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(BIOLOGIA VEGETAL)

FATORES EDÁFICOS QUE LIMITAM A GERMINAÇÃO, O ESTABELECIMENTO E O CRESCIMENTO DE ESPÉCIES ARBÓREAS EM CAMPOS ÚMIDOS DE CERRADO

JONATHAN WESLEY FERREIRA RIBEIRO

Tese apresentada ao Instituto de Biociências do Câmpus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Doutor em Ciências Biológicas, ênfase em Biologia Vegetal.

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RESUMO

Os campos úmidos são formações herbáceo-arbustivas que ocorrem sobre solos mal drenados e lençol freático superficial, sendo frequentemente encontrados entre formações savânicas e florestais no Cerrado. Há poucas informações sobre como os fatores edáficos interferem no estabelecimento de espécies arbóreas, impedindo sua colonização por árvores das vegetações adjacentes. Assim, o objetivo desta tese foi elucidar a influência de fatores edáficos dos campos úmidos sobre as estratégias de estabelecimento de espécies arbóreas típicas de formações savânicas e florestais do Cerrado, permitindo a manutenção da fisionomia campestre e, portanto, conferindo resiliência aos campos úmidos. Foram realizados experimentos em laboratório e casa de vegetação para avaliar a tolerância de sementes e de plantas jovens ao alagamento, respectivamente. Além disso, conduzimos experimentos em diferentes áreas de campos úmidos na Floresta Estadual de Assis, Assis, SP, onde introduzimos sementes e mudas das espécies *Calophyllum brasiliense* (espécie da floresta, exclusiva de ambientes úmidos), *Hymenaea stigonocarpa* (espécie da savana, exclusiva de solos bem drenados), e *Tapirira guianensis* (espécie generalista, indiferente à umidade do solo). No capítulo 1, avaliamos o grau de tolerância ao alagamento em condições de laboratório utilizando sementes de doze espécies arbóreas, sendo quatro savânicas, quatro florestais e quatro generalistas. Adicionalmente, avaliamos a emergência de plântulas de duas dessas espécies em condições de campo (campos úmidos). Os resultados obtidos sugerem que sementes de espécies típicas da floresta de galeria e espécies generalistas apresentam maior tolerância ao alagamento do que sementes de espécies savânicas. Entretanto, o nível de tolerância e a capacidade de germinar em condições de alagamento dependem da espécie e do tempo de exposição ao estresse. A alta tolerância ao alagamento em algumas espécies pode favorecer a emergência de plântulas em campos úmidos. No capítulo 2, avaliamos, em casa de vegetação, como o encharcamento e o alagamento do solo do campo úmido interferem na fisiologia fotossintética, crescimento e mortalidade de plantas jovens das espécies *C. brasiliense*, *H. stigonocarpa* e *T. guianensis*. O regime de alagamento do solo afetou de maneiras distintas as espécies estudadas. A espécie *C. brasiliense* apresentou alta tolerância aos regimes de alagamento, sendo capaz de manter sua fisiologia fotossintética e crescimento mesmo nas condições mais severas de alagamento do solo. A espécie *T. guianensis* apresentou tolerância intermediária, apresentando redução da capacidade fotossintética apenas em condições mais severas de alagamento, porém sem prejuízos ao crescimento. E a espécie

savânica *H. stigonocarpa* foi menos tolerante às condições de encharcamento e alagamento do solo, com acentuada redução da capacidade fotossintética, do crescimento radicular e maior mortalidade. No capítulo 3, avaliamos como o estabelecimento inicial (estágio de plântula) e tardio (mudas) de árvores no campo úmido é afetado pelo tipo funcional das espécies e como fatores edáficos (profundidade mínima do lençol freático, teor de argila e saturação de bases) interferem em sua fisiologia foliar. Os resultados demonstraram que a capacidade de estabelecimento varia de acordo com o tipo funcional, sendo a espécie de floresta e a generalista aquelas que apresentam maior capacidade de estabelecimento, devido à maior capacidade de estabelecimento de plântulas (*C. brasiliense*) ou sobrevivência das mudas (*T. guianensis*). Além disso, mudas de *C. brasiliense* e *T. guianensis* apresentaram maiores taxas de assimilação de carbono e melhor balanço hídrico, resultando em maiores taxas de crescimento e sobrevivência em comparação com a espécie de savana *H. stigonocarpa*. Por outro lado, mudas da espécie savânica apresentaram menores taxas de assimilação de carbono e maior déficit hídrico foliar. Como resultado, as mudas não cresceram e apresentaram menores taxas de sobrevivência. O alagamento do solo gerado pelo lençol freático superficial foi o principal fator edáfico limitante da assimilação de carbono e balanço hídrico de espécies arbóreas no campo úmido, impondo restrições ao crescimento e estabelecimento, especialmente para a espécie da savana.

Palavras-chave: Alagamento, germinação de sementes, fisiologia fotossintética, assimilação de carbono, emergência de plântulas.

ABSTRACT

Neotropical wet grasslands from the Brazilian Cerrado are herbaceous-shrub vegetation, which occur on poorly drained soils with surface groundwater, and frequently found between savanna and forest vegetation. Little is known about how edaphic factors regulate wet grasslands and constrain colonization of tree species from the adjacent arboreal vegetation. Therefore, this thesis aimed to elucidate the effects of edaphic variables of wet grasslands on the establishment of typical tree species from savanna and forest vegetation. Experiments were carried out in the laboratory and greenhouse to assess seed and seedling tolerance to flooding in tree species. In addition, we performed field experiments in different areas of wet grasslands at Assis State Forest, Assis, SP, Brazil, where we introduced seeds and saplings of the species *Calophyllum brasiliense* (a gallery forest species, typical of humid soils), *Hymenaea stigonocarpa* (a savanna species, typical of well-drained soils), and *Tapirira guianensis* (generalist species, indifferent to soil humidity). In chapter 1, we evaluated seed tolerance to flooding of savanna (4 species), forest (4 species) and generalist (4 species) tree species. Additionally, we evaluated seedlings' establishment of two from these species under field conditions (wet grasslands). Our results suggest that the flood tolerance was low or absent in seeds of the savanna species, while it tended to increase among the generalist and gallery forest species, which were able to keep the germinability capacity after been submerged in water during different periods. Seedling emergence over wet grasslands was, in general, higher for the forest species. However, the level of tolerance and the ability to germinate while submerged depended on the species and time of exposure to stress. The high seed tolerance to flooding favors seedling emergence in wet grasslands. In chapter 2, we evaluated in semi-controlled experiments how soil waterlogging and flooding of grasslands affect photosynthetic physiology, growth and mortality of *C. brasiliense*, *H. stigonocarpa* and *T. guianensis* tree saplings. Soil waterlogging and flooding differentially affected the species under study. The species *C. brasiliense* presented high tolerance to soil waterlogging and flooding, being able to maintain its photosynthetic physiology and growth even under flooding conditions. The species *T. guianensis* presented an intermediate pattern of tolerance, exhibiting reduction in photosynthetic capacity only under flooding conditions, but without impacts on growth. The savanna species *H. stigonocarpa* presented low tolerance to soil waterlogging and flooding; in these conditions, their saplings presented reduction in photosynthetic capacity and root growth, and higher mortality. In chapter 3, we evaluated how

tree establishment is affected by the functional type of species in wet grasslands and how edaphic factors interfere in leaf physiology of the species. The results showed that tree establishment varies according to the type of species, with the species *C. brasiliense* and *T. guianensis* exhibiting highest establishment potential, due to the greater ability of seedling establishment (*C. brasiliense*) or sapling growth and survival (*T. guianensis*). In addition, saplings of *C. brasiliense* and *T. guianensis* showed higher carbon assimilation rates and better water balance in plants, resulting in higher saplings growth and survival rates than observed for *H. stigonocarpa*. On the other hand, saplings of *H. stigonocarpa* exhibited lower carbon assimilation rates and greater leaf water deficit, leading to inhibition of sapling growth and lower survival rates. Soil waterlogging produced by surface groundwater was the main edaphic factor limiting leaf carbon assimilation and water balance of saplings in wet grasslands, imposing restrictions on plant establishment, especially for the savanna species.

Key words: Flooding, seed germination, photosynthetic physiology, carbon assimilation, seedling emergence.

SUMÁRIO

Introdução geral 12
Referências 15
Capítulo 1: Seed tolerance to flooding in Cerrado tree species: implications to tree colonization of tropical wet grasslands 21
Abstract 22
Introduction 23
Material and methods 25
Results 29
Discussion 33
References 36
Capítulo 2: Respostas morfofisiológicas ao alagamento do solo de campo úmido em espécies arbóreas do Cerrado 42
Resumo 43
Introdução 44
Material e métodos 46
Resultados 49
Discussão 54
Referências 56
Anexos 62
Capítulo 3: Shallow water table and soil properties alone do not constrain tree colonization in tropical wet grasslands 63
Abstract 64
Introduction 65
Materials and methods 67
Results 73
Discussion 79
References 83
Supplementary data 90
Conclusões gerais 92

INTRODUÇÃO GERAL

Na região tropical, biomas abertos (savanas e campos) e florestas úmidas constituem estados alternativos estáveis, mantidos principalmente por feedbacks positivos entre distúrbios do fogo e a vegetação (Warman e Moles, 2009; Dantas et al., 2013). O conceito de estados alternativos estáveis em comunidades biológicas pressupõe que ecossistemas podem apresentar diferentes configurações após serem submetidos a distúrbios, e na dependência do nível de distúrbio e de outros fatores determinantes, o ecossistema se mantém estável, independentemente de qual seja sua configuração/estado final (Scheffer et al., 2001; Beisner et al., 2003). A compreensão dos estados alternativos estáveis depende do entendimento de como os fatores ambientais interferem na estrutura, composição e resiliência das comunidades, a partir dos efeitos sobre os indivíduos e das interações interespecíficas (Scheffer et al., 2001; Beisner et al., 2003; Hoffmann et al., 2012). Neste contexto, compreender quais e como os fatores ecológicos limitam a distribuição de espécies arbóreas é crucial para o entendimento dos estados alternativos estáveis em regiões tropicais (Bond et al., 2005; Bond, 2008). Quando se trata de savanas e campos, mudanças na cobertura arbórea relacionam-se com a alteração do estado e a dinâmica das vegetações (Warman e Moles, 2009; Dantas et al., 2013), podendo culminar em perdas de biodiversidade e serviços ecossistêmicos essenciais (Honda e Durigan, 2016; Abreu et al., 2017).

A ocorrência de savanas em detrimento de florestas é determinada, em escala global, pela precipitação (Hirota et al., 2011). Entretanto, em regiões com níveis de precipitação intermediária e sazonalidade amena, o fogo é o principal fator que limita a expansão da cobertura arbórea (Staver et al., 2011). No Cerrado brasileiro, considerado uma savana úmida, formações savânicas podem transicionar para formações florestais após a supressão do fogo (Durigan e Ratter, 2006; Hoffmann et al., 2012; Dantas et al., 2013) e vice-versa em caso de incêndios muito frequentes (Medeiros e Miranda, 2005). O fogo, portanto, é o principal distúrbio que governa a estabilidade das vegetações savânicas e florestais no Cerrado, através de interações complexas entre esse fator de distúrbio, os recursos minerais e as características das espécies (Hoffmann et al. 2012; Dantas et al. 2013). Espécies arbóreas típicas de savana e floresta representam grupos funcionais distintos, que determinam diferentes respostas aos recursos, distúrbios e condições de estresse (Hoffmann, 2000; Hoffmann e Franco, 2008; Rossatto et al., 2009). Árvores da savana acumulam mais rapidamente uma casca espessa do que árvores da floresta, apresentando maiores probabilidades de se tornarem resistentes ao

fogo; ao passo que árvores da floresta acumulam área foliar mais rapidamente do que espécies das savanas, acelerando a transição para o estado de floresta (Hoffman et al., 2009; 2012).

Apesar do papel fundamental do fogo na distribuição de formações campestres, savânicas e florestais do Cerrado (Hoffmann et al. 2009; 2012; Dantas et al., 2013), em algumas circunstâncias a mudança de estado parece não ocorrer mesmo mediante a supressão do fogo (Leite et al., 2018; Xavier et al., 2019). Nestas circunstâncias, fatores edáficos relacionados à disponibilidade de água e nutrientes e drenagem do solo podem ser determinantes para a variação fisionômica da vegetação (Marimon-Júnior e Haridasan, 2005; Rossi et al., 2005; Assis et al., 2011; Rossatto et al., 2012; Villalobos-Vega et al., 2014). Fatores edáficos são particularmente determinantes para a ocorrência de campos úmidos no Cerrado, em locais onde o lençol freático é superficial, provocando condições de alagamento do solo e estabelecendo restrições para o estabelecimento de plantas lenhosas (Tannus, 2007; Leite et al., 2018; Xavier et al., 2019).

Os campos úmidos são formações essencialmente herbáceo-arbustivas de solos encharcados sazonal ou permanentemente, em locais onde há o afloramento do lençol freático (Ribeiro e Walter, 2008). Ocorrem em diversos tipos de solos hidromórficos, sendo geralmente rasos, ácidos, de baixa fertilidade, alta saturação de alumínio (Munhoz et al., 2008; Mendes et al., 2012), frequentemente encontrados nas encostas, nas chapadas, nos olhos d'água, circundando veredas e na borda de florestas de galeria, ou mesmo em áreas planas contíguas aos rios (Ribeiro e Walter, 2008). Gradientes topográficos podem ocorrer em maior ou menor grau, ocasionando variações nas características edáficas, como granulometria, profundidade do lençol freático, teor de umidade, de matéria orgânica e propriedades químicas do solo (Tannus, 2007). Podem ocorrer dois tipos fitofisionômicos de campo úmido: o campo limpo, fisionomia predominantemente herbácea, com a presença de alguns subarbustos; e o campo sujo, onde ocorrem indivíduos arbustivos e arbóreos de pequeno porte entremeados pelo estrato herbáceo (Ratter et al., 1997; Ribeiro e Walter, 2008) (Fig. 1). Tratam-se de ecossistemas com elevada diversidade florística, sendo as famílias Poaceae, Cyperaceae e Asteraceae de grande representatividade (Tannus e Assis, 2004; Munhoz e Felfili, 2007; Ribeiro e Walter, 2008). A vegetação do campo úmido é composta por mosaicos florísticos, determinados principalmente pela variação na umidade do solo, relacionada a diferenças nas variações físicas do solo e profundidade do lençol freático (Meirelles et al., 2002; Tannus, 2007; Munhoz et al., 2008; Mendes et al., 2012).

