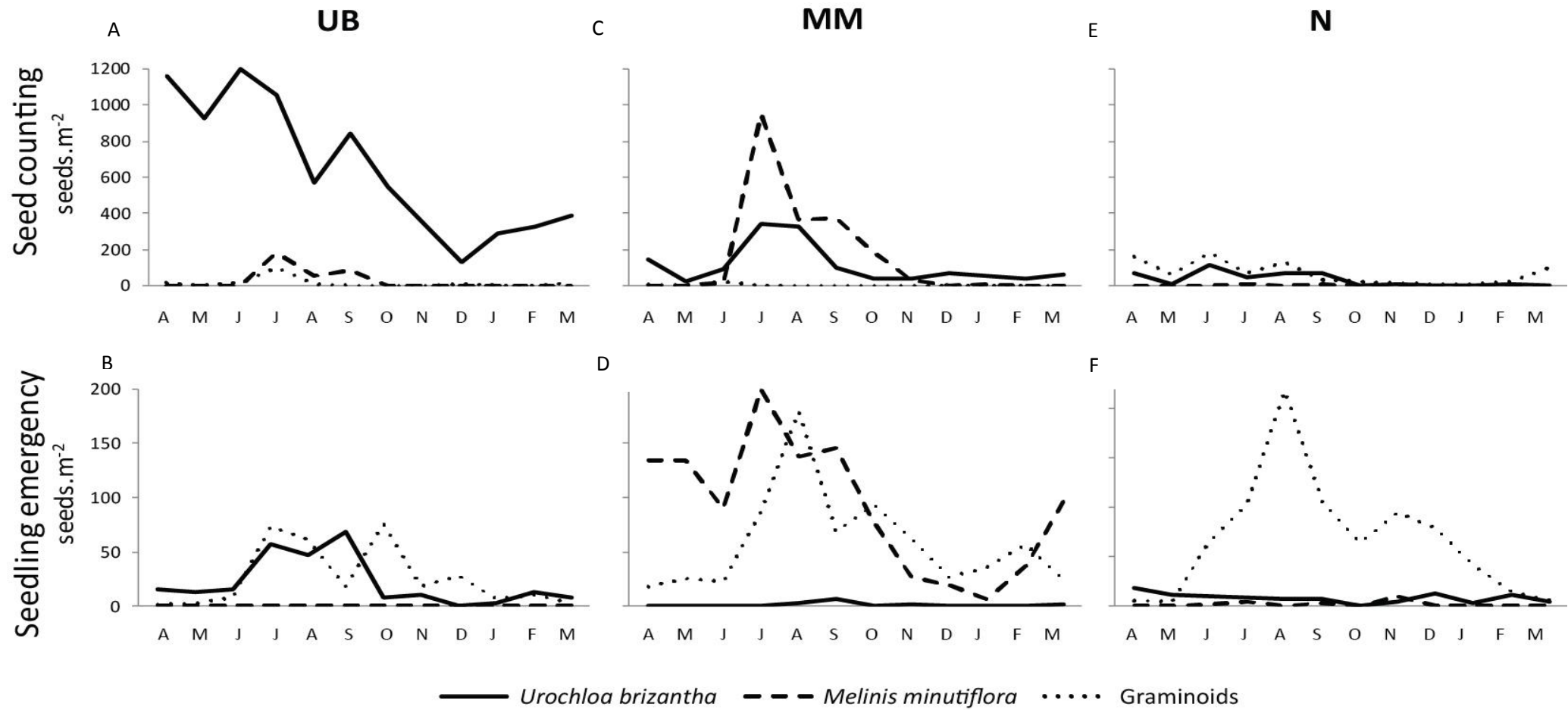


Figure 2. Dynamics of the soil seed bank (mean±EP) throughout the year of invasive grasses (*Urochloa brizantha* and *Melinis minutiflora*) and native graminoids (Poaceae and Cyperaceae) in areas invaded by *Urochloa brizantha* (UB), *Melinis minutiflora* (MM) and dominated by native species (N) in Cerrado areas using two methods: seed counting and seedling emergence.

The peak of *M.minutiflora* seeds in the soil seed bank of UB was in July (184 seeds.m⁻², Fig.2A), using the seed counting method. However, no seedlings were observed in the seedling emergence method for this species in areas invaded by *Urochloa brizantha* (Fig.2B). Both seed counting and seedling emergence methods did not detect a significant variation in the density of *M. minutiflora* in the treatment UB ($p > 0.05$, Fig2A-B). The highest density of native species seeds was found in July (102 seeds.m⁻², Fig.2A), represented only by *Gymnopogon foliosus* seeds, showing a significant variation throughout the year ($p=0.008$).

In areas invaded by *Melinis minutiflora* (MM), the soil seed bank was mainly composed by the invasive species, *M. minutiflora* and *U. brizantha* (Fig. 2C). Seeds of *M. minutiflora* dominated nearly 60% (285±161 seeds.m⁻²) of the seed bank with higher seed deposition in July (949 seeds.m⁻², Fig.2C), being the same pattern observed using the seedling emergence method (92±60 seeds.m⁻², Fig 2D). Both seed counting and seedling emergence were capable to detect the density variation of *M. minutiflora* seed bank throughout time ($p < 0.001$). *U.brizantha* seeds represented 40% (112±108seeds.m⁻²) of the total density using the seed counting method, with the highest density recorded in July (339 seeds.m⁻², Fig.2C), showing a significant variation along the year ($p < 0.001$). However, seedlings of *U. brizantha* emerging from the seed bank represented only 1% of the total density (2±1 seeds.m⁻², Fig 2D) in these areas (MM). The native species corresponded to 2% (7±4 seeds.m⁻²) of the soil seed bank when seeds were counted, and 38% (58±46 seeds.m⁻²) when the seedling emergence method was used, with a peak of density between June and August (180 seeds.m⁻², Fig.2D).

Native graminoids dominated 65% (68±61 seeds.m⁻²) of the soil seed bank in non-invaded areas, mainly represented by *Axonopus pressus* and

Gymnopogon foliosus seeds, with higher deposition in June (180 seeds.m⁻², Fig.2E). Both methods detected a variation on density throughout time ($p \leq 0.001$). *U. brizantha* seeds represented 33% (38 ± 35 seeds.m⁻²) of the seed bank, showing a significant variation throughout time ($p < 0.001$, Fig.2E). However, when the seedling emergency method was used, only 10% of the total seedlings were from *U. brizantha* (8 ± 5 seeds.m⁻², Fig.2F). *M. minutiflora* seeds comprised 2% (4 ± 2 seeds.m⁻²) of the total density and a significant variation along time was only detected when the seed counting method was used ($p = 0.04$, Fig.2F-E).