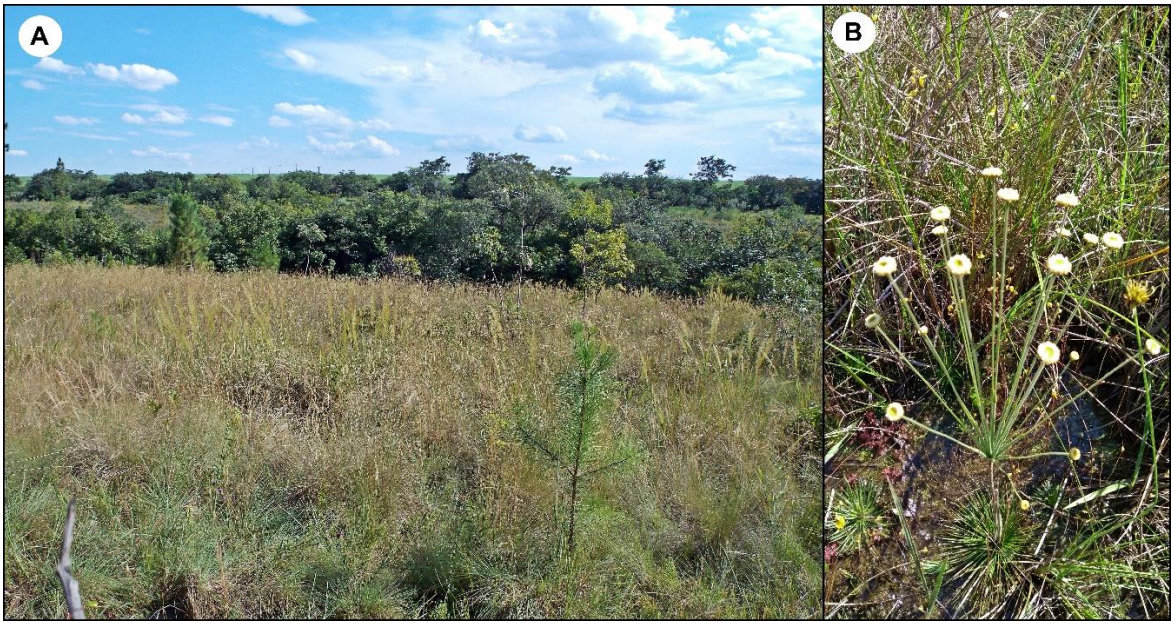


Figura 1. Campo úmido na Floresta Estadual de Assis, Assis, São Paulo, Brazil. (A) Fotografia panorâmica de uma área de campo úmido, delimitada pela floresta de galeria. (B) Detalhe da vegetação herbácea e solo alagado.

O lençol freático superficial pode reduzir a diversidade, a densidade e a área basal de espécies lenhosas de comunidades savânicas do Cerrado (Rossatto et al., 2012; Villalobos-Vega et al., 2014; Leite et al., 2018; Xavier et al., 2019). Estudos recentes demonstram que, em áreas campestres protegidas contra incêndios, em geral, apenas campos úmidos ocorrem onde a profundidade do lençol freático não ultrapassa 2 metros (Leite et al., 2018; Xavier et al., 2019). Fatores ambientais relacionados ao alagamento estão frequentemente ligados à mortalidade de plantas lenhosas em áreas úmidas do Cerrado (Durigan et al., 2004; Oliveira et al., 2015). Entretanto, a ocorrência de florestas de galeria sujeitas a alagamentos sazonais ou permanentes dos solos, frequentemente adjacente aos campos úmidos sugere que o alagamento do solo pode não restringir completamente o estabelecimento de árvores nos campos úmidos (Silva, 2015). Espécies arbóreas raramente se estabelecem nos campos úmidos, mesmo aquelas que comumente ocorrem nas matas de galeria adjacentes (Durigan et al. 2004). Entretanto, os fatores e mecanismos envolvidos na montagem das comunidades nesses ambientes, especialmente os que limitam o estabelecimento de árvores nos campos úmidos, ainda não foram devidamente esclarecidos. Neste sentido, o objetivo desta tese foi elucidar a influência de fatores edáficos sobre as estratégias de estabelecimento de espécies arbóreas típicas de formações savânicas e florestais do Cerrado, permitindo a manutenção da fisionomia campestre e, portanto, conferindo resiliência aos campos úmidos.

Partindo da premissa de que os fatores edáficos, principalmente aqueles relacionados ao alagamento do solo, interferem significativamente na dinâmica de colonização de espécies arbóreas no campo úmido (Tannus, 2007; Leite et al., 2018; Xavier et al., 2019), as seguintes questões nortearam o desenvolvimento desta tese: (I) As respostas morfofisiológicas às variações edáficas do campo úmido diferem entre espécies funcionalmente distintas do Cerrado (exclusivas, indiferentes ou intolerantes a ambientes úmidos)? (II) Espécies arbóreas, introduzidas por sementes ou mudas, são capazes de se estabelecer em áreas de campo úmido? (III) Qual o principal fator edáfico que interfere nas estratégias de estabelecimento e limita a colonização do campo úmido por espécies arbóreas?

A presente tese está dividida em três capítulos, sendo que no primeiro avaliamos o grau de tolerância ao alagamento em sementes de espécies arbóreas savânicas, florestais e generalistas (que ocorrem em ambos os tipos de vegetação). Adicionalmente, avaliamos a emergência e sobrevivência de plântulas de duas espécies em campos úmidos, uma espécie da savana com baixo grau de tolerância ao alagamento, e uma espécie da floresta com alto grau de tolerância ao alagamento. No segundo capítulo, avaliamos, através de experimentos em casa de vegetação, como o regime de alagamento do solo do campo úmido interfere na fisiologia fotossintética, crescimento e sobrevivência de plantas jovens de três espécies: *Calophyllum brasiliense* (espécies da floresta, tolerante ao alagamento), *Hymenaea stigonocarpa* (espécie da savana, intolerante ao alagamento), e *Tapirira guianensis* (espécie generalista, indiferente ao alagamento). No terceiro capítulo, avaliamos como o estabelecimento das espécies arbóreas no campo úmido é afetado pelo tipo funcional (tolerante, intolerante e indiferente ao alagamento) e como fatores edáficos interferem na fisiologia foliar das espécies.

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CAPÍTULO 1

Seed tolerance to flooding in Cerrado tree species: implications to tree colonization of tropical wet grasslands



Malha de algodão envolvendo sementes de *Calophyllum brasiliense*, sob pedriscos para evitar a flutuação das sementes (esquerda). Sementes de *Stryphnodendron rotundifolium* com protrusão radicular no tratamento controle (centro). Sementes de *Genipa americana* germinadas (formando plântulas) após tratamento de submersão em água (direita).

Cotton mesh enclosing seeds of *Calophyllum brasiliense*, under gravel to prevent seeds from floating (left). Seeds of *Stryphnodendron rotundifolium* with root emerging in the control treatment (center). Germinated seeds of *Genipa americana* (forming seedlings) after submersion treatment in water (right).

Seed tolerance to flooding in Cerrado tree species: implications to tree colonization of tropical wet grasslands

ABSTRACT

Soil waterlogging can negatively affect tree colonization of flood-sensitive species, especially during seed germination and seedling stages. The ability of seeds to germinate during or after water submergence may be a key adaptation strategy in flood-prone ecosystems, helping trees to withstand hypoxia/anoxia stress. Soil waterlogging is claimed as the main environmental filter constraining tree colonization in Neotropical wet grasslands from the Brazilian Cerrado. However, wet grasslands frequently form abrupt boundaries with gallery/swamp forests, and many tree species occur on waterlogged soils of these forests. In this context, we aimed to investigate the effects of experimental flooding on seed germination of different Cerrado tree species. We performed laboratory germination trials to investigate seed tolerance to flooding in savanna (4), forest (4) and generalist (4) – that occurs both in savannas and forests – tree species. We also evaluated seedling emergence, emergence success and seedling survival in wet grasslands of a savanna (*Hymenaea stigonocarpa*) and a forest (*Calophyllum brasiliense*) species. Eight of the 12 species under study exhibited some degree of seed tolerance to flooding. Flood tolerance (FT) was low or absent in seeds of the savanna species, while there was great variation in FT to generalist species, and most forest species showed seeds with high flood tolerance. Despite low emergence success (≤ 19.8%) in the grasslands for both tested species, the first-year seedling survival was high (≥ 80.8%) for both species. Our results indicate that soil waterlogging is an important environmental filter constraining seed germination of trees in wet grasslands, especially for species with flood intolerant and sensitive seeds. However, soil waterlogging did not completely prevent seedling establishment in the grasslands. Thus, grasslands' waterlogging probably interact with other constraint factors to prevent tree colonization over Cerrado wet grasslands, especially to those species with high tolerance to flooding in the seeds.

Key words: Flood tolerance, seed germination, tropical savannas, seedling emergence, Cerrado.

INTRODUCTION

Many of the seasonal tropical landscapes are composed by vegetation mosaics of grassy (grasslands and savannas) and forest patches (Bond and Parr, 2010). Such vegetation mosaics are a common landscape of the Brazilian savanna (known as Cerrado), with a range of vegetation types varying from grasslands, savannas and forest physiognomies (Oliveira and Ratter, 2002). Most of these mosaics occur under suitable climates to forest expansion (Bond, 2010). In these scenarios, understanding the reasons that tree failure to establish in grassy ecosystems have crucial implications to comprehend tropical grassland dynamics and promote their conservation (Bond, 2008; Wakeling et al., 2012; Joshi et al., 2020), once tree encroachment of tropical grassy biomes is a worldwide phenomenon (Van Auken, 2009; Durigan and Ratter, 2006) that may result in the loss of species diversity and ecosystem functions associated with open grassy vegetations (Ratajczak et al., 2012; Honda and Durigan, 2016; Pinheiro et al., 2016; Rossatto and Rigobelo, 2016).

The expansion of trees over grasslands depends, in a first instance, on the availability and dispersal of viable seeds from local populations. Since tree seeds arrive in grasslands, they need suitable environmental conditions to seed germination and seedling establishment (Grubb, 1977; Donohue et al., 2010; Salazar et al., 2012). Environment conditions such as high temperatures, drought, as well as fire disturbances often limit tree regeneration in tropical savannas (Hoffmann, 1996; Hoffmann et al., 2004; 2012). However, seeds of savanna tree species are often more resistant to desiccation, heat shocks and high temperatures than forest species, which may explain at least in part, the low recruitment of forest trees into dry grassy physiognomies (Ribeiro and Borghetti, 2014). By other hand, waterlogging is a major environmental filter in wetlands, selecting plant regeneration strategies associated with seed tolerance to flood (e.g., submersed germination) and or seed scape from anoxic conditions (e.g., dispersal time, buoyance, seed longevity in stand water) (Keddy, 1992; van der Valk, 1981; Urquhart, 2004).

Poor oxygen supply of the waterlogged soils of wetlands impairs the aerobic metabolism of buried seeds (Crawford, 1992; Kozlowski, 1997). Some species readily germinate while submerged, while others do not germinate submerged, but may remain viable in water during variable periods and germinate after submergence (Marques and Joly, 2000; Kolb and Joly, 2010; Pires et al., 2018). Some species can poorly germinate or died when maintained in waterlogged conditions (Paz et al., 2017; Oliveira et al., 2019). In the

Neotropics, seed tolerance to flooding is common among species from flooded and swamp forests (Wittmann et al. 2007; Melo et al. 2015; Paz et al., 2017), but some degree of tolerance may also be present in some savanna tree species, allowing them to expand their distribution range into savanna wetlands (Oliveira et al., 2019).

In the Cerrado, wetlands are often associated with grassy vegetation, such as wet savannas and grasslands (Cianciaruso et al., 2005; Munhoz et al., 2008; Bijos et al., 2017). These wet grasslands occur on water-satured, organic but poor-nutrients soils, where vegetation is characterized by a rich herbaceous-subshrub layer and low presence or even absence of trees (Munhoz et al., 2008; Ribeiro and Walter, 2008). This vegetation frequently occurs as a belt between seasonal savannas upslope and gallery forests downslope (Ribeiro and Walter, 2008), where soil waterlogging supposed constrain tree invasion of adjoint savannas and gallery forests into wet grasslands (Villalobos-Vega et al., 2014; Leite et al., 2018; Xavier et al., 2019). However, the knowledge about how flooding conditions of tropical grasslands constrain the regeneration niches of the Cerrado trees is scarce (but see Oliveira et al., 2019), limiting our ability to predict possible changes in tropical vegetation in response to changing ecological factors.

We analyzed the effects of experimental flooding on the germination process of different Cerrado tree species. Additionally, we performed a field experiment to evaluate tree seedling emergence along wet grasslands in two tree species with distinct seed tolerance to flooding. We expected that species typical of seasonally flooded soils should present higher flooding tolerance in their seeds, whereas species restricted to well-drained soils would have lower seed tolerance to flooding and would lose germination capacity even in short flooding periods. On the other hand, generalist species that occur both in well-drained and waterlogged soils would show an intermediate seed tolerance to flooding. We also expected that seedling emergence in wet grasslands should be higher for the tree species with high seed tolerance to flooding rather than the species with low seed tolerance. We focus on germination requirements regarding flooding tolerance because is an indirect indicative of anoxia tolerance (Fagerstedt and Crawford, 1987), allowing us to infer how waterlogging of tropical wet grasslands may interfere on regeneration niches of trees through seed germination limitations.

MATERIAL AND METHODS

Study site

This study was conducted at the Assis State Forest (ASF, 22º 36’ 50’’S and 50º 24’ 52’’W), a conservation area for sustainable use located in the western region of the state of São Paulo, Brazil. The wet grasslands studied are characterized by a dense graminoid vegetation layer (40-cm tall), and hydromorphic soils with elevated organic matter levels. The grasslands were surrounded by woody savannas upslope (in well-drained soils) and gallery forests downslope (in permanently wet soils). The regional vegetation is classified as cerrado *sensu lato* (Ribeiro and Walter, 2008) with transitions to seasonal semideciduous forests (Secretaria do Meio Ambiente, 2010). Savanna physiognomies with variable tree density predominate on the well-drained interfluves sites (Assis et al., 2011). In the riparian zone, there are different types of vegetation, such as gallery and swamp forests and wet grasslands (Durigan et al., 1999). Soils in the region are often sandy with high aluminum concentration and low nutrient availability, classified from the summit down to the footslope, as rhodic haplustox, typic haplustox and epiaquic haplustult (Juhász et al., 2006). With elevations ranging from 500 to 588 m, the local climate is Cfa (subtropical humid with hot summer) following the Köppen classification, with an annual rainfall of approximately 1,400 mm concentrated in the summer (December to March) and a dry season of variable duration in the winter (Alvares et al., 2013). The annual average temperature is 21.8°C with an average minimum of 8.4°C and average maximum of 32.4°C (Secretaria do Meio Ambiente, 2007).

Study species

Seeds were collected from several individuals (> 10) from different vegetation types at the western region of the state of São Paulo, Brazil (more details in Table 1). The species for this study were selected according to the vegetation type in which each species typically occurs, in savanna, forest or in both vegetation types. Species that occur both in savanna and forest vegetation were classified as generalist species (Table 1). The savanna species selected are among the most common tree species found in the cerrado *sensu stricto* (Ratter et al., 2003), which is a savanna type vegetation with small trees and shrubs often 3–8 m tall with approximately 30% of crown cover and a rich herbaceous layer. This vegetation occurs on well-drained and low-fertility soils, with deep water table and seasonal water deficit at topsoil level (Oliveira-Filho and Ratter, 2002). The generalist species selected are tree species with a large geographical range occurrence, being found in cerrado *sensu stricto*, closed woodlands

(cerradão), gallery forests and semideciduous seasonal forest along the Neotropical region (Durigan et al. 1999; Ribeiro and Walter, 2008). The forest species selected are often found in gallery and riverine forests with waterlogged soils and/or semideciduous seasonal forests with well-drained soils.

Table 1. Study species according to their family, preferred habitat (forest; generalist; savanna), presence of seed dormancy (ND: non-dormant; PY: physical dormancy), date and location of collection. cerrado: cerrado *sensu stricto*; GF: gallery forest; SSF: seasonal semideciduous forest; RF: riparian forest.

Species	Family	Dormancy	Date of collection	Municipality (vegetation type)
Savanna				
Dimorphandra mollis Benth.	Fabaceae	PY	Sep/18	Birigui (cerrado)
Hymenaea stigonocarpa Mart. ex Hayne	Fabaceae	PY	Oct/16	Assis (cerrado)
Stryphnodendron rotundifolium Mart.	Fabaceae	PY	Jul/17	Assis (cerrado)
Tabebuia aurea (Silva Manso) Benth. & Hook.f. ex S.Moore	Bignoniaceae	ND	Oct/18	Penápolis (cerrado)
Generalist				
Copaifera langsdorffii Desf.	Fabaceae	PY	Sep/18	Assis (cerrado)
Guazuma ulmifolia Lam.	Malvaceae	PY	Aug/17	Birigui (SSF)
Moquiniastrum polymorphum (Less.) G. Sancho	Asteraceae	ND	Feb/18	Assis (cerrado)
Tapirira guianensis Aubl.	Anacardiaceae	ND	Feb/17	Assis (GF)
Forest				
Calophyllum brasiliense Cambess.	Calophyllaceae	PY	Jul/16	Assis (GF)
Genipa americana L.	Rubiaceae	ND	Jan/18	Panorama (RF)
Handroanthus heptaphyllus (Vell.) Mattos	Bignoniaceae	ND	Oct/18	Araraquara (SSF)
Styrax pohlii A.DC.	Styracaceae	ND	Mar/18	Assis (GF)

Submergence treatments

Seeds of the species *D. mollis*, *H. stigonocarpa*, *S. rotundifolium*, *C. langsdorffii*, *G. ulmifolia* and *C. brasiliense* were mechanically scarified before germination tests. Seeds of each study species were subjected to normoxia condition (control) and three treatments with different periods of submergence: seeds were maintained submerged during 15 (s15), 30 (s30) and 45 (s45) days. After that period, non-germinated seeds of each treatment were removed from the water to be incubated in the same conditions as the control test during 30 days.

Normoxia conditions were obtained by placing seeds in plastic boxes with filter paper saturated with distilled water. Flooding conditions were obtained by keeping the seeds in bags of cotton fiber, forcing the seeds to stand under the water surface (from 1 cm below).

In all treatments, four plastic boxes with 25 seeds each were used. All treatments (normoxia and submerged seeds) were carried out at 25 ± 1ºC with 12 h of white light photoperiod (60 µmol m^{-2} s^{-1}). The number of germinated seed was recorded at the end of each submergence period, and then a second record was done after 30 days of normoxia to evaluate seed germination after submergence period. Seeds were considered germinated after the radicle reached 1 mm in length. For each replicate, in all experiments, we calculated the final germination percentage (germinability; G% = $(\sum n_i N^{-1})100$), where Σ_{ni} is the amount of germinated seeds in relation to the number of seeds (N) placed to germinate (Ranal and Santana, 2006). In the case of submergence treatments, the final germination percentage accounted for seeds germinated during and after submergence treatments.

To evaluate seed tolerance to flooding, we calculated the Flooding Tolerance Index (FTI) to each replicate of submergence treatments ($s15$, $s30$, and $s45$) using the following equation:

$$\text{FTI} = \left(s15\,\frac{(Gc-Gs)}{Gc} + s30\,\frac{(Gc-Gs)}{Gc} + s45\,\frac{(Gc-Gs)}{Gc}\right) \div 3,$$

where Gc is the mean germination percentage of control and Gs is the germination percentage of the submergence treatments. FTI vary from 0 (maximum tolerance to flooding) to 1 (maximum intolerance to flooding). We attributed 0 value for those replicates that presented a higher germination percentage than their respective control.

Soil water content of grasslands

Soil water contents were measured during dry (September 2016) and rainy (January 2017) season in three wet grassland sites at ASF. In each site, we established 10 rows of 6 m in length each, positioned parallel to the stream margin (gallery forest tree line) with 1.5 m to 4.5 m apart from each other (depending on each site; see block dimensions below). This distance allowed us to sample the entire moisture gradient of the site. In each row, we collected four fresh soil samples, every 2 meters, at 0-5 cm depth. Each soil sample was placed in a sealed plastic bag and temporarily stored in thermal boxes (6ºC) to avoid evaporation. Shortly after that, samples were weighed with a 0.1 mg precision balance. Dry soil mass was determined by oven-drying of the samples at 105°C to constant mass, and the

soil water content was calculated as $(Wm - Dm/Dm) \times 100$, where Wm is the soil wet mass and *Dm* is the soil dry mass.

Seedling emergence in grasslands

The experiment was conducted in three sites of wet grasslands at ASF, following a factorial block (3 blocks) design with one variation level, species (*C. brasiliense* – forest species; and *H. stigonocarpa* – savanna species). We established three blocks (one per study site); the size of blocks varied among study sites: block 1, 15 m x 20 m; block 2 and 3, 45 m x 20 m. Each block contained 20 parallel rows, which were considered as replicates (10 for each species, interspersed with each other), 1 m distant from each other. The rows were perpendicular to the stream margin with one end close to the savanna and the other close to the gallery forest. Each row contained 10 holes containing 3 seeds each of the same species. There was at least 1-m distance between holes. Seeds were sown in the period of seed dispersal as follows: August and December 2016 for *C. brasiliense* and *H. stigonocarpa*, respectively. Seeds were sown at a 1-cm depth to avoid predation and removal by animals, totalling 900 per species. The location of each hole (in total, 300 per species) was marked with a colored flag. We did not treat seeds to overcome dormancy before seeding. The viability of the seed batches was assessed by germination trials using sand as substrate at greenhouse conditions, being high to both species (*C. brasiliense*: 90%; *H. stigonocarpa*: 93%). Seedling emergence in each hole of seeds was verified monthly after seeding during 12 months (September 2016 to December 2017). We considered that seedlings emerged when there was an emergence of any vegetative part above ground. The average percentage of seedling emergence (PSE%) for each replicate (rows) and species was calculated using the equation: SE% = $((\sum ni\ N^{-1}) \times 100))$; where Σn_i is the number of emerged seedlings in each replicate, and N is the total number of seeds placed to germinate per replicate. We calculated the emergence success as the emergence rate in the field (per species within each replicate) relative to the germination for the species in the laboratory (Passaretti et al. 2020). Seedling survival was calculated as the percentage of emerged seedlings in each replicate surviving after 12 months (Passaretti et al. 2020).

Data analysis

Differences in germination between each submergence treatment and control were tested using generalized linear models (GLMs) with a binomial error distribution and a logit link function with GLMs performed separately for each species. Differences in soil water

content between the dry and rainy seasons for each different position of each wet grassland site were tested using t-test ($p < 0.05$). Differences in the average percentage of seedling emergence, emergence success and seedling survival between species, were tested using a generalized linear mixed model (GLMM) with a binomial distribution (proportion data) and blocks as random effects. The t-test analyzes were performed in Bioestat 5.0. The GLM and GLMMs analyzes were performed in R software (R Core Team, 2018), where we used the "*lme4*" package (Bates et al., 2015), and "*multcompView*" (Graves et al., 2019) and "*lsmeans*" (Lenth, 2018) package for multiple comparisons. When significant differences were detected for species performances, we performed contrast analysis by "*emmeans*" functions from "*emmeans*" package (Russell, 2019).

RESULTS

Tolerance to submergence

Seeds of the species *H. stigonocarpa*, *H. heptaphyllus* and *T. aurea* germinated only after being removed from water in the short-time submergence (s15) treatment, with a significant decrease in relation to the control and FTI varying from 0.90 to 0.97 (low tolerance to flooding) (Table 2, 3). For the species *M. polymorphum*, seeds germinated during submergence and after being removed from water, but in all submergence treatment germination significantly decreased in relation to control (Table 2, 3). Seeds of the species *T. guianensis* germinated in short and long-time (s-30) submergence treatments, but only after removal from water and with a significant decrease in germination in relation to control (Table 2, 3). Thus, the species *M. polymorphum* and *T. guianensis* presented intermediate FTI values of 0.52 and 0.72, respectively (Table 3).

For the species *G. ulmifolia*, *G. americana* and *S. pohlii* germination occurred during submergence and after removal from water, with no loss of germination capacity in relation to control, irrespective of submergence period (Table 2, 3). Seeds of *C. brasiliense* germinated only after removal from water, with no loss of germination capacity in relation to control, irrespective of submergence period (Table 2, 3). Therefore, seeds of the species *G. ulmifolia*, *G. americana*, *S. pohlii* and *C. brasiliense* showed high tolerance to flooding, with the lowest FTI values, varying from 0.01 to 0.1 (Table 3). Seeds of *D. mollis*, *S. rotundifolium* and *C. langsdorffii* did not germinate in any submergence treatments and were dead (in decomposing state) after the submergence periods; with FTI values of 1.0 (maximum intolerance to flooding) (Table 3).

Table 2. Parameters estimated by GLM models for final seed germination of tree species in the submergence treatments s-15, s-30, and s-45, which consisted of 15, 30, and 45 days of submergence in water plus 30 days of normoxic conditions. Control treatment (normoxia) was used as intercept. S.E.: Standard error.

Species	Fixed effects	Estimate	S.E.	*P*-value
H. stigonocarpa	Intercept	-0.405	0.102	<0.001
	s-15	-1.939	0.204	<0.001
T. aurea	Intercept	1.208	0.237	<0.001
	s-15	-3.959	0.483	<0.001
G. ulmifolia	Intercept	0.405	0.102	<0.001
	s-15	0.441	0.149	0.003
	s-30	0.213	0.146	0.144
	s-45	-0.041	0.144	0.773
M. polymorphum	Intercept	1.450	0.127	<0.001
	s-15	-0.741	0.166	<0.001
	s-30	-2.158	0.166	<0.001
	s-45	-3.108	0.186	<0.001
T. guianensis	Intercept	3.178	0.255	<0.001
	s-15	-2.424	0.276	<0.001
	s-30	-4.993	0.293	<0.001
C. brasiliense	Intercept	0.823	0.108	<0.001
	s-15	0.083	0.154	0.588
	s-30	0.834	0.174	<0.001
	s-45	0.911	0.177	<0.001
G. americana	Intercept	0.405	0.102	<0.001
	s-15	0.441	0.149	0.003
	s-30	0.213	0.146	0.144
	s-45	-0.041	0.144	0.773
H. heptaphyllus	Intercept	-0.619	0.104	<0.001
	s-15	-1.471	0.191	<0.001
S. pohllii	Intercept	0.160	0.100	0.110
	s-15	-0.160	0.141	0.258
	s-30	-0.230	0.141	0.141
	s-45	-0.200	0.141	0.157

Table 3. Flooding tolerance index (FTI) and means (± standard error) of seed germination percentage in control (unflooded condition) and in the submergence treatments s-15, s-30, and s-45, which consisted of 15, 30, and 45 days of submergence in water plus 30 days of normoxic conditions, respectively. FTI vary from 0 (maximum tolerance to flooding) to 1 (maximum intolerance to flooding). During: means seed germination during submergence treatments; After: means seed germination after being removed from water; (FG) Final germination: sum of seed germination (During + After). Asterisks indicate significant differences between the final germination of treatments and the control according to GLM models; –: absence of germination.

				s-15			s-30			s-45		
	Species	FTI	Unflooded	During	After	FG	During	After	FG	During	After	FG
Savanna	*D. mollis*	1.00	79 ± 3	–	–	–	–	–	–	–	–	–
	H. stigonocarpa	0.93	40 ± 9	0	9	9 ± 3*	–	–	–	–	–	–
	S. rotundifolium	1.00	69 ± 3	–	–	–	–	–	–	–	–	–
	T. aurea	0.97	77 ± 3	0	6	6 ± 6*	–	–	–	–	–	–
Generalist	*C. langsdorffii*	1.00	84 ± 5	–	–	–	–	–	–	–	–	–
	G. ulmifolia	0.01	57 ± 6	70	7	77 ± 9*	61	0	61 ± 3	59	0	59 ± 3
	M. polymorphum	0.52	81 ± 2	53	14	67 ± 3*	32	1	33 ± 5*	16	0	16 ± 6*
	T. guianensis	0.72	96 ± 2	0	68	68 ± 5*	0	14	14 ± 2*	–	–	–
Forest	*C. brasiliense*	0.02	69 ± 3	0	71	71 ± 5	0	84	84 ± 6*	0	85	85 ± 1*
	G. americana	0.01	60 ± 2	40	30	70 ± 2*	52	13	65 ± 6	56	3	59 ± 2
	H. heptaphyllus	0.90	35 ± 6	0	11	11 ± 9*	–	–	–	–	–	–
	S. pohlii	0.10	54 ± 3	0	50	50 ± 4	3	45	48 ± 2	10	39	49 ± 2

Soil water content and seedling emergence in grasslands

The percentage of soil water content was quite variable among sites, varying from 31.0 to 160.0% in site 1, 410.0 to 1361.6% in site 2 and 22.0 to 444.5% in site 3 (Fig. 1a – c). In general, soil water content was little influenced by the season, with few exceptions (Fig. 1a – c).