N treatment showed the highest species richness when compared to the other treatments (Table 1). Using the seed counting method, we found six species: *Andropogon bicornis*, *Andropogon lateralis*, *Aristida megamapotamica*, *Axonopus pressus*, *Gymnopogon foliosus* and *Sporobolus aeneus*, and a sedge species (Table 1). Only *Andropogon bicornis* did not germinate in the seedling emergency method. On the other hand, areas with dominance of invasive species (UB and MM), a lower number of species was found (Table 1). In these areas, *Andropogon lateralis* and *Sporobolus aeneus* were not found, and *Andropogon bicornis* was observed only when the seed counting method was used in areas dominated by *U. brizantha* (Table 1).

Table1. Percentage, density (mean $\pm$ SD) and richness of graminoid species found in the soil seed bank in different treatments: dominance of *Urochloa brizantha* (UB), *Melinis minutiflora* (MN) and native species (N), obtained using different methods, seed counting (Sc) and seedling emergence (Se), in the soil seed bank in *Campo sujo* in natural regeneration.

Treatment	Percentage (%)		Density		Richness	
	Sc	Se	Sc	Se	Sc	Se
UB	17	17	28 $\pm$ 14	28 $\pm$ 26	5	4
MM	5	39	7 $\pm$ 4	57 $\pm$ 46	4	4
N	78	44	68 $\pm$ 61	67 $\pm$ 61	7	6

Discussion

The high potential of regeneration of invasive species, through high seed production (Freitas & Pivello 2005; Martins et al. 2006) and germination percentage (Aires et al. 2013; Gorgone-Barbosa et al. 2016) allows the species to remain in the community, implying an increase in the deposition of their seeds in the soil seed bank (Brooks et al. 2010). The biotic and/or abiotic changes resulting from invasive species entering the community have a direct effect on soil seed bank dynamics (Brooks et al. 2010; Nguyen et al. 2017; Galloway et al. 2017), limiting resources for germination and establishment of seedlings of other species that are stored in the seed bank (Brooks et al. 2010; Bao et al. 2015).

Grass seeds need light to germinate (Zaidan & Carreira 2008; Musso et al. 2014; Kolb et al. 2016), and the accumulation of biomass by invasive species prevents the entry of light into the system, making it difficult for native species to germinate and establish (Clifton, 2004; Brooks et al. 2010). Germination and reproduction are the most important traits used to evaluate the dynamics of the soil seed bank (Bonis et al. 1995). Thus, the dominance of invasive species in the soil seed bank would lead to a higher probability of invasion success, producing a positive feedback on the invasion process.

The invasive species present adaptive advantages that allow these species to stand out in the regeneration niche, altering the dynamics and establishment of native species seed as a consequence of changes in the environment. Thus, the soil seed bank of invaded areas presents less species richness when compared to non-invaded areas (see Bao et al. 2014; Bao et al. 2015; Nguyen et al. 2017; Galloway et al. 2017). In general, our study showed that areas with the dominance of invasive species had higher densities of invasive species seeds than native ones in the soil seed bank, as we had expected, and similar as found in other studies (Nguyen et al. 2017; Galloway et

al. 2017). Although it is a common pattern in invaded communities, environmental filters can alter this dynamics. Flooding can be a factor that will prevent or at least decrease the establishment success of an invasive species, *Urochloa humidicola*, favoring thus, native species (Bao et al. 2014, 2015).

The local soil seed bank is dominated mainly by *Urochloa brizantha*. *Melinis minutiflora* showed to have a seed bank with lower density when compared to the other invasive species, showing that *Urochloa brizantha* is producing and dispersing more seeds, or its seeds may have a higher longevity than *Melinis minutiflora* seeds. In general, perennial species are known to have late germination and low seed production (Gillespie and Volaire 2017), but this pattern is not found in invasive species, which are able to invest in high reproduction rates and germination even when environmental resources are limited, allowing them to rapid establish in the area (Pyšek & Richardson 2007). Although a portion of their seeds can show physiological dormancy, daily temperature fluctuation experienced in the gaps after disturbance showed to break this dormancy and enhance germination of both invasive species (Carmona & Martins 2010; Aires et al. 2013; Gorgone-Barbosa et al. 2016). Moreover, the dormant seeds tend to form a persistent soil seed bank (Thompson et al. 2003), maintaining thus this species in the community throughout space and time (Carmona 1995; Silva & Dias-Filho 2001). In addition, these species have present low mean germination time (Aires et al. 2013; Gorgone-Barbosa et al. 2016, submitted), which gives them the advantage of germinating fast in newly open areas. Because of that, *Urochloa brizantha* can be pointed as an aggressive invasive species, which produces lots of seeds, with high percentage of germination, part of dormant seeds which forms a persistent soil seed bank, that can regenerate as soon as a gap is opened in the

vegetation. Thus, a seed bank dominated by invasive species would lead to a higher chance of invasion success after disturbance events.

Although a high density of *U. brizantha* was detected in the seed bank using the seed counting method, when the emergence method was used, the same density was not found, probably because part of the seeds has physiological dormancy (Adkins et al. 2002) and/or many seeds have no embryo (pers. observation). In areas invaded by *U. brizantha*, the presence of *M. minutiflora* and native species seeds in the soil seed bank was low probably because the presence of *U. brizantha* reduces the aboveground cover of native species and also, prevent native species to germinate due to allelopathy. (Barbosa et al. 2008; Oliveira et al. 2016). Even seedlings from *Melinis minutiflora* were not observed in the seedling emergence method in the UB areas, despite their presence when the seed counting method was used, confirming thus, that the presence of *Urochloa brizantha* also prevented the germination and establishment of the other invasive species.

In areas where native species dominated, the density of native species was higher than invasive ones, being represented mainly by one annual species: *Gymnopogon foliosus*, which produces seeds with high initial viability and germination percentage (Dairel & Fidelis, unpublished date). The abundance of annual native species in the seed bank favors the high capacity of regeneration and maintenance of species in the ecosystem, because they are able to persist in the system until favorable conditions for colonization occur (Bao et al. 2014; Barton et al. 2016). Despite the dominance of native graminoids in non-invaded areas, the presence of invasive species could already be observed in the soil seed bank, whilst this presence is insignificant in the aboveground vegetation (pers.obs.). Thus, there is a propagule pressure from neighboring areas, which

are invaded, in the patches of native vegetation, showing a potential new invasion occurring from the soil seed bank if a disturbance occurs, for example.

Native grasses are characterized by high production of unviable or empty seeds and some species can present dormancy, as observed in previous studies (Ramos et al. 2016; Kolb et al. 2016; Dairel & Fidelis, unpublished data). Thus, from the few species found in the local soil seed bank, only a small part would be able to germinate and establish. Therefore, grass regeneration from the soil seed bank can be difficult, since some species may form a transient soil seed bank (Andrade et al. 2002; Aires et al. 2013; Musso et al. 2014). *Gymnopogon foliosus* is the dominant native grass found in the soil seed bank, being also observed establishing in gaps within the aboveground vegetation (pers. obs.). Therefore, this species can be considered a potential competitor for the invasive species and can be used in restoration of open savannas.