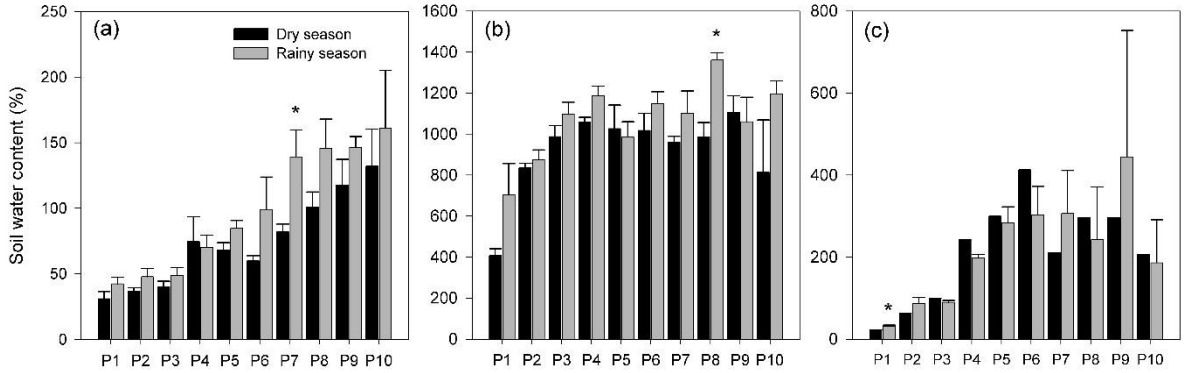


Figure 1. Mean (± standard error; n = 4) values of soil water content measured in different positions of three wet grasslands during the dry and rainy season, at Assis State Forest, São Paulo state, Brazil. (a) site 1; (b) site 2 and (c) site 3. P1 to P10 represent different positions in the wet grasslands where soil samples were collected, where P1 represent the position close to the savanna edge whereas P10 is the position close to the gallery forest. Asterisks indicate significant differences between the dry and rainy seasons according to t-test ($p < 0.05$).

Seedling emergence in the field was low for both species, not surpassing 12.0 and 19.0% of the seeds sown for *H. stigonocarpa* and *C. brasiliense*, respectively (Fig. 2a). Emergence in the field was much lower than it could be related to germination rates in the laboratory, with emergence success differing among the species ($\chi 2 = 52.1$, df = 1, $P < 0.0001$, Fig. 2b), being higher for *C. brasiliense* (19.8%) relative to *H. stigonocarpa* (12.9%). From the emerged seedlings, the percentage of those surviving after 12 months from sowing did not differ among the species ($\chi 2 = 1.64$, df = 1, $P = 0.199$; Figure 2c).

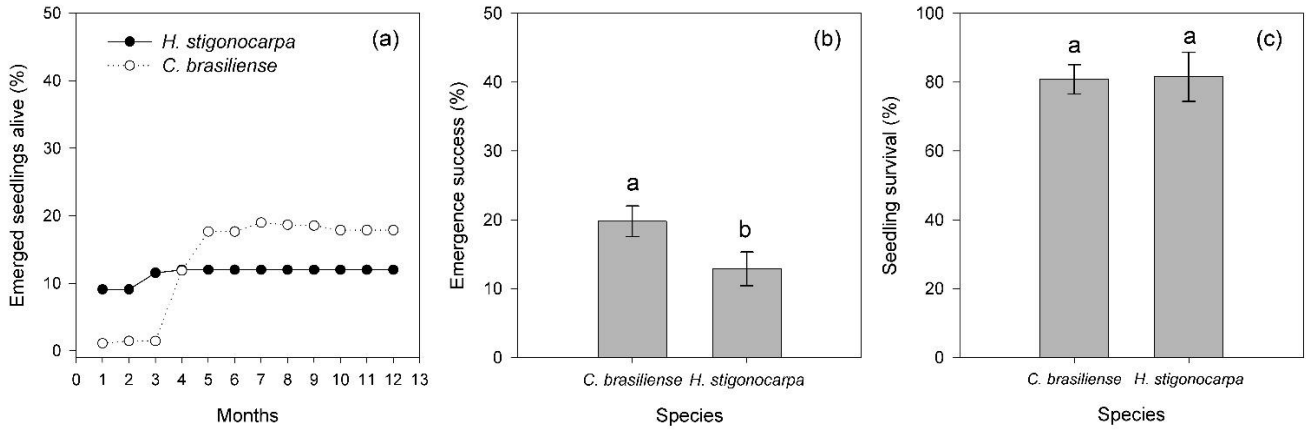


Figure 2. Emerged seedlings alive (percentage related to the seeds sown; n = 30) over 12 months after sowing (a), emergence success (emergence in the field relative to the germination in the laboratory; n = 30) (b), seedling survival (percentage of emerged seedlings surviving after 12 months; n = 24–30) (c), in wet grasslands from southeastern Brazil. Bars represent means ± standard error. Different letters on the top of the bars indicate significant differences between species by contrast analysis ($P < 0.05$).

DISCUSSION

The soil water content of the study grasslands was high with most soil samples presenting values above 60%, and reached values above 1000% at site 2. Similar values of soil water content were found in a wet grassland from the central Cerrado region, where values of soil water content ranged from 90 to 637.5% (Munhoz et al., 2008). Such a huge soil water content observed here is due to both groundwater dynamic, which remains superficial year-round, and the highly organic soils (Chapter 3), which retain water even during the dry season (van der Valk et al., 1999). These results indicate that grassland soils were waterlogged in most parts of the grasslands and throughout the year, imposing a serious limitation on tree regeneration of species with flood-sensitive seeds (Oliveira et al., 2019).

The 12 study species presented different germination responses to experimental flooding. This large variation in flooding response was similar to those reported in other studies of tropical species susceptible to flooding (Joly and Crawford, 1982; Lopez and Kursar, 2003; Oliveira et al., 2015). As expected, seeds of trees that can occur in flooded habitats (*G. ulmifolia*, *G. americana*, *S. pohlii* and *C. brasiliense*) showed high tolerance to flooding ($FTI \geq 0.01 \leq 0.1$). Between the high tolerant species, *G. ulmifolia* and *G. americana* showed tolerance to flooding through germination capacity in stand water, while the species *C. brasiliense* and *S. pohlii* showed a flood-scape strategy, with most of their seed

germination occurring after water drawdown (Urquhart, 2004). For these species, other studies have suggested that their high seed tolerance to flood contributes to coping with soil waterlogging and flooding in flooded and swampy tropical forests (Scarano et al., 1997; Marques and Joly, 2000; Kissmann and Habermann, 2014; Pires et al., 2018; Urquhart, 2004).

The species *M. polymorphum* (FTI = 0.52) and *T. guianensis* (FTI = 0.72) showed intermediate tolerance to flooding. Their seeds germinated during or after flooding, however, as the flooding time went on, germination decreased for *M. polymorphum* and was completely inhibited at 45 days for *T. guianensis*. These species naturally occur both in well-drained and poorly drained soils across the Cerrado region (Durigan et al., 1999; Xavier et al., 2019). *M. polymorphum* shows high plasticity in leaf structure and seed germination patterns, which has been associated with its occurrence in both Neotropical savannas and forests (Rossatto and Kolb, 2010; Ribeiro and Kolb, 2016). *T. guianensis* is widespread with a distribution pattern extending from the Amazon to the Atlantic forest (Oliveira-Filho and Ratter, 1995), being frequently associated with waterlogged soils of the gallery forests (Sampaio et al., 2000). Although these species can germinate in some flooding conditions, and colonize humid habitats, our results suggest that long periods of soil waterlogging (>45 days) will reduce or prevent seed germination of *M. polymorphum* and *T. guianensis* in permanently waterlogged soils of wet grasslands (Pérez-Ramos and Marañón, 2009).

Although our small number of species do not allow us to affirm that all savanna species has flood intolerant seeds, here, as initially expected, most tree species with low tolerance or intolerance were savanna species (*D. mollis*, S. rotundifolium, *H. stigonocarpa* and *T. aurea*) associated with well-drained soils. The low seed tolerance to flooding observed here for the savanna species are in agreement with the consensus that savanna species restricted to well-drained soils are flooding intolerant (Joly and Crawford, 1982; Oliveira et al., 2015; Xavier et al., 2017). In the Neotropics, waterlogged prone grasslands had less plant species richness than dry grasslands and savannas, suggesting that soil waterlogging restricts the species distribution over hydrological gradients (Tannus and Assis, 2004; Vilalobos-Vega et al., 2014; Leite et al., 2018; Xavier et al. 2019). Flooding is rarely experienced by typical savanna species, which majority occur in deep well-drained soils, except to headwater, riverine and hyperseasonal sites (Ribeiro and Water, 2008; Cianciaruso et al., 2005). Most of the Cerrado flora evolved in free flooded areas under frequent fire disturbance (Ribeiro and Walter, 2008; Simon et al., 2009), therefore, plants are well adapted to seasonal drought and fires instead flooding (Meinzer et al., 1999; Rossatto et al., 2012).

Here, seeds of the savanna species *T. aurea* and *H. stigonocarpa* poorly germinated after 15 days of water submergence (6.0 and 9.0%, respectively). However, seeds of the same species from flooded savannas of the Brazilian Pantanal successfully germinated during (*T. aurea*) or after (*H. stigonocarpa*) 61 days of flooding (Oliveira et al., 2019). Such distinct intraspecific responses to flooding found by Oliveira et al. (2019) and our results reflect differences in both local population adaptations and the methodology used. Tree populations inhabiting flooded sites may present better performance under flooding than populations inhabiting dry sites (Pires et al., 2018). This especially seems to be the case of *T. aurea* from the Brazilian Pantanal, where periodically flood regimes may select those individuals with better responses to flooding (Oliveira et al., 2019).

In the case of *H. stigonocarpa*, Oliveira et al. (2019) subjected seeds with physical dormancy to flooding, while in this study we chose to overcome physical dormancy before flooding treatments. Physical dormancy prevents seeds from water imbibition, maintaining its metabolism in a quiescent state. Such a mechanism may preserve germination capacity during flooding periods, and seeds can germinate after water recedes (Paz et al., 2017). However, we believe that physical dormancy has little biological significance to deal with waterlogging in our grassland's sites because they show little or no variation in the soil waterlogging regime year-round. In this way, we expect that physical dormant seeds will inevitably face hypoxic or anoxic conditions even when dormancy is released. This germination pattern of physical dormant seeds in flooding treatments also applies to the savanna species *D. mollis*, *S. rotundifolium* and the generalist species *C. langsdorffii*, which died even after the shortest time of flooding.

In relation to *C. langsdorffii*, our result is similar to those founded by Oliveira et al. (2019). *C. langsdorffii* is among the species with a wide habitat distribution range, occurring from dry to periodically flooded sites. The lack of germination in flood conditions indicate that adult individuals of *C. langsdorffii* can withstand flooding but seeds did not (Siebel and Blom, 1998). This also is the case of the forest species *H. heptaphyllus*, which can occur as adults in flooded forest patches (Souza et al., 2019), however, their seeds did not tolerate medium (30 days) and long (45 days) flooding periods.

In our field experiment, we found strong constraints for tree seedling emergence, even for the species with high seed tolerance to flood *C. brasiliense* (19.8% of the potential germination) and worse for the species with low seed tolerance to flood *H. stigonocarpa* (12.9%). Although seeds of *C. brasiliense* can remain viable in waterlogged soils up to 3

months (Marques and Joly, 2000), the permanent soil waterlogging throughout the year observed in our grassland sites probably impairs seed germination, since *C. brasiliense* seeds cannot germinate when submerged in water (Marques and Joly, 2000; Urquhart, 2004). In fact, we observed for both study species that most seedlings emerged in less flooded places over the grasslands. Once emerged, seedling survival over the first year was high (80.8% for *C. brasiliense* and 81.5% for *H. stigonocarpa*).

Therefore, at least for the two study species, environmental filters of the grasslands act primarily hindering seed germination rather than seedling survival. Together, our results indicate that soil waterlogging is a strong environmental filter constraining the early steps of tree colonization in the wet grasslands, however, waterlogging did not completely prevent tree seedling establishment for our study species in grasslands. We believe that soil waterlogging may be the primary environmental filter constraining tree establishment for those species with flooding intolerant or flooding-sensitive seeds (Oliveira et al., 2019). On the other hand, long time of waterlogging and other factors, such as fire and rare frost events (Hoffmann et al., 2012; 2019), should interact to constrain tree colonization of species with high flood tolerant seeds.

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CAPÍTULO 2

Respostas morfofisiológicas ao alagamento do solo de campo úmido em espécies arbóreas do Cerrado



Condições experimentais em casa de vegetação (esquerda) e detalhe das plantas de *Hymenaea stigonocarpa* no tratamento de solo alagado (direita).

Respostas morfofisiológicas ao alagamento do solo de campo úmido em espécies arbóreas do Cerrado

RESUMO

Campos úmidos compreendem vegetações essencialmente herbáceo-arbustivas sobre solos sazonal ou permanentemente saturados ou até mesmo alagados pela elevação do lençol freático. Apesar de se localizarem frequentemente entre formações savânicas e florestas de galeria no Cerrado, é rara nos campos úmidos a ocorrência de espécies arbóreas das vegetações adjacentes. Tendo em vista que a saturação hídrica do solo exerce forte pressão seletiva sobre a distribuição de espécies arbóreas, o objetivo deste estudo foi avaliar como o excesso de água no solo de campos úmidos interfere em parâmetros fotossintéticos e no crescimento de espécies arbóreas funcionalmente distintas do Cerrado. Para isso, conduzimos experimentos em casa de vegetação utilizando plantas jovens das espécies arbóreas *Calophyllum brasiliense* (espécie da floresta de galeria), *Hymenaea stigonocarpa* (espécie da savana) e *Tapirira guianensis* (espécie generalista). Submetemos as plantas a três tratamentos: solo controle (sem excesso ou falta d'água), solo encharcado (solo com saturação hídrica) e solo alagado (solo com lâmina d'água acima do solo), utilizando solo de campo úmido como substrato. Com exceção da massa foliar, o encharcamento ou alagamento do solo não prejudicou nenhum dos outros parâmetros avaliados em plantas de *C. brasiliense*. A espécie *T. guianensis* apresentou redução da assimilação de carbono em solo alagado, mas não no solo encharcado. Além disso, as espécies *C. brasiliense* e *T. guianensis* foram as únicas a apresentarem raízes adventícias e hipertrofia de lenticelas em resposta ao alagamento. Plantas da espécie *H. stigonocarpa* apresentaram redução acentuada da assimilação máxima de carbono, condutância estomática, e de parâmetros da fluorescência da clorofila *a* nos tratamentos encharcado e alagado. Além disso, o solo alagado reduziu a massa das raízes e causou maior mortalidade em plantas de *H. stigonocarpa*. Os resultados sugerem que o regime hídrico dos solos de campos úmidos interfere de forma diferente no estabelecimento de espécies arbóreas de diferentes formações vegetais, sendo a espécie da savana a que apresentou menor capacidade de se ajustar ao estresse imposto pelo aumento da saturação hídrica dos solos de campos úmidos.

Palavras-chave: Fisiologia fotossintética, assimilação de carbono, crescimento, savana, mata de galeria.

INTRODUÇÃO

O alagamento do solo é um importante fator ecológico que limita a distribuição de plantas vasculares em áreas úmidas em todo o mundo (Crawford, 1992; Kozlowski, 1997; Fraaije et al., 2015). O estresse por alagamento é especialmente relevante para plantas terrestres na região Neotropical, onde extensas áreas do território são cobertas por áreas úmidas, que variam desde florestas tropicais a savanas e campos com solos alagados permanente ou sazonalmente (Junk et al., 2011; 2014). O conhecimento sobre a adaptação das plantas ao alagamento está bem estabelecido para espécies de florestas tropicais sujeitas ao alagamento (Lobo e Joly, 1998; Parolin, 2001; Lopez e Kursar, 2003; Mielke et al., 2003; Rengifo et al., 2005; Kolb e Joly, 2009; Parolin et al., 2010; Wittmann et al., 2013), entretanto, o tema tem sido pouco abordado para espécies da savana (Kissmann et al., 2014; Oliveira et al., 2015; Oliveira e Gualtieri, 2017).

Uma das principais limitações ao estabelecimento de plantas lenhosas em áreas úmidas é a redução da disponibilidade de oxigênio no solo para suas raízes, devido à baixa difusão de O_2 em solos alagados (Greenwood, 1961; Ernst, 1990). Nestas condições, processos microbianos dependentes de O_2, como a nitrificação, são afetados, prejudicando a absorção de nutrientes pelas plantas (Kozlowski, 1997). A regeneração de ATP através da respiração aeróbica é cessada, resultando em mudanças no metabolismo energético (Kolb et al., 2002; Colmer e Voesenek, 2009) e alterações hormonais nas plantas (Voesenek et al., 2006). O alagamento geralmente é seguido por decréscimos no crescimento da planta (Kissmann et al., 2014; Oliveira et al., 2015), o que pode representar uma estratégia de sobrevivência, pois permite a economia de energia através da redução da respiração, para manter um baixo metabolismo (Wiedenroth, 1993). A redução do crescimento geralmente está associada com a redução nas taxas de assimilação de carbono, que por sua vez, pode ser influenciada pela redução da condutância estomática, que limita o acesso ao CO_2 (Mielke et al., 2003; Oliveira e Joly, 2010; Kissmann et al., 2014), e/ou por danos diretos aos fotossistemas, que reduzem a eficiência da fase fotoquímica da fotossíntese (Reginfo et al., 2005; Oliveira e Joly, 2010).

A tolerância ao alagamento varia grandemente entre as espécies (Kozlowski, 1997; Siebel e Blom, 1998). A persistência em áreas sujeitas ao alagamento geralmente está ligada à capacidade de manifestar alterações morfoanatômicas que facilitam a oxigenação dos órgãos submersos, como formação de tecidos aerenquimatosos, produção de lenticelas hipertróficas e

raízes adventícias (Blom e Voesenek, 1996; Kozlowski, 1997). A presença de tais adaptações está relacionada com a manutenção do crescimento e sobrevivência sob condições de alagamento, pois permitem a redução dos danos causados pelo decréscimo na concentração de O_2 em solos alagados (Crawford, 1992; Kolb et al., 1998; Kolb e Joly, 2009; Pimentel et al., 2014; Oliveira et al., 2015). Assim, espera-se encontrar uma alta plasticidade nesses atributos em espécies que estão sujeitas ao alagamento em seus habitats característicos (Kolb et al., 1998; Kissmann et al., 2014), porém, a tolerância ao alagamento nem sempre está relacionada diretamente com a ocorrência das espécies em áreas alagadas (Lopez e Kursar, 2003).

O Cerrado brasileiro (uma savana neotropical) é composto por um mosaico de vegetações, que variam desde campos abertos e savanas até florestas úmidas densas (Oliveira e Ratter, 2002). Embora fisionomias savânicas predominem na paisagem, devido à predominância de solos bem drenados e de baixa fertilidade, muitas áreas do Cerrado são ocupadas por áreas úmidas, como campos úmidos, savanas úmidas ou florestas de galeria (Oliveira e Ratter, 2002; Cianciaruso et al., 2005; Munhoz e Felfili, 2006; Bijos et al., 2017). Os campos úmidos são áreas úmidas dominadas por vegetação herbáceo-arbustiva sobre solos ácidos, com alto teor de matéria orgânica, sazonalmente ou permanentemente alagados. São ecossistemas com rica diversidade de espécies de plantas herbáceas tolerantes ao alagamento do solo (Tannus e Assis, 2004; Munhoz e Felfili, 2006; Munhoz et al., 2008; Amaral et al., 2013). Mesmo sendo frequentemente encontrados entre as savanas e florestas de galeria (Oliveira e Ratter 2002), é rara a ocorrência de árvores nos solos orgânicos e alagados desta vegetação (Tannus e Assis, 2004).

Espécies arbóreas savânicas são geralmente intolerantes ao alagamento, tendo sua distribuição restrita aos solos bem drenados da savana nos interflúvios (Oliveira e Ratter, 2002; Oliveira et al., 2015). Florestas de galeria por sua vez, ocorrem sobre solos úmidos e frequentemente sujeitos a inundação, abrigando espécies arbóreas tolerantes ao alagamento (Felfili, 1995; Sampaio et al., 2000; Oliveira e Ratter 2002; Guarino e Walter 2005; Kissmann et al., 2012, 2014). Entretanto, as espécies da floresta raramente se estabelecem nos campos adjacentes, com solos permanentemente alagados (Durigan et al. 2004).

Uma vez que a saturação hídrica dos solos pode ser considerada como o principal fator de filtragem de espécies de árvores nos campos úmidos do Cerrado (Cianciaruso et al., 2005; Leite et al., 2018; Xavier et al., 2019), pretendemos responder como a saturação hídrica e o alagamento do solo de campo úmido podem afetar o estabelecimento de plantas jovens em três espécies arbóreas com diferentes níveis de tolerância ao alagamento (tolerante, intolerante

e indiferente ao alagamento). Nossa hipótese é que plantas intolerantes não serão capazes de ajustar suas características fisiológicas sob condições de saturação hídrica e alagamento do solo, o que trará prejuízo ao crescimento e levará a uma mortalidade elevada. Por sua vez, plantas de espécies tolerantes e indiferentes serão capazes de ajustar suas características fisiológicas, especialmente as trocas gasosas, o que levará a melhores desempenhos no acúmulo de biomassa e a uma elevada sobrevivência sob condições de saturação hídrica e alagamento do solo.

MATERIAL E MÉTODOS

Material vegetal e condições experimentais

Frutos das espécies *Calophyllum brasiliense* Cambess. (Calophyllaceae), *Hymenaea stigonocarpa* Mart. ex Hayne (Fabaceae) e *Tapirira guianensis* Aubl. (Anacardiaceae) foram coletados na Floresta Estadual de Assis (FEA), localizada entre as coordenadas geográficas 22º36’50’’S e 50º24’52’’W, no município de Assis, estado de São Paulo, Brasil. As três espécies selecionadas para este estudo ocorrem naturalmente no domínio do Cerrado (Oliveira-Filho e Ratter, 1995; Ratter et al., 2003), porém, diferem tanto na distribuição, considerando os principais tipos de vegetação do Cerrado, quanto na tolerância à saturação hídrica dos solos (Durigan et al., 2004). Frutos maduros das espécies estudadas foram coletados de diferentes árvores matrizes (n = 5 – 10) que ocorriam em seus ambientes naturais: *H. stigonocarpa* (exclusiva de solos bem drenados) em vegetação savânica (cerrado *sensu stricto*); *C. brasiliense* (exclusiva de ambientes úmidos) e *T. guianensis* (indiferente à umidade do solo) em florestas de galeria.

As sementes foram extraídas dos frutos e a polpa aderida às sementes foi removida manualmente, em água corrente. As sementes das espécies *C. brasiliense* e *H. stigonocarpa* foram escarificadas mecanicamente com auxílio de lixas para madeira nº 80. As sementes foram dispostas sobre papel filtro em caixas plásticas, umedecidas com água destilada e mantidas em câmaras de germinação regulada à temperatura constante de 25 ºC e sob fotoperíodo de 12 horas de luz branca fluorescente (60 µmol m^{-2} s^{-1}). Sementes que apresentavam de 1 a 2 cm de comprimento da raiz primária foram colocadas em caixas de areia lavada fina em casa de vegetação, sob tela com 50% de sombreamento (aprox. 679 µmol m^{-2} s^{-1}), e regadas diariamente. Plantas com aproximadamente 15 cm de altura foram transplantadas para sacos plásticos com cerca de 1.100 cm^3, contendo solo de campo úmido.

O solo de campo úmido foi coletado a uma profundidade de 0-20 cm, na FEA e as características físico-químicas do solo são apresentadas na Tabela A1 (Anexos). Feito o transplante, as plantas permaneceram mais 15 dias sob sombreamento de 50%. Após este período, as plantas foram expostas e aclimatadas à luz plena (aprox. 1395 µmol m^{-2} s^{-1} ao meio-dia) durante 5 dias antes de serem submetidas a três tratamentos com diferentes condições hídricas: (I) solo alagado, (II) solo encharcado [com excesso de umidade] ou (III) solo controle [sem excesso ou falta de água]. Cada planta foi considerada como uma réplica, e o número de réplicas variou entre os tratamentos e espécies em função da disponibilidade de sementes e plantas, sendo que o número de réplicas variou de 15 a 30 no tratamento alagado e de 10 a 15 nos tratamentos encharcado e controle.

A condição de alagamento foi simulada através da disposição dos sacos de polietileno em tanques plásticos (65 x 55 x 20 cm; 71,5 L), onde o nível da água permaneceu a 2-3 cm acima do solo. Para a condição de solo encharcado, os sacos de polietileno foram mantidos em tanques (dimensões citadas acima) com o nível da água cobrindo ¾ da altura dos sacos, com a lâmina d'água abaixo da superfície do solo. As concentrações de O_2 dissolvido no volume de água foram obtidas com um medidor multiparâmetro de qualidade da água (U-50, HORIBA Advanced Techno Co., Ltd., Kyoto, Japão). Cinco medidas de O_2 dissolvido foram realizadas em cada tanque, sendo as medidas realizadas nos cantos e no centro, à uma profundidade que correspondia à metade da altura da lâmina de água em cada tanque. No tratamento alagado a água apresentava concentração de O_2 dissolvido de 6,6 ± 0,9 mg L^{-1} à temperatura de 22,1 ± 0,1 ºC. E no tratamento encharcado a água apresentava concentração de O_2 dissolvido de 5,1 ± 0,3 mg L^{-1} à temperatura de 23,6 ± 0,3 ºC. Para o tratamento controle, as plantas foram regadas uma vez ao dia. Os experimentos foram conduzidos em desenho inteiramente casualizado, logo após o período de dispersão natural das sementes, de outubro de 2016 a março de 2017 para as espécies *C. brasiliense* e *T. guianenses*, e de fevereiro a abril de 2018 para a espécie *H. stigonocarpa*. A umidade do solo em cada tratamento foi aferida pelo método gravimétrico, em cinco réplicas de cada tratamento. A quantidade média de água no solo em cada tratamento foi de 0,36 g H_2O g $solo^{-1}$ no controle, 0,70 g H_2O g $solo^{-1}$ no encharcado e 0,94 g H_2O g $solo^{-1}$ no alagado.

Parâmetros fotossintéticos

Após 20 dias desde o início do experimento foram realizadas medidas fisiológicas foliares de fluorescência da clorofila *a* e trocas gasosas em folhas jovens, porém

completamente expandidas, em cada indivíduo (n = 3 – 5 por espécie), sendo ambas as medidas realizadas na mesma folha. As medidas foram realizadas durante o período da manhã (08:30 - 11:00 h). As medidas de fluorescência da clorofila *a* foram realizadas em folhas pré-adaptadas ao escuro por 30 minutos, através do uso de um fluorômetro portátil (PAM Fluorometer – Junior; Walz, Alemanha). Diferentes variáveis fotoquímicas foram obtidas a partir de parâmetros da fluorescência da clorofila *a*: F_M e F_0 (fluorescência máxima e mínima de folhas adaptadas ao escuro, respectivamente), e F_M' e F_0' (fluorescência máxima e mínima de folhas adaptadas à luz, respectivamente). Utilizamos o software WINCONTROL (V.3.18) (Walz, Alemanha) (Genty et al., 1989) para obter os valores do rendimento quântico máximo do fotossistema II (F_v/F_m) e a taxa máxima de transporte de elétrons ($ETR_{max} = \Delta F/F_M' \times PAR \times 0,5 \times \alpha$). O valor médio de 0,84 foi utilizado para absorbância foliar (α) (Maxwell e Johnson, 2000; Baker, 2008). As trocas gasosas foram medidas através de um sistema aberto e portátil de trocas gasosas por infravermelho (LcProSD; ADC BioScientific Ltd., Hoddesdon, Reino Unido), para determinação dos valores de taxa de assimilação de carbono (*A*) e condutância estomática (g_s). Essas medidas foram tomadas sob uma temperatura constante de aproximadamente 26 °C e uma densidade constante de fluxo de fótons saturante de 1.400 μmol $m^{-2}s^{-1}$, quantidade considerada suficiente para saturar o aparato fotossintético de espécies arbóreas savânicas e florestais do Cerrado (Franco e Lüttge, 2002).