The peak of seeds in the soil seed bank may be related to the reproductive phenology of the species present in the aerial plant community (Aires et al 2013). Using the seedling emergence method, we observed that the dynamics of the soil seed bank varied with the time of dispersion of the dominant species in each treatment (UB, MM and N), justifying the peaks found between June to September. The flowering of grasses found in the area follows the seasonal pattern already described in Cerrado, occurring in the wet season (Munhoz & Felfili 2007; Ramos et al. 2014) and their seeds were stored in the soil seed bank, waiting the next rainy season, conferring advantages to their establishment (Almeida 1995; Ramos et al. 2017). On the other hand, the invasive species have disperse later, and together with the high germination percentages, they have an advantage in the colonization of a new area (Aires et al. 2013), increasing thus, the propagule pressure in non-invaded areas.

Finally, the use of two different methods to evaluate the soil seed bank showed to be very efficient, mostly to identify species with dormancy, that would not germinate using the seedling emergence method alone. According to Prince et al. (2010), the use of two methods (seed counting and seedling emergency) is more efficient to estimate species richness and seed density, since it is possible to detect seeds of species that does germinate, either because they are empty or dormant. In our study, we were able to infer factors that are preventing germination (e.g. allelopathy) when the seedling emergence method was used, as observed in areas invaded by *Urochloa brizantha*. Therefore, we recommend the use of two methods for the study of soil seed banks, mostly in ecosystems with high dominance of species with dormant species, such as tropical savannas (Dayrell et al. 2016).

Conclusion

The invasion of African grasses in Cerrado open savannas is changing the composition and dynamics of the soil seed bank from both invaded and non-invaded areas, affecting thus vegetation regeneration by seeds in this system. The efficiency of seed production and the high germination percentage found in invasive species resulted in a soil seed bank dominated by these species in invaded areas. In addition, the aboveground dominance of invasive species hinders the entry of light and probably of native species seeds into the system. Thereby, the presence of invasive grasses may be compromising the reestablishment of native grasses through of the soil seed bank. Therefore, it is urgently needed that management practices are applied in invaded areas, in order to control the entrance of *Urochloa brizantha* and *Melinis minutiflora* in both above- and belowground parts of the plant community.

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What influences grass germination in Cerrado?

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Abstract

Studies about dormancy and factors influencing the germination of native grasses in Cerrado are recent and many gaps still need to be filled. In Brazilian Savannas, fire is common and extremely important to maintain structure, diversity and vegetation physiognomy. In Savanna vegetation as Cerrado fire is thus, a frequent disturbance and its occurrence opens gaps in the herbaceous layer. The temperature fluctuation in the soil surface, especially after fire events when more gaps are available is also an important environmental filter for species germination. Therefore, this study aims to evaluate the germination of native grasses, using daily temperature fluctuations and heat shock, since most grasses may have physiological dormancy. To evaluate the effects of daily temperature fluctuations, ten grass species were collected in the field, placed to germinate in germination chambers simulating thermal fluctuation of the day (19°C to 55°C), for 45 days (25 seeds/replicate, 4 replicates). Control seeds were kept in room temperature for the same period. For the heat shock experiments, nine grass species were submitted to the following heat shock treatments: 100°C for 1 minute, 100°C for 3 minutes, 200°C for 1 minute and a control without heat shock, which was not exposed to the heat shocks (20 seeds/replicate, 5 replicates). After both treatments, temperature fluctuations and heat shock, seeds were put to germinate for 30 days (27°C, 12/12hs-light) and non-germinated seeds were tested for viability with tetrazolium tests. Germination percentages were low for all study species, except for *Andropogon lateralis*, *Aristida riparia*

and *Gymnopogon foliosus*, which showed germination >50%. *Axonopus aureus*, *Loudetiopsis chrysothrix* and *Sporobulos cubensis*, those that had physiological dormancy were stimulated to germinate when submitted to daily temperature fluctuations, and *S. cubensis* resist to heat shock of 200°C. For all other species, the treatments did not change germination percentages.

Keywords: Cerrado, native grass, seed germination, physiological dormancy, temperature fluctuation, heat shock

Introduction

Tropical savannas are characterized by a continuous herbaceous layer, with C4 grasses being one of the most representative group and the main fuel load for savanna fires (Trollope 1982; Bourliere & Hadley 1983; Veldman et al. 2015). Fire is a common disturbance in these ecosystems (Bond & Van Wilgen 1992) and extremely important to maintain structure, diversity and vegetation physiognomy (Bond & Keeley 2005). Furthermore, grasses have a great variability in morphological and physiological traits (Risser 1985; Sarmiento 1992), which leads to a high resilience to fire (Bond 2004), and are considered the major component of tropical savannas (Sarmiento 1992).

Grasses have different strategies that allow their dominance in fire-prone ecosystems, such as the C4 photosynthetic pathway, that facilitates the accumulation of biomass, inducing high photosynthetic rates and efficiency in the use of nutrients (Osborne 2008; Edwards et al. 2010). Because of the high accumulation of biomass, it has a positive feedback in cycle grass-fire (D'Antonio & Vitousek 1992). Furthermore, fire-prone species usually have protected buds and storage reserve that allow them to quickly resprout after defoliation

(resprouters, Clarke et al. 2013), whilst some species persist in the post-fire system by seedling recruitment (seeders, Whelan 1995; Bond & Van Wilgen 1996). Therefore, species depending on seeds to regenerate after fire must have seed and germination traits related to post-fire regeneration that will enable them to persist in these ecosystems (Grubb 1977; Jimenéz-Alfaro et al. 2016).

Despite the high production of seeds, grass species have low recruitment by seeds (Foster 2001; Foster & Tilman 2003, Foster 2007), and less is known about germination traits of grasses (Aires et al. 2013, Commander et al. 2017). Dormancy, environmental factors (such as water, light, fire), as well as competition and longevity can affect the establishment of grass species by seeds (Cole et al. 2005, Commander et al. 2017). In tropical savannas, grasses usually produce a high proportion of unviable seeds or even seeds with no embryo (Carmona et al. 1999; Wright et al. 2014; Dayrell et al. 2016) and generally have physiological dormancy (Baskin & Baskin 2014; Erickson et al. 2016; Dayrell et al. 2016; Commander et al. 2017), that can be broken through the exposure to high temperatures (see Adkins et al. 2002; Ramos et al. 2016; Commander et al. 2017). Thus, tropical savanna grasses usually present low percentage of germination (LeStradic et al. 2015; Ramos et al. 2016; Kolb et al. 2016).