Desenvolvimento e biomassa

Ao longo do experimento foram observadas alterações morfológicas que facilitam a oxigenação dos tecidos submersos frente ao excesso de água, como a formação de lenticelas hipertróficas e desenvolvimento de raízes adventícias (Blom e Voesenek, 1996; Kozlowski, 1997). Após 60 dias de experimento, selecionamos aleatoriamente cinco plantas de cada tratamento para avaliar a massa seca do caule, da raiz e das folhas. Para determinação da massa seca, os materiais foram coletados, acondicionados separadamente em sacos de papel e mantidos em estufa a 80 ºC durante 48 horas. Após secagem, foram pesados separadamente em balança analítica. A mortalidade das plantas foi avaliada após 60 dias de tratamento, e o número de réplicas consideradas variou conforme a espécie: *C. brasiliense* (n = 10 – 25), *H. stigonocarpa* (n = 5 – 10) e *T. guianensis* (n = 9 – 23).

Análise dos dados

O efeito dos tratamentos sobre a ETR_{max}, F_v/F_m, *A*, g_s e biomassa das plantas foi analisado através de análises de variância simples, separadamente para cada espécie, e seguida pelo teste de Tukey ($P < 0,05$) para a comparação das médias. Todos os dados foram testados quanto a normalidade (teste de Shapiro-Wilk, $\alpha=0,05$) e homogeneidade das variâncias (teste de Levene, $\alpha=0,05$), e quando não atenderam estes pressupostos foram transformados para Log10, porém são apresentados nas figuras e tabelas os dados originais.

RESULTADOS

Respostas fisiológicas

Em plantas de *C. brasiliense*, o tratamento encharcado proporcionou melhor desempenho na taxa de transporte de elétrons (ETR_{max}) em relação aos tratamentos controle e alagado, os quais não diferiram entre si ($F = 8,0$; $GL = 2$; $P < 0,001$) (Fig. 1A). Plantas de *H. stigonocarpa* apresentaram menor ETR_{max} no tratamento alagado em relação aos tratamentos controle e encharcado (Fig. 1C), que não diferiram entre si ($F = 30,5$; $GL = 2$; $P < 0,001$). Os tratamentos não afetaram significativamente a ETR_{max} em plantas de *T. guianensis* ($F = 1,4$; $GL = 2$; $P = 0,28$) (Fig. 1E). Não houve efeito significativo dos tratamentos sobre o F_v/F_m em plantas de *C. brasiliense* ($F = 2,43$; $GL = 2$; $P = 0,12$) e *T. guianensis* ($F = 1,38$; $GL = 2$; $P = 0,29$) (Fig. 1B e 1F, respectivamente). Entretanto, houve redução dessa variável no tratamento alagado em plantas de *H. stigonocarpa* em relação aos tratamentos controle e encharcado, que não diferiram entre si ($F = 32,09$; $GL = 2$; $P < 0,001$) (Fig. 1D).

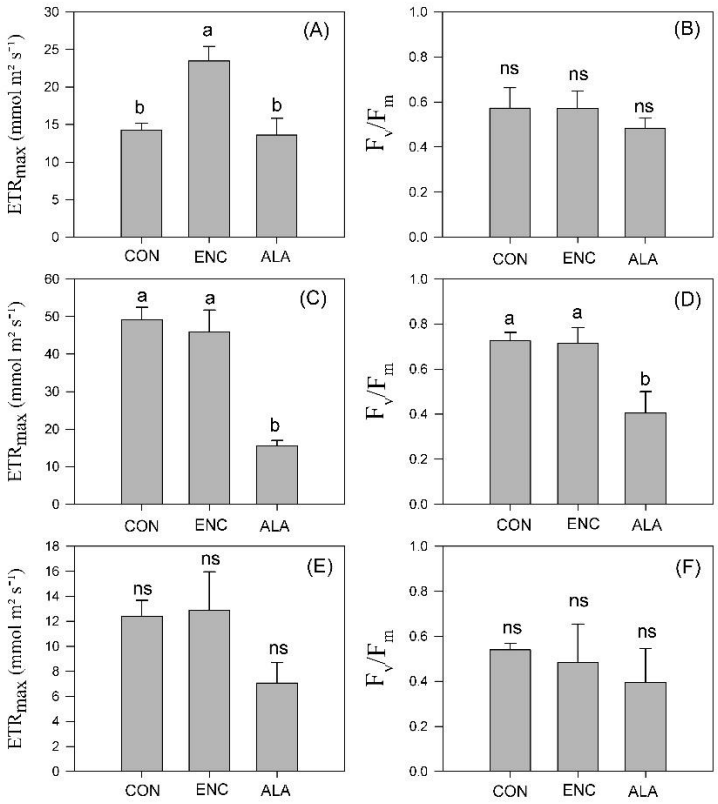


Figura 1. Valores médios ± erro padrão (n = 5) da taxa máxima de transporte de elétrons (ETR_{max}) e rendimento quântico máximo do fotossistema II (F_v/F_m) em folhas de *Calophyllum brasiliense* (A e B), *Hymenaea stigonocarpa* (C e D) e *Tapirira guianensis* (E e F) 20 dias após serem submetidas aos tratamentos controle (CON), Encharcado (ENC) e Alagado (ALA). Letras diferentes indicam diferença estatística entre as médias de acordo com o teste de Tukey ($P < 0,05$).

Não houve efeito significativo dos tratamentos aplicados sobre a taxa de assimilação de carbono (*A*) ($F = 0,99$; $GL = 2$; $P = 0,40$) e g_s ($F = 0,15$; $GL = 2$; $P = 0,85$) em plantas de *C. brasiliense* (Fig. 2A e B). Para plantas de *H. stigonocarpa*, os tratamentos encharcado e alagado reduziram drasticamente *A* ($F = 57,1$; $GL = 2$; $P < 0,0001$) e g_s em relação ao controle ($F = 24,8$; $GL = 2$; $P < 0,0001$) (Fig. 2C e D). Em *T. guianensis*, os valores de *A* foram menores no tratamento alagado em relação aos tratamentos controle e encharcado, que não diferiram entre si ($F = 43,9$; $GL = 2$; $P < 0,0001$) (Fig. 2E). E os valores de g_s foram reduzidos nos tratamentos encharcado e alagado em relação ao tratamento controle ($F = 12,5$; $GL = 2$; $P < 0,001$) (Fig. 2F).

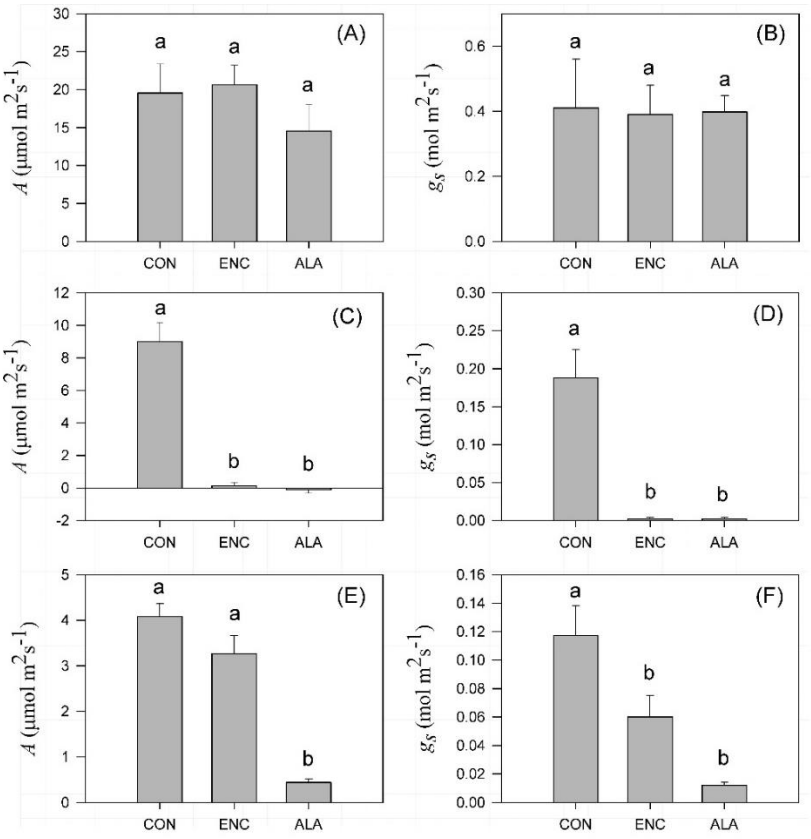


Figura 2. Valores médios ± erro padrão (n = 5) de taxa de assimilação de carbono (*A*) e condutância estomática (g_s) em plantas de *Calophyllum brasiliense* (A e B), *Hymenaea stigonocarpa* (C e D) e *Tapirira guianensis* (E e F) após serem submetidas aos tratamentos controle (CON), Encharcado (ENC) e Alagado (ALA) durante 20 dias. Letras diferentes indicam diferença estatística entre as médias de acordo com o teste de Tukey ($P < 0,05$).

Crescimento, respostas morfológicas e mortalidade

Os tratamentos afetaram significativamente todas as variáveis de crescimento em plantas de *C. brasiliense* (Tabela 1). Para esta espécie, o solo encharcado proporcionou maior média de biomassa total, devido ao melhor desenvolvimento das raízes nessa condição (Tabela 2). Já o tratamento alagado reduziu significativamente a acumulação de massa foliar em comparação com o controle, entretanto, a massa total das plantas não diferiu significativamente do tratamento controle (Tabela 2). Para a espécie *H. stigonocarpa* houve efeito significativo dos tratamentos apenas para a massa das raízes (Tabela 1), com plantas alagadas apresentando redução de cerca de 75% da massa em relação ao controle (Tabela 2). Para *T. guianensis* houve efeito significativo dos tratamentos apenas para a massa das raízes (Tabela 1). Plantas do tratamento encharcado apresentaram maior biomassa das raízes em relação ao tratamento alagado, porém não diferiram significativamente do tratamento controle (Tabela 2).

Tabela 1. Resultados da análise de variância (anova) para o efeito dos tratamentos sobre a biomassa seca do caule, folhas, raízes e da planta (total) para as espécies estudadas.

	gl	*F*	*P*
Calophyllum brasiliense			
Caule	2	4,68	0,030
Folhas	2	18,6	0,0002
Raiz	2	21,7	0,0001
Total	2	34,9	0,0000
Hymenaea stigonocarpa			
Caule	2	0,07	0,93
Folhas	2	1,80	0,21
Raiz	2	6,70	0,01
Total	2	3,05	0,08
Tapirira guianensis			
Caule	2	3,57	0,060
Folhas	2	1,73	0,217
Raiz	2	4,33	0,038
Total	2	3,36	0,068

Tabela 2. Valores médios ± erro padrão (n = 5) de massa seca do caule, folhas, raízes e massa total (total) de plantas após serem submetidas durante 60 dias a tratamentos com diferentes conteúdos de água no solo. Letras diferentes indicam diferença estatística entre as médias nas colunas, de acordo com o teste de Tukey ($p < 0,05$).

Espécie/tratamento	Caule (g)	Folhas (g)	Raízes (g)	Total (g)
Calophyllum brasiliense				
Controle	0,80 ± 0,17 ab	0,97 ± 0,10 a	0,39 ± 0,04 b	1,64 ± 0,18 b
Encharcado	1,17 ± 0,10 a	1,17 ± 0,05 a	0,68 ± 0,05 a	3,02 ± 0,06 a
Alagado	0,71 ± 0,10 b	0,57 ± 0,07 b	0,26 ± 0,02 b	1,54 ± 0,17 b
Hymenaea stigonocarpa				
Controle	0,70 ± 0,12 a	1,28 ± 0,17 a	1,33 ± 0,29 a	3,32 ± 0,56 a
Encharcado	0,75 ± 0,05 a	1,07 ± 0,15 a	0,64 ± 0,17 ab	2,47 ± 0,32 a
Alagado	0,69 ± 0,14 a	0,88 ± 0,15 a	0,33 ± 0,04 b	1,90 ± 0,28 a
Tapirira guianensis				
Controle	0,15 ± 0,05 a	0,65 ± 0,14 a	0,59 ± 0,11 ab	1,40 ± 0,28 a
Encharcado	0,26 ± 0,03 a	0,87 ± 0,09 a	0,70 ± 0,08 a	1,83 ± 0,17 a
Alagado	0,14 ± 0,02 a	0,63 ± 0,03 a	0,36 ± 0,04 b	1,13 ± 0,06 a

Ao final do período experimental, todos os indivíduos das espécies *C. brasiliense* e *T. guianensis* apresentaram alterações morfológicas em resposta ao excesso de água no solo. A formação de lenticelas hipertróficas caulinares, após 15 dias de experimento, foram as primeiras alterações morfológicas observadas nos tratamentos encharcado e alagado nas duas espécies (Fig. 3B, C, H e I). A formação de raízes adventícias no tratamento encharcado (*C. brasiliense*) e alagado ocorreu após 15 e 30 dias de experimento para as espécies *T. guianensis* e *C. brasiliense* respectivamente (Fig. 3B, C e I). Plantas da espécie *H. stigonocarpa* não apresentaram alterações morfológicas em resposta à saturação hídrica (Fig. 3E e F), e apresentando clorose foliar no tratamento alagado (dado não mostrado).

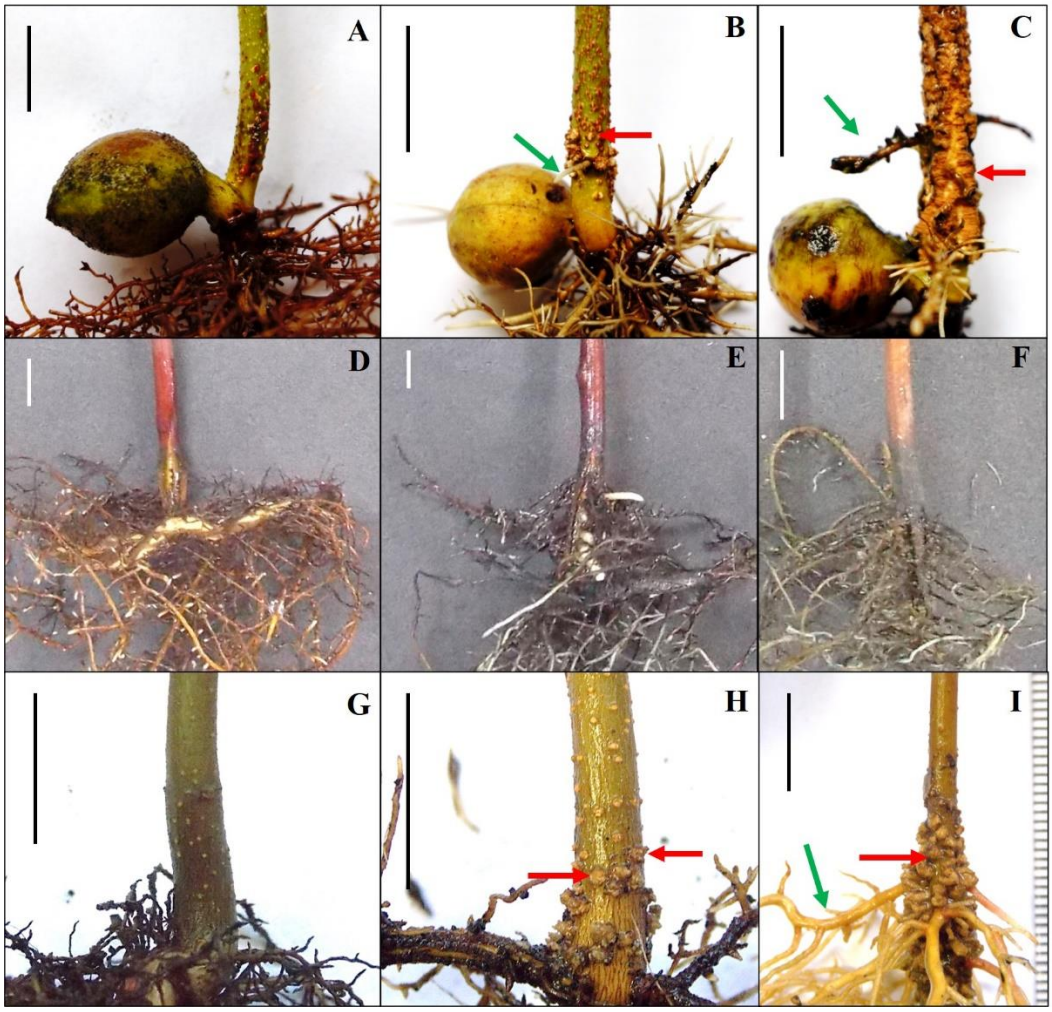


Figura 3. Detalhes da base do caule em plantas submetidas aos tratamentos controle (coluna da esquerda), encharcado (coluna central) e alagado (coluna da direita) após 60 dias. *Calophyllum brasiliense* (A, B e C), *Hymenaea stigonocarpa* (D, E e F) e *Tapirira guianensis* (G, H e I). Setas verdes: raízes adventícias; Setas vermelhas: lenticelas hipertróficas; Barras: 1 cm.

Para a espécie *C. brasiliense* não houve mortalidade de plantas nos tratamentos aplicados. Para a espécie *H. stigonocarpa* não houve mortalidade entre as plantas no tratamento controle, entretanto, houve mortalidade de uma (20%) e quatro (40%) plantas nos tratamentos encharcado (n = 5) e alagado (n = 10), respectivamente. Para a espécie *T. guianensis* não houve mortalidade nos tratamentos controle e encharcado, e uma planta morreu no tratamento alagado (4,3%; n = 23).

DISCUSSÃO

Nossos resultados demonstram que as espécies estudadas exibem respostas distintas ao estresse por alagamento em solos de campos úmidos. Com exceção da massa foliar, nenhum dos outros parâmetros avaliados foi prejudicado pela saturação hídrica do solo em plantas de *C. brasiliense*, demonstrando sua alta tolerância ao alagamento. Nas plantas de *T. guianensis* o alagamento reduziu a assimilação de carbono, porém sem prejuízos para a acumulação de biomassa. E como esperado, o desempenho da espécie *H. stigonocarpa* foi bastante prejudicado pela saturação hídrica dos solos, com a inibição da capacidade fotossintética.

Como a sobrevivência das plantas é dependente de um balanço de carbono positivo, a manutenção da capacidade fotossintética é um aspecto chave para a persistência em solos alagados (Chen et al., 2005). A redução da assimilação de carbono é uma resposta comum em plantas alagadas (Kozlowski, 1997; Rengifo, et al., 2005; Parent et al., 2011), sendo geralmente causada pela redução da g_s, que limita a disponibilidade de CO_2 para as reações de fixação do carbono (Lopez e Kursar, 2003; Mielke et al., 2003; Kissmann et al., 2014). No presente estudo, plantas de *H. stigonocarpa* apresentam forte inibição da taxa de assimilação de carbono (*A*) nos tratamentos encharcado e alagado, devido à alta redução da condutância estomática (g_s). A redução da g_s em plantas alagadas geralmente é atribuída a uma diminuição na hidratação foliar (Pezeshki, 2001) e/ou a fatores não hidráulicos, como a sinalização hormonal entre as raízes alagadas e o caule (Zhang e Zhang, 1994; Else et al., 2009). Plantas alagadas de *H. stigonocarpa* também apresentaram menor eficiência fotoquímica, evidenciada pela redução da taxa máxima de transporte de elétrons (ETR_{max}) e do rendimento quântico máximo do fotossistema II (F_v/F_m), indicando a presença de danos nos fotossistemas em plantas alagadas (Oliveira e Joly, 2010). Além disso, observou-se clorose foliar nas plantas alagadas dessa espécie, provavelmente devido à degradação de clorofilas (Kozlowski, 1997).

Apesar da redução generalizada da capacidade fotossintética de *H. stigonocarpa* em condições de alagamento, não houve redução da biomassa total das plantas. Contudo,

observou-se acentuada redução da biomassa das raízes em plantas alagadas, indicando a degeneração de raízes provocada pela saturação hídrica do solo (Parent et al., 2011). O tratamento encharcado, de maneira geral, proporcionou maior acúmulo de massa das raízes em plantas das espécies *T. guianensis* e *C. brasiliensis*, o que levou a maior massa total em plantas *C. brasiliense* neste tratamento. Estes resultados sugerem que plantas da espécie *C. brasiliense* tem preferência por solos saturados de água, evidenciado pelos maiores valores de ETR_{max} e maior massa das raízes no tratamento encharcado. O incremento de biomassa em situações de saturação hídrica é uma resposta pouco relatada. Porém, resultados similares foram encontrados para *Tabebuia cassinoides*, espécie tolerante ao alagamento e típica de florestas inundadas no domínio da floresta tropical atlântica (Kolb e Joly, 2009).

A ocorrência de raízes adventícias e hipertrofia de lenticelas induzidas pelo alagamento são mecanismos adaptativos frequentemente encontrados em plantas tolerantes ao alagamento (Parent et al., 2011; Kissmann et al., 2014; Pires et al., 2018). Essas estruturas podem facilitar as trocas de gases entre as partes aéreas e submersas das plantas, e auxiliar na eliminação de metabólitos potencialmente tóxicos (Kozlowski, 1997; Medri et al., 1998). Assim, estão geralmente associadas com a melhor manutenção da capacidade fotossintética e crescimento das plantas em condições de alagamento, uma vez que aliviam os efeitos negativos do estresse (Kozlowski, 1997; Kolb et al., 1998; Parent et al., 2011; Oliveira e Joly, 2010; Kissmann et al., 2014). Assim, o desenvolvimento dessas estruturas pode explicar a melhor performance fotossintética e a menor mortalidade em plantas de *T. guianensis* e *C. brasiliense* em tratamentos com elevada saturação hídrica do solo, quando comparadas à performance da espécie savânica *H. stigonocarpa*.

Espécies arbóreas da savana e da floresta de galeria diferem essencialmente em seus atributos funcionais envolvidos na utilização de recursos, provavelmente devido à história evolutiva das espécies em resposta as diferentes pressões seletivas presentes em seus ambientes típicos de ocorrência (Hoffmann et al., 2004; Hao et al., 2008; Rossatto et al., 2009; Capuzzo et al., 2012). Neste sentido, é provável que as respostas ao alagamento também sejam distintas entre espécies da savana e da floresta de galeria, uma vez que a sobrevivência em condições de alagamento está intimamente ligada à evolução das adaptações morfofisiológicas que conferem tolerância e sustentam o crescimento e a reprodução das plantas em ecossistemas sujeitos ao alagamento (Jackson et al., 2009; Parolin e Wittmann, 2010; Wittmann et al., 2013), embora algumas espécies conservem tais adaptações mesmo habitando áreas menos sujeitas ao alagamento (Pires et al., 2018).

A alta tolerância ao alagamento em plantas de *C. brasiliense* está de acordo com o fato dessa espécie ser frequentemente encontrada em solos alagados em matas de galeria (Scarano, 1998; Oliveira e Joly, 2010). A espécie generalista *T. guianensis* apresentou níveis intermediários de tolerância ao alagamento, o que pode explicar sua ampla distribuição na região Neotropical, desde solos bem drenados da savana até solos mal drenados da floresta de galeria, e em outros tipos de florestas ripárias (Oliveira-Filho e Ratter, 1995). Em contrapartida, plantas de *H. stigonocarpa* apresentaram baixa capacidade de realizar ajustes morfofisiológicos em resposta à saturação hídrica e ao alagamento do solo, indicando que, uma vez que suas sementes sejam dispersas sobre os campos úmidos, a colonização por esta espécie será limitada pelo excesso de água no solo. Estes resultados encontram suporte em estudos recentes, os quais demonstraram que a saturação hídrica dos solos limita a ocorrência de espécies lenhosas de comunidades savânicas em locais onde há o afloramento do lençol freático, como nos campos úmidos do Cerrado (Cianciaruso et al., 2005; Leite et al., 2018; Xavier et al., 2019).

Nós levantamos a hipótese de que a elevada saturação hídrica dos solos seja o fator primário que impede a colonização de espécies arbóreas típicas da savana nos campos úmidos (Leite et al., 2018; Xavier et al., 2019). Entretanto, acreditamos que apenas a saturação hídrica dos solos não seja suficiente para impedir a colonização dos campos úmidos por espécies de florestas de galeria adjacentes (Silva, 2015), especialmente espécies tolerantes ao alagamento, capazes de realizar ajustes morfofisiológicos que garantem sua persistência em solos hipóxicos (Blom e Voesenek, 1996; Kozlowski, 1997). No caso de espécies tolerantes ao alagamento, é provável que outros fatores atuem em conjunto com a saturação hídrica dos solos para limitar o avanço destas espécies sobre os campos úmidos adjacentes, como os regimes de incêndios naturais (Hoffmann et al., 2012), e geadas esporádicas (Hoffmann et al., 2019), que ocorrem principalmente nos limites ao sul no domínio do Cerrado.

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ANEXOS

Tabela A1. Características físico-químicas do solo de campo úmido utilizado nos experimentos.

Determinações	
Fósforo resina (P)	4,0 mg/dm^3
Matéria orgânica (M.O.)	34,0 g/dm^3
Carbono orgânico (C)	20,0 g/dm^3
Potencial hidrogeniônico (pH)	4
Potássio (K)	0,7 mmolc/dm^3
Cálcio (Ca)	4,0 mmolc/dm^3
Magnésio (Mg)	1,0 mmolc/dm^3
Acidez trocável (Al)	17,0 mmolc/dm^3
Saturação por base (V%)	7,3 %
Saturação por Al (m%)	74,9 %
Enxofre (S)	4,0 mg/dm^3
Sulfato (SO_4)	12,0 mg/dm^3
Areia	828,0 g/kg
Argila	123,0 g/kg
Silte	49,0 g/kg
Classificação textural	Arenoso

CAPÍTULO 3

Shallow water table and soil properties alone do not constrain tree colonization in tropical wet grasslands



Indivíduos de *Calophyllum brasiliense* emergidos a partir de semeadura direta (esquerda) e de muda (direita), plantadas em janeiro de 2016 em campo úmido na Floresta Estadual de Assis, SP. Altura dos indivíduos ao redor de 1,60 m, em junho de 2019.

Individuals of *Calophyllum brasiliense* emerged from direct seeding (left) and nursery-raised seedling (right), planted in January 2016 in a wet grassland at Assis State Forest, SP, Brazil. The height of the individuals was approximately 1.60 m in June 2019.

Shallow water table and soil properties alone do not constrain tree colonization in tropical wet grasslands

**Artigo submetido ao periódico Oecologia*

ABSTRACT

Open grassy vegetation and forests share riparian zones across the Neotropical savannas, characterizing alternative stable-states. The factors determining the occurrence and maintenance of each vegetation type, however, are yet to be elucidated. To disentangle the role of environmental factors constraining tree establishment in wet grasslands, we evaluated tree establishment during early (seedlings) and late (saplings growth and survival) stages. We also analysed the role of soil properties and groundwater depth in leaf gas exchange and leaf water potential of tree saplings introduced in Cerrado wet grasslands. Three functionally distinct tree species were evaluated: (I) a flood-tolerant species characteristic of gallery forests, (II) a flood-intolerant species characteristic of seasonally dry savannas, and (III) a generalist species found in both gallery forests and seasonally dry savannas. The gallery forest species had better potential to seedling establishment with germination better adapted to waterlogging than the other species. During the sapling stage, both the gallery forest and the generalist species presented growth and survival significantly higher than the savanna species. The surface groundwater depth restricted stomatal conductance and leaf water potential in savanna saplings, resulting in low carbon assimilation, slow growth and high mortality. Despite better growth and survivorship, gas exchange rates of the gallery forest and generalist trees were also impaired by surface groundwater depth. Waterlogging caused by surface groundwater is an important factor constraining tree establishment in wet grasslands, but in especial flood-tolerant trees can survive and grow, even slowly, in waterlogged grasslands, thus potentially overcoming this constraint.

Key words: Alternative stable-states, Cerrado, grassland-forest boundary, groundwater depth, flood tolerance, seedling establishment.

INTRODUCTION

Tropical grassy biome is characterized by the codominance of C_4 grasses and trees; when combined in different proportions, this biome forms a mosaic of vegetation types ranging from open grasslands to woody savannas (Scholes and Archer, 1997; Parr et al., 2014; Veldman et al., 2015) differentiated by tree cover, a major driver of ecosystem function (Sankaran et al., 2005; Hoffmann et al., 2012). Open vegetation types largely occur under suitable climate and soil conditions for forest expansion (Bond et al., 2005) and are often maintained by natural disturbances (Lehmann et al., 2011). Currently, there is an emerging consensus that, globally, water availability, fire, soil properties and herbivory interact to maintain grassy and forest biomes as alternative stable states in much of the tropics (Sankaran et al., 2005; Bond, 2008; Hirota et al. 2011; Lehmann et al. 2011; 2014).