Species from fire-prone ecosystems may have adaptive germination traits to fire (Keeley et al. 2011; Lamont & He 2017), such as breaking of dormancy by the exposure to high temperatures (see Moreira et al. 2010) or temperature fluctuation (see Santana et al. 2013). Dormancy can be affected indirectly and directly by fire. Indirectly by exposure by daily temperature fluctuation as a result of the opening vegetation and creation of gaps (Santana et al. 2013; Daibes et al. 2017) or directly with the heat shock (Moreira et al. 2010).

According to Ramos et al. (2016), grasses with dormant seeds are more tolerant to the exposure to high temperatures than non-dormant seeds, being

able to tolerate a great temperature. For example, *Aristida riparia* and *Aristida setifolia*, two common grasses found in Cerrado, showed to be resistant to 110°C for 5 minutes. Kolb et al. (2016) concluded that grass species show low germinability and exposing seeds to alternating temperatures (30°C and 20°C) increased germination, but in low percentage. Moreover, these species can have their germination enhanced by the exposure to smoke (see Moreira et al. 2010).

Therefore, the aim of our study is to evaluate the germination and viability of Cerrado native grasses seeds when submitted to daily temperature fluctuation and heat shock treatments. Our hypothesis is that these treatments will be able to break the physiological dormancy of these seeds and that seeds of native grasses will tolerate the exposure to high temperatures. We also evaluated seed dormancy and longevity in order to elucidate the process involving seed germination in a fire-prone ecosystem.

Material e methods

Study area and seed collection

Seeds of the native grasses were sampled in two different areas of Cerrado: Estação Ecológica de Itirapina (EEI, Southeastern Brazil, 47° 51'-48'W and 22° 11'-22° 15'S, 2300ha) and Reserva Natural Serra do Tombador (RNST), located in Central Brazil (47° 45'-51'W and 13° 35'-38'S, 8900ha). Both areas have seasonal climate, with a well-marked dry season (from May to September), and a wet season from October to April. At the EEI, average annual temperature is 22° C and average annual precipitation, 1459mm (Zanchetta et al. 2006). The RSNT shows an average temperatures ranging from 22° to 25°C and annual precipitation from 1300 to 1500mm (Fundação Grupo Boticário, 2011). The main vegetation type where seeds were collected was *campo sujo* (Table 1), which is

an open savanna dominated by a rich herbaceous layer with scattered shrubs and small trees (Coutinho 1982). However, two species were collected in both *campo sujo* (CS) and wet grasslands (WG, Table 1).

Table 1. Grass species sampled in the two sites (EEI: Estação Ecológica de Itirapina, Southeastern Brazil, and RNST: Reserva Natural da Serra do Tombador, Central Brazil), in different vegetation types (CS: campo sujo; WG: wet grassland), with the date of collection and different treatments applied: TF- daily temperature fluctuation, HS- heath shock, and the species which had their longevity (L) tested.

Species	Site	Vegetation Type	Collection date	TF	HS	L
<i>Andropogon lateralis</i> L.	EEI	CS/WG	April/2016	x	x	x
<i>Andropogon bicornis</i> L.	EEI	CS/WG	April/2016	x	x	x
<i>Aristida megapotamica</i> Spreng.	EEI	CS	April/2016	x	x	x
<i>Aristida riparia</i> Trin.	EEI/RNST	CS	April/2016	x	x	x
<i>Aristida jubata</i> (Arechav.) Herter	EEI	CS	February/2016	x	x	
<i>Axonopus aureus</i> P.Beauv	RNST	CS	October/2016	x		
<i>Gymnopogon foliosus</i> (Willd.) Nee	EEI	CS	June/2016	x	x	x
<i>Loudetiopsis chrysotrix</i> (Nees) Conert	EEI	CS/WG	April/2016	x	x	x
<i>Sporobolus aeneus</i> R.Br.	EEI	CS/WG	April/2016	x	x	x
<i>Sporobolus cubensis</i> Hitchc.	RNST	CS	October/2016	x	x	

We collected seeds of eleven native grass species that are dominant in the areas (Table 1). Seeds were collected from different populations and individuals (>15 individuals) to guarantee the genetic variability. They were sorted in the lab and empty seeds were counted (from a total of 100 seeds from each species) and not used for germination trials. Seeds were not stored for more than three months before germination trials and only undamaged and full seeds were used.

Germination experiments

Daily temperature fluctuation (TF)

To evaluate the effects of daily temperature fluctuation on grass seeds germination, four replicates of 25 seeds of each species were placed in different germination chambers, which simulated the temperature variation of the day (ranging from 19° to 55°C) for 45 days (Table 1). These temperatures were previously measured in the field for 90 days and we used the average temperature of each hour of the day (for more information, see Daibes et al. 2017). Each replicate was placed in a different chamber, to avoid pseudoreplication. Four replicates with 25 seeds of each species were not submitted to treatment (control), and left for the same period in room temperature.

Heat shock treatment (HS)

For the heat shock experiments, we used nine grass species, due to the amount of available seeds (see Table 1). We had five replicates with 20 seeds for each species that were submitted to the following heat shock treatments: 100°C for 1 minute, 100°C for 3 minutes, 200°C and a control, which was not exposed to the heat shocks. We chose these temperatures following the studies of Miranda et al. (1993), that measured fire temperatures in different Cerrado vegetation types, and Fichino et al. (2016), that performed heat shock experiments using Cerrado species. Heat shocks were performed in a pre-heated electronic muffle and each replicate was placed separately to avoid pseudoreplication.

Longevity (L)

Seeds of seven grass species (Table 1) were stored after collection for 6 and 12 months in paper bags and ziplock at room temperature for further

germination experiments, to evaluate their longevity. We used four replicates of 25 seeds for each species.

Germination procedures

After the treatments (temperature fluctuation, heat shocks, longevity), seeds were placed in Petri dishes with two filter papers imbibed in distilled water, and they were placed to germinate in germination chambers with constant temperature (27°C) and light regime of 12 / 12h (Fichino et al. 2016). We performed observations three times a week, for 30 days. Seeds that showed radicle or cotyledons were considered to be germinated and removed from the Petri dishes. At the end of the germination trials, ungerminated seeds were submitted to the tetrazolium test (1%) to verify their viability (Lakon, 1949).

Data Analyses

To analyze differences among germination and viability percentages for each treatment and species, we used generalized linear models (GLM), with a quasi-binomial distribution (values in percentage of germinated seeds and their viability) and the Post hoc function in the Tukey's test was used to compare the effect of the treatments with the control for each treatment (daily temperature fluctuation, heat shock and longevity). All analyzes were done in software R 3.2.5 (R Development Core Team, 2011) with the packages *vegan* (Oksanen et al. 2017), *lme* (Bates et al. 2015), *multcomp* (Hothorn et al. 2008), *lattice* (Sarkat et al. 2015), *graphics*.