Water availability drives tree cover under drier climates (Sankaran et al., 2005; Lehmann et al., 2011). In contrast, in mesic climates, fire has a pivotal role in the suppression of trees, acting via positive grass-fire feedback (Hoffmann et al., 2012; Dantas et al., 2013). In these systems, fire decreases tree cover, which subsequently favours the light-dependent grassy layer, promoting fuel for fire spread and maintaining an open-canopy vegetation state (Sankaran et al., 2005; Bond, 2008; Hoffmann et al., 2009; 2012; Dantas et al., 2013; Lehmann et al., 2014). Herbivory also exerts great influence on the vegetation structure (Levick et al., 2009; Staver and Bond, 2014), with grazers enhancing and browsers decreasing woody cover (Van Auken, 2009; Venter et al., 2018). On a global scale, soil properties poorly predict tree cover (Bond, 2010; Lehmann et al., 2011; 2014), but soil fertility is reported to determine the rates of tree growth after disturbance (Hoffmann et al., 2009; 2012; Dantas et al., 2013).

The previously depicted scenarios are useful to explain the treeless state of grassy vegetation on well-drained soils and at a large scale; however, many savannas and grasslands occur on poorly-drained, shallow groundwater and waterlogged soils across the Neotropical region (Sarmiento, 1984; Cianciaruso et al., 2005; Munhoz et al., 2008) on a rather fine-scale mosaic. In this situation, the superficial groundwater depth is noted as a constraining environmental factor of savanna woody vegetation along slope gradients across the Neotropical region (Rossatto et al., 2012; Villalobos-Vega et al., 2014; Leite et al., 2018). At the bottom of such slope gradients, wet grasslands from Brazilian savannas (known as Cerrado) form boundaries with gallery forests (swamp forests) (Ribeiro and Walter, 2008). It

has been proposed that soil waterlogging generated by surface groundwater prevents tree colonization in these grasslands (Villalobos-Vega et al., 2014; Leite et al., 2018; Xavier et al., 2019). However, many gallery forests are subjected to extended periods of waterlogging (Ribeiro and Walter, 2008; Teixeira et al., 2011), which may indicate that waterlogging alone does not prevent tree colonization on wet grasslands (Silva, 2015). Few studies have addressed tree establishment performances in natural conditions for the Neotropical grassy biomes (Hoffmann, 1996; 2000; Hoffmann et al., 2004; Kanegae et al., 2000), and to our best knowledge none has provided evidence based on field experiments assessing the extent to which abiotic variables limit tree establishment in tropical wet grasslands.

The major consequence of soil waterlogging is a decline in oxygen availability, which inhibits aerobic ATP production in seeds and roots (Crawford, 1992; Kozlowski, 1997; Kolb et al., 2002). For seeds, anoxia and hypoxia conditions reduce or completely inhibit germination and seedling emergence (Pérez-Ramos and Marañón, 2009; Kolb and Joly, 2010; Oliveira et al., 2019). For established seedlings, waterlogging imposes restrictions on stomatal conductance, which may limit water uptake and leaf carbon assimilation, thereby reducing plant growth and survival (Parent et al., 2011; Kissmann et al., 2014). Gallery forest trees that are subjected to soil waterlogging frequently show a suite of morphophysiological adaptations that alleviate anaerobic stress (e.g. adventitious roots, stem hypertrophic lenticels), allowing plants to maintain high enough carbon assimilation rates to sustain growth and survival under waterlogging conditions (Lobo and Joly, 1998; Oliveira and Joly, 2010; Kissmann et al., 2014). In contrast, savanna trees that rarely experience root waterlogging poorly express such adaptive responses and are expected to be more susceptible to waterlogging stress (Joly and Crawford, 1982; Oliveira et al., 2015).

Here, we investigated tree establishment in waterlogged soils of tropical grasslands, and how groundwater depth and soil properties affect leaf gas exchange and water potential of tree saplings, which are leaf functional traits related to flood tolerance and persistence. We performed field experiments to assess tree establishment in early (seedlings) and late (saplings) plant stages of three functionally distinct species: (1) a flood-tolerant species originating in gallery forest, (2) a flood-intolerant species originating in seasonal dry savannas and (3) a flood-tolerant generalist tree species that can occur at both gallery forest and seasonally dry savannas. Additionally, we analysed leaf gas exchange and leaf water potential in tree saplings to evaluate the possible effects of soil properties and groundwater depth on these leaf functional traits. We expected that seedling establishment and sapling growth and

survival would be higher for the gallery forest and generalist species that are adapted to waterlogged soils than for the savanna species, which are restricted to well-drained soils. We predicted that the higher sapling growth and survival of the gallery forest and generalist species would be related to higher carbon assimilation rates and better leaf rehydration of saplings year-around. In contrast, we expected lower carbon assimilation rates and lower leaf rehydration for savanna saplings, in close association with their lower sapling growth and survival. We also expected that low surface groundwater depth, which results in soil waterlogging, would be the main environmental factor hampering carbon assimilation and water status of saplings since soil waterlogging rather than soil properties is the most common factor constraining tree abundance in similar grasslands across the Brazilian Cerrado (Villalobos-Vega et al., 2014; Leite et al., 2018; Xavier et al., 2019).

MATERIALS AND METHODS

Study sites

We established three study sites on three patches of wet grasslands with no recent records of fire occurrence, at Assis State Forest (ASF), Assis municipality, São Paulo state, Brazil (22°36’1.03’’S and 50°25’41.47’’W, 22°36’3.36’’S and 50°25’37.07’’W, and 22°36’49.50’’S and 50°24’52.76’’W). The wet grasslands were surrounded by woody savannas upslope (in well-drained soils) and gallery forests downslope (in permanently wet soils) with altitudes ranging from 515 to 530 m. This transition savanna-wet grassland-gallery forest is a common feature in Cerrado landscapes (Munhoz and Felfili, 2008). The local climate is Cfa (subtropical humid with hot summer) following the Köppen classification, with an annual rainfall of approximately 1,400 mm concentrated in the summer (December to March) and a dry season of variable duration in the winter (Alvares et al., 2013). The annual average temperature is 21.8°C with an average minimum of 11°C and average maximum of 31°C. Soils in the region are often sandy with high aluminum concentration and low nutrient availability, classified from the summit down to the footslope, as rhodic haplustox, typic haplustox and epiaquic haplustult (Juhász et al., 2006). The waterlogged condition, which occurs in the entire Cerrado region in wet grasslands and veredas (i.e. wet grassland with the palm tree *Mauritia flexuosa* L.), is determined by groundwater fluctuations attributed to rain infiltration over the entire watershed and not related to floods, which are unusual in the Cerrado. The wet grasslands where the experiments were performed are characterized by a

dense graminoid vegetation layer (40-cm tall) with ground cover surpassing 90%. Soils in the experimental areas are hydromorphic with elevated organic matter levels, which is typical of wetlands in the Cerrado region (Munhoz et al., 2008).

Study species

The tree species selected for this study are abundant and naturally occur in the adjacent arboreal vegetation of the study wet grasslands, producing a large number of viable seeds annually, but exhibit different waterlogging tolerances: (I) *Calophyllum brasiliense* Camb. (Calophyllaceae) is a species widely distributed in gallery forests, being typical of moist soils, flood-tolerant, and rarely found in well-drained soils of savanna vegetation (Oliveira-Filho and Ratter, 1995; Oliveira and Joly, 2010); (II) *Hymenaea stigonocarpa* Mart. ex Hayne (Fabaceae) is a typical savanna species that grows exclusively in well-drained soils subjected to drought (Durigan et al., 1999); and (III) *Tapirira guianensis* Aubl. (Anacardiaceae) is a generalist species adapted to both gallery forests and savanna vegetation (Durigan et al., 1999).

We introduced the study species in wet grasslands by direct seeding and planting saplings to evaluate interspecific differences in seedling establishment, growth and survival under field conditions. Before planting, saplings of each species were grown in tubes (290 cm^3) with a commercial substrate (Carolina soil®) composed of peat, vermiculite, organic waste and limestone. Seeds were collected from at least 10 individuals per species in dry savannas (H. stigonocarpa) and gallery forests (*C. brasiliense* and *T. guianensis*). Seeds of *H. stigonocarpa* and *C. brasiliense* present physical dormancy and, although seedling emergence can occur naturally in the field, they take a long time to germinate. Therefore, to ensure the possibility of seedling emergence in the field during the experimental period, we mechanically scarified seeds of *C. brasiliense* and *H. stigonocarpa* before sowing. To verify the viability of seed lots used in the field experiment, we performed germination tests under semi-controlled conditions using the techniques summarized in the Table S1 (Supplementary data). The viability of the seed lots was high for all species (≥ 90%, Table S1).

Experimental design

The study was set up in a block design, with one block established in each wet grassland site (3 blocks in total). In each block, we carried out two experiments: (I) In the first experiment (henceforth, SS-experiment), we assessed the seedling emergence, as well as

growth and survival of saplings, and (II) in the second experiment (henceforth Phy-experiment), we assessed leaf physiological parameters of saplings. In the SS-experiment, for each block, 30 lines were perpendicularly positioned to the stream margin with one end close to the savanna and the other close to gallery forest (Fig. 1a). Each line consisted of 10 planted saplings and 10 seed holes containing 3 seeds each of the same species (Fig. 1b). There was at least 1-m distance between saplings or seed holes within lines and 1-m distance between lines. Inside each line, saplings and seed holes were placed 50 cm from each other. In the Phy-experiment, we introduced only saplings within each block, planting five lines of each species with 10 saplings per line (Fig. 1c). The distance between saplings and lines was the same described for the SS-experiment. In all blocks, species were systematically alternated among planting lines (Fig. 1b, c) to encompass the soil heterogeneity within sites.

Saplings (approximately 50 cm in height) were planted in January 2016 in holes opened with a digger in both experiments, totalling 450 individuals per species in each block. Seeds were sown in their dispersal period as follows: January, August and December 2016 for *T. guianensis*, *C. brasiliense* and *H. stigonocarpa*, respectively. Three seeds per hole were sown at a 1-cm depth to avoid predation and removal by animals, totalling 900 seeds per species. The location of each seed hole was marked by a coloured flag.

Soil and groundwater depth variables

One line of five piezometric wells was installed to monitor the groundwater depth in Phy-experiment of each block (Fig. 1a), 15 in total. Wells were opened with an auger deep enough to expose the groundwater even in the dry season. In each well, we installed a 2" PVC pipe with perforations and covered by a geotextile blanket to allow the passage of water and avoid the passage of soil particles. The lines of piezometric wells were perpendicular to the stream margin with one end close to the savanna and another end close to the gallery forest. Each line was positioned at the middle of each Phy-experiment (Fig. 1a, c). During data collection, wells near the savanna corresponded to position 1, and those close to the gallery forest corresponded to position 5 within each line of wells (Fig. 1c). The groundwater depth was recorded monthly from January 2016 to December 2017 with a piezometer, which consists of a graduated grid connected to a sensor that emits a sound signal when in contact with water. From these data, we obtained the minimum, maximum and the range of groundwater depth during the observation period.

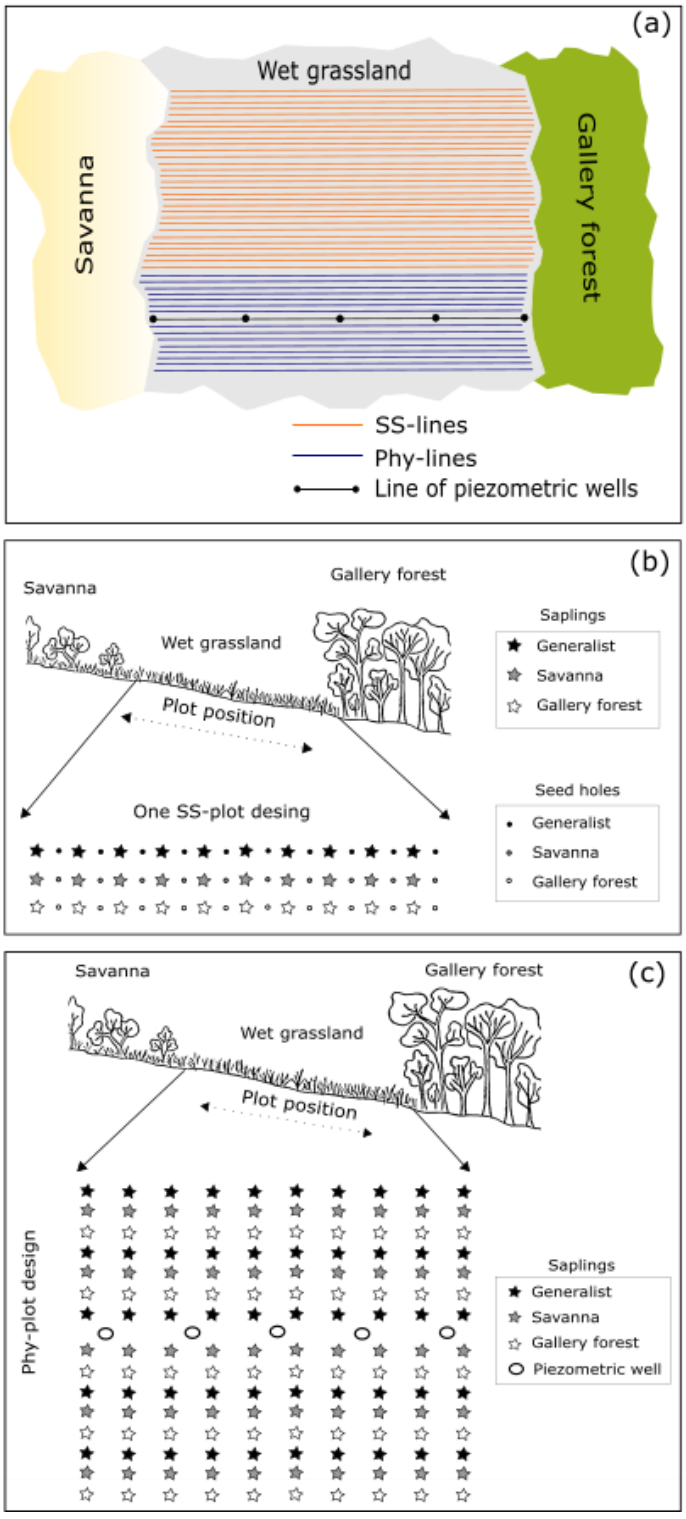


Figure 1. Physiognomic profile of vegetation in the experimental sites and a general description of experimental design. (a) Schematic experimental design along an experimental wet grassland site, where SS-lines contained saplings and seed holes and Phy-lines contained only saplings with one line of piezometric wells. (b) SS-plot position in the field and the

arrangement of saplings and seeds holes within each line. (c) Phy-plot position in the field and the arrangement of the saplings within each line and piezometric wells.

We collected five surface-soil subsamples (0–20 cm) around each piezometric well, which were mixed and homogenized to obtain a composite soil sample. In total, 15 soil samples were subjected to physical and chemical analyses. The particle size distribution (soil fractions of clay and sand) was determined using the pipette method (Camargo et al., 