Results

In general, species showed low percentages of empty seeds, with only two species having more than 50% of their seeds with no embryo: *Andropogon*

bicornis (69%) and *Loudetiopsis chrysothrix* (70%, Table 2). Moreover, two species had seed viability lower than 50% at the beginning of the experiments: *Andropogon bicornis* (21%) and *Aristida megapotamica* (35%, Table 2). Only three species showed to have physiological dormancy: *Axonopus aureus*, *Loudetiopsis chrysothrix* and *Sporobolus cubensis* (Table 2).

Table 2. Percentage of initial viable and empty seeds of native grasses of Cerrado. Classification of species in non-dormant (ND) and dormant species (Physiological Dormancy – PD).

Species	Initial viability (%)	Empty seeds (%)	Dormancy type
<i>Andropogon lateralis</i> .	84	11	ND
<i>Andropogon bicornis</i>	21	69	ND
<i>Aristid amegapotamica</i>	35	40	ND
<i>Aristida riparia</i>	61	37	ND
<i>Aristida jubata</i>	56	31	ND
<i>Axonopus aureus</i>	79	28	PD
<i>Axonopus pressus</i>	14	89	ND
<i>Gymnopogon foliosus</i>	88	20	ND
<i>Loudetiopsis chrysothrix</i>	69	70	PD
<i>Sporobolus aeneus</i>	51	12	ND
<i>Sporobolus cubensis</i>	98	10	PD

Seed germination of most species was not affected by daily temperature fluctuations (Fig.1). However, the species that showed to be dormant had their germination percentage increased by this treatment. *Axonopus aureus* showed an increase of 45% in relation to the control ($p=0.001$, Fig.1), *Sporobolus cubensis* and *Loudetiopsis chrysothrix* had an increase of 57% and 25%, respectively (Fig.1, $p=0.006$ and $p=0.03$, respectively). The viability of all study species was not affected by temperature fluctuation (Fig.1, $p>0.05$).

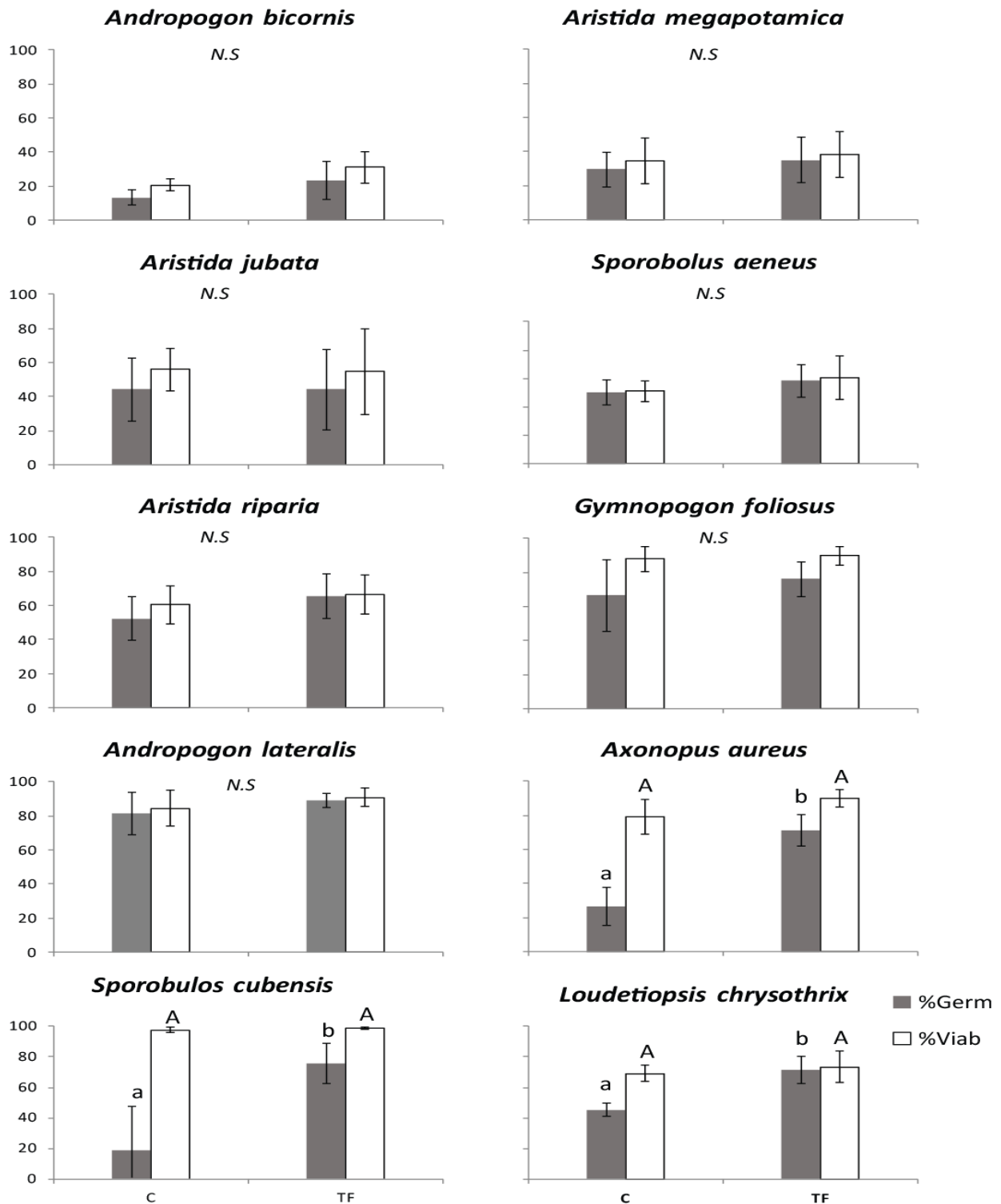


Figure 1. Effects of daily temperature fluctuation on seeds germination (gray bar) and viability (white bar) of 10 native grass species from Cerrado (mean $\pm$ SE) when exposed to the different treatments: C (control, no exposure to temperature fluctuation) and TF (temperature fluctuation). Different letters mean significant differences between treatments for seed germination ($P \leq 0.05$). There were no significant differences between treatments for seed viability.

All species resisted up to 100° for 1 minute, having neither germination nor viability affected by the treatments (Fig. 2, $p>0.05$). *Aristida megapotamica* and *Gymnopogon foliosus* seeds resisted to the exposure to high temperatures, having their germination and viability percentage not affected by the heat shock treatments (Fig.2, $p>0.05$). However, the exposure to 100°C for 3 minutes led to a decrease in 62% of germination of *Andropogon lateralis* seeds ($p=0.002$, Fig.2) and 66% of viability ($p=0.005$, Fig.2) in relation to the control. Seeds of *Sporobolus aeneus* also decreased in germination and viability when exposed to 100°C 3'(p=0.02, Fig.2).

Most species showed a decrease in both germination and viability when exposed to 200°C. Two species did not germinate at all at this temperature, and all seeds showed to be dead after the treatment (*Andropogon lateralis* and *Sporobolus aeneus*, Fig.2). Other species showed a significant decrease in germination and viability percentage: *Loudetiopsis chrysothrix*, *Aristida jubata*, *Aristida riparia*, ($p\leq0.05$, Fig.2). *Andropogon bicornis* showed a decrease in 21% in viability ($p<0.001$), but germination percentages did not differ when seeds were exposed to 200°C. Finally, one species had its dormancy broken when seeds were exposed to 200°C: *Sporobolus cubensis* showed an increase of 48% in germination ($p<0.001$, Fig.2).

Seeds stored for six months usually had their germination and viability not affected by time ($p>0.05$, Table 3). *Aristida megapotamica* showed an increase of 50% in germination and viability after six months ($p<0.001$ and $p=0.006$, respectively, Table 3). On the other hand, seeds of *Aristida riparia* decreased in germination and viability percentage after being stored for six months ($p=0.014$ and $p=0.003$, respectively, Table 3).

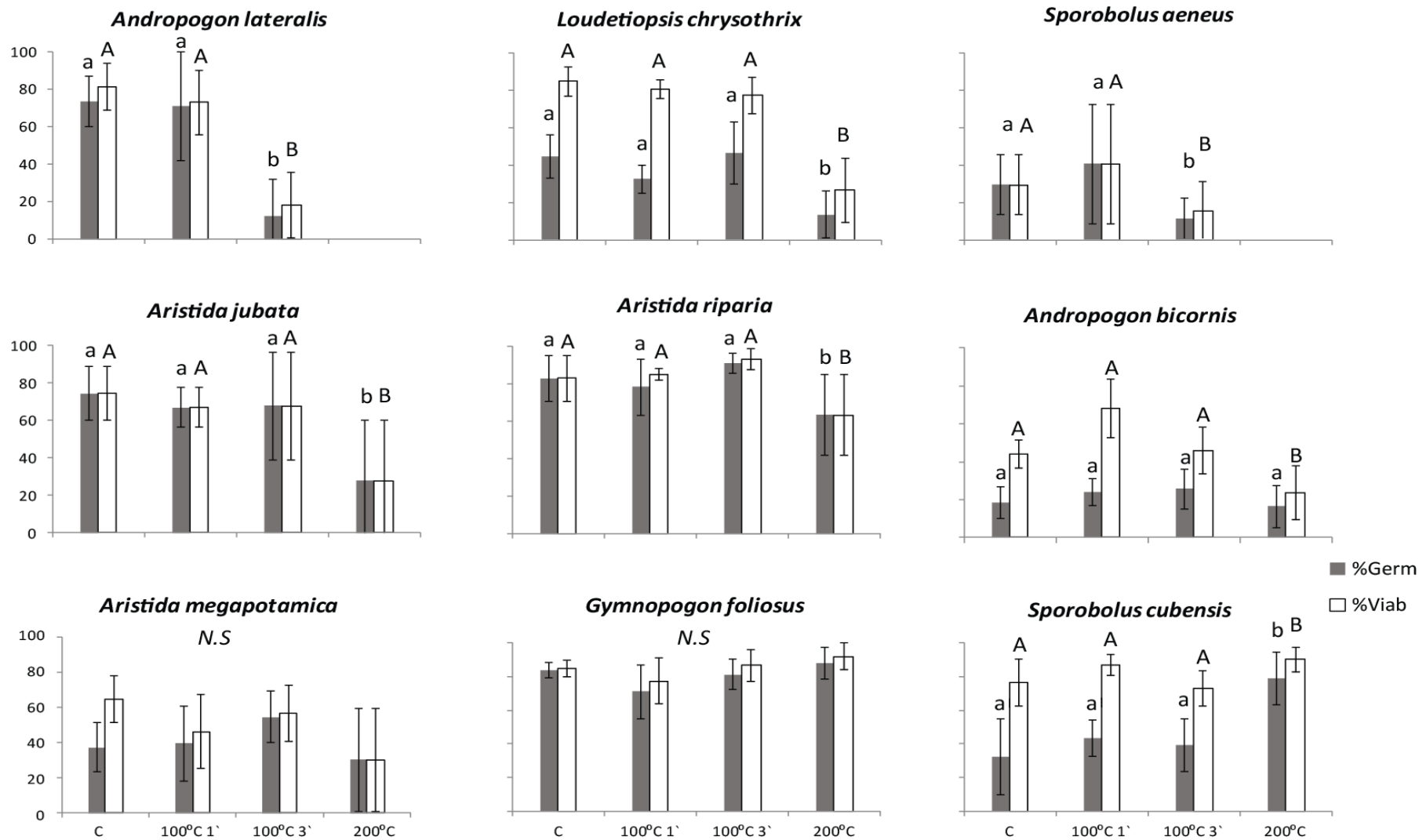


Figure 2. Germination percentage (mean±SE) on 9 native grass species of Cerrado, according to the different heat shock treatments: Control (C), 100°C 1', 100°C 3' and 200°C.

One year after seed storage, seeds of *Andropogon bicornis* and *Gymnopogon foliosus* showed the same germination and viability when compared to the beginning of the experiments ($p>0.05$, Table 3). However, some species showed a significant decrease in germination and viability, such as *Andropogon lateralis* (34% in germination percentage and 29% in viability), *Aristida riparia* (24% in germination and 23% in viability), and *Sporobolus aeneus* (19% in germination and 18% in viability, Table 3). However, seeds of *Aristida megapotamica* increased in 66% in germination ($p<0.001$) and 60% in viability in relation to the beginning of the experiments ($p=0.003$, Table 3). Moreover, *Loudetiopsis chrysotrix* seeds showed higher germination percentages and viability one year after being stored ($p\leq 0.05$, Table 3).

Table 3. Germination and viability percentages (mean $\pm$ SD) for seeds from different native grass species of Cerrado just after seed collection (0) and 6 and 12 months after storage.

Species	Germination (%)			Viability (%)		
	0	6	12	0	6	12
<i>Andropogon bicornis</i>	13 $\pm$ 4.41	7 $\pm$ 5.08	0	21 $\pm$ 3.6	13 $\pm$ 11.1	0
<i>Andropogon lateralis</i>	81 $\pm$ 12.54	72 $\pm$ 11	47 $\pm$ 17.7**	84 $\pm$ 10.5	76 $\pm$ 8.5	55 $\pm$ 15.8**
<i>Aristida megapotamica</i>	29 $\pm$ 10.05	84 $\pm$ 4.7**	95 $\pm$ 5**	35 $\pm$ 13.5	88 $\pm$ 3.5**	95 $\pm$ 5**
<i>Aristida riparia</i>	52 $\pm$ 10.6	10 $\pm$ 6.75*	28 $\pm$ 13.7**	61 $\pm$ 12.1	13 $\pm$ 7.7**	38 $\pm$ 18*
<i>Gymnopogon foliosus</i>	66 $\pm$ 21	85 $\pm$ 5.1	42 $\pm$ 17.1	88 $\pm$ 7.1	86 $\pm$ 6.2	53 $\pm$ 19.2
<i>Loudetiopsis chrysotrix</i>	45 $\pm$ 4.11	-	75 $\pm$ 10.32**	69 $\pm$ 5.2	-	80 $\pm$ 12**
<i>Sporobolus aeneus</i>	50 $\pm$ 8.7	47 $\pm$ 5.98	31 $\pm$ 13.21*	51 $\pm$ 7.2	47 $\pm$ 6	33 $\pm$ 12.4*

(*) $p \leq 0.05$; (**) $p \leq 0.001$;

Discussion

Dormancy is a mechanism that prevents germination of seeds in a certain period of time on unfavorable environmental conditions such as temperature, light or water availability (Baskin & Baskin 2004), which can be an efficient strategy for the maintenance of species in the ecosystem. Savanna ecosystems have the higher percentage of dormant species, when compared to tropical forests. Moreover, grasses and sedges showed to have the highest percentages of

dormancy within the monocots (Dayrell et al. 2016), and thus, factors that could break dormancy should be considered. Environmental conditions, such as the range of temperature, are known to be essential for grass germination (see Carmona et al. 1998; Zaidan & Carreira 2008; Musso et al. 2014; Kolb et al. 2016). Therefore, studies elucidating the mechanisms involved on seed germination are crucial for the understanding of post-fire plant community regeneration by seeds.

In fire-prone ecosystems, fire events are fundamental for vegetation dynamics (Bond & Keeley 2005), opening gaps within the vegetation (Fidelis et al. 2012), and creating new sites for species to establish (Grubb 1977). In these gaps, conditions may be different, showing a higher daily temperature fluctuation in the soil (Santana et al. 2013; Fidelis & Blanco 2014; Daibes et al. 2017), which may directly influence plant establishment by seeds. Moreover, fire itself influences seed germination by increasing temperatures, which could have positive (break of dormancy, smoke, see Keeley et al. 2011) and negative (kill the seeds) effects on seed germination. The pattern found can favor recolonization by grass species through seeds, taking into account the trade-off between seed trait (resistance) and environmental factors (high temperatures) that the seeds are submitted.

In this study, three species showed to have physiological dormancy: *Axonopus aureus*, *Loudetiopsis chrysothrix* and *Sporobolus cubensis*. These species had their dormancy broken by daily temperature fluctuation, showing that this is an important mechanism for grass germination in Cerrado. At the same time, this treatment did not alter the germination percentage of non-dormant species, showing that these species have seeds that tolerate a high range of temperature in the soil. Similarly, the viability of non-dormant seeds was also not affected. Some studies using Cerrado grasses showed that alternating two

temperatures (e.g. 15°C/25°C or 20°C/30°C) affected negatively seed viability (Musso et al. 2014; LeStradic et al. 2015).

Many grass species are tolerant to the exposure to high temperatures (Scott et al. 2010, Gonzalez and Ghermandi 2012, Ramos et al. 2016), and had their dormancy broken as observed for *Sporobolus cubensis*. This species had its dormancy broken by both daily temperature fluctuation and heat shock at 200°C, indicating that this species have a great advantage in post-fire environments. Indeed, this species resprout and flowers rapidly after fire (Zirondi et al., in preparation), releasing its seeds whilst gaps are still available. Thus, seedling establishment of this species would be from the recruitment from the seed bank, that would be fulfilled after each fire event. Probably, this might be the strategy used by other grass species with fire-related cues for germination in Cerrado areas, such as *Mesosetum ferrugineum*, which also had its dormancy broken after being exposed to high temperatures (Ramos et al. 2016), resprouting and flowering vigorously after fire (Zirondi et al., in preparation). Furthermore, species with dormant seeds had their germination triggered by the exposure to both daily temperature fluctuation, as well as to high temperatures. These seeds may present higher contents of heat shock proteins (Ramos et al. 2016), which could be related to fire events (adaptive traits, Keeley et al. 2011, Lamont & He 2017) or to dehydration (exaptation, Bradshaw et al. 2011).

Fire-prone species can be stimulated to germinate after fire or be tolerant, with no changes in their capacity to germinate and decreased in viability (Paula & Pausas 2008; Fichino et al. 2016). In our study, all species, with dormant and non-dormant seeds, resisted up to 100°C. In open savannas, fire is usually fast with low temperatures at the surface and even lower in the first 3 cm of the soil (Miranda et al. 1993; Schmidt et al. 2017; Rissi et al. 2017), showing that seeds would survive the passage of fire in these areas. Thus, to resist high

temperatures is also an important trait in fire-prone ecosystems (Fichino et al. 2016; Jiménez-Alfaro et al. 2016), because it will assure the seed bank to be fulfilled if fire occurs during seed dispersal and also, it would enable seedling recruitment from seed bank in post-fire environments.

Only three species had no fire-resistant seeds (*Andropogon lateralis*, *Loudetipis chrysothrix* and *Sporobolus aeneus*). Seeds of these species were collected in both wet grasslands and open savanna, showing that the environment would influence germination traits and responses to fire. Indeed, grass species from wet grasslands had lower germination percentages and also lower resistance to the exposure to fire temperatures than grass species from open savannas (Ramos et al. 2016). Fires in wet grasslands usually have lower maximum temperature than other open savannas in Cerrado, with a short residence time at 1cm (Schmidt et al. 2017) and thus, seeds of species from these grasslands are usually not exposed to hot fires, as the ones from open savannas, and thus, the evolution of fire-resistant seeds might be related to habitat moisture (Ramos et al. 2016).

Finally, to understand how seed and germinations might be affected by fire, we should also understand their longevity, since most fires in Cerrado occur during the dry season (Pivello 2011) and most grass species disperse during the rainy season (Munhoz & Felfili 2007; Ramos et al. 2014). Therefore, dormant seeds might be in the seed bank when fire occurs and if they are still viable, grass seedlings might be recruited after fire events, when the first rains occur in the system. Most of the study species had seeds with low longevity, since viability percentages decline after storage in comparison to freshly collected seeds. In general, native grass seeds have low longevity, reinforcing the formation of a transient soil seed bank for perennial species (Aires et al. 2013; Andrade & Miranda 2014), with many seeds losing their ability to germinate after the first

year of dispersion (Thompson & Grime 1979; Aires et al. 2013). However, some grass species had both germination and viability percentage increased as they were stored for longer periods (Aires et al. 2013; Kolb et al. 2016). *Aristida megapotamica* showed an increase of 25% in seed viability, according to Martin (1946), grasses have seeds with fully developed embryos and therefore, they cannot present morphological dormancy. Thus, the increase in germination and viability in these species could indicate that our tetrazolium tests were not efficient to identify viable embryos in freshly sampled seeds in species with high dormancy levels, as observed in other fire-prone ecosystems (Ooi, pers. comm.).

Conclusion

Our results showed that some grass species showed low germination percentage, but high initial viability, indicating the presence of physiological dormancy. These species had their dormancy broken by the exposure to daily temperature fluctuation and heat shock, being an important strategy in the post-fire environment. Despite the break of dormancy, most grass species showed to have fire-tolerant seeds (to 100°C), except the species from wet grasslands, showing the importance of the environment on the trait responses to fire. Finally, species showed to have low longevity, forming thus, a transient seed bank. We showed the importance of fire-related cues for germination in Cerrado grass species, showing the importance not only to study the direct effects of fire (heat shock), but also the indirect ones (daily temperature fluctuation) and relating these results to the presence of dormancy and environment where seeds were collected. Studies that elucidate the mechanisms involved in seedling recruitment and persistence of species in post-fire environments are of crucial importance for the better understanding of plant assembly. Since grasses are the dominant group in open savannas, further studies using this group should be carried out.

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CONSIDERAÇÕES FINAIS

O processo de invasão biológica se tornou uma das maiores preocupações para conservação do ecossistema atualmente (Callaway and Maron 2006; Bajwa et al. 2016). A dinâmica natural de comunidades vegetais vem sendo fortemente modificada pela presença de espécies invasoras, sendo a invasão por gramíneas africanas uma das principais ameaças ao Cerrado (Pivello et al. 1999; Durigan et al. 2007). As áreas sob regeneração natural podem ser mais facilmente invadidas e, após eventos de distúrbio, novos indivíduos podem ser recrutados a partir de um banco de sementes viável.

Nesse sentido, o objetivo geral do presente trabalho foi analisar a dinâmica do banco de sementes de gramíneas invasoras e nativas de uma área em regeneração de campo sujo elucidando, assim, o potencial de regeneração de gramíneas do banco de sementes. Para tal, os objetivos específicos se basearam nas perguntas: 1) Como é a dinâmica do banco de sementes de gramíneas invasoras e nativas em áreas com e sem invasão biológica, em uma área de campo sujo em regeneração? e 2) Como os efeitos de flutuação térmica e choques de temperatura afetam a germinação de gramíneas nativas?

No primeiro capítulo encontramos que o banco de sementes do solo na área de estudo é dominado principalmente por espécies invasoras. *Melinis minutiflora* apresentou o dobro da densidade de sementes no solo quando comparada às sementes de espécies nativas, enquanto *Urochloa brizantha* apresentou uma densidade 4x maior do que espécies nativas, representando mais da metade das sementes presentes no banco de sementes do solo da área de estudo. Áreas invadidas apresentaram alta densidade de sementes invasoras, *Urochloa brizantha* e *Melinis minutiflora*, no banco de sementes do solo. As espécies invasoras dominantes na comunidade vegetal aérea apresentaram alta densidade de sementes no solo. Desta forma, parcelas

invasoras por *Urochloa brizantha* apresentaram alta densidade de sementes dessa espécie no solo, e o mesmo ocorreu para áreas com dominância de *Melinis minutiflora*. Apenas em áreas não invadidas apresentaram densidade de sementes de espécies nativas superior a 50%. No entanto, a presença de uma grande quantidade de propágulos de espécies invasoras no banco de sementes do solo foi encontrada. Assim, a entrada de espécies invasoras no sistema tem dificultado, por uma série de fatores, o estabelecimento de espécies nativas, tanto na vegetação aérea, quanto já no banco de sementes do solo. Portanto, havia necessidade de se entender os mecanismos que envolviam a germinação de sementes de espécies nativas em relação a um distúrbio. Nesse sentido, o segundo capítulo teve o intuito de analisar os fatores diretos e indiretos, provocados pelo fogo, que influenciam a germinação de gramíneas nativas, inferindo o potencial dessas espécies em se estabelecerem na comunidade via banco de sementes do solo após distúrbio como o fogo.

No segundo capítulo, a grande maioria das gramíneas nativas estudadas apresentou baixa porcentagem de germinação nos três tratamentos aplicados (controle, flutuação diária de temperatura e choques térmicos). Apesar desse padrão, foi encontrada baixa porcentagem de sementes vazias e alta viabilidade inicial, sugerindo que as baixas porcentagens de germinação estariam relacionadas à dormência fisiológica encontrada em algumas espécies. As espécies, em sua maioria, não responderam à amplitude térmica diária. Porém, espécies com dormência fisiológica (*Axonopus aureus*, *Loudetipsis chrysothrix* e *Sporobulos cubensis*) tiveram essa dormência quebrada submetidas aos tratamentos de flutuação térmica diária, e *Sporobulos cubensis* também teve sua dormência quebrada quando submetida aos choques de temperatura de 200°C.

Com os resultados, notamos que áreas com invasão biológica apresentam o banco de sementes do solo com alta densidade de *Urochloa*

brizantha e *Melinis minutiflora* e em áreas onde não há dominância de invasoras na comunidade área, muitas sementes de *Urochloa brizantha* e *Melinis minutiflora* já fazem parte do banco de sementes do solo. Contudo, é de extrema importância práticas de manejo e controle de espécies invasoras, tanto no quesito de comunidade vegetal aérea, como na propagação de sementes que se encontram disponível no banco de sementes do solo, meio pelo qual, *Urochloa brizantha* e *Melinis minutiflora* são capazes de reconstituir a biomassa aérea da comunidade após eventos de distúrbio.

O nicho de regeneração das espécies invasoras sobressai quando comparado ao das espécies nativas, conferindo-lhe vantagens adaptativas à colonização do ambiente (Hughes & Vitousek 1993; Levine et al. 2003). Sendo assim, a regeneração de gramíneas nativas via banco de sementes é dificultada. Tendo em vista a longevidade das sementes de gramíneas nativas no solo, a presença de dormência fisiológica detectada em algumas espécies e ainda serem espécies com alta viabilidade inicial, podemos inferir que o banco de sementes de gramíneas nativas do solo é uma fonte viável para manutenção dessas espécies no sistema. Porém, alguns estímulos externos devem ocorrer para quebrar a dormência das espécies com dormência fisiológica e acelerar a germinação das demais espécies. As espécies nativas suportam os filtros ambientais encontrados no sistema, como passagem de fogo e aumento da amplitude térmica sobre o solo, sendo algumas delas estimuladas a germinar quando submetidas a tais condições.

Contudo, a invasão por *Urochloa brizantha* e *Melinis minutiflora* altera toda dinâmica do banco de sementes do solo, dificultando o processo de estabelecimento das espécies nativas, ressaltando assim, a importância do plano de conservação dessas áreas. O controle de gramíneas invasoras, *Urochloa brizantha* e *Melinis minutiflora*, é uma prática essencial, uma vez que

essas espécies estão estabelecidas no sistema, através da elaboração e aplicação técnicas de manejo que visam à conservação de formações campestres do Cerrado.

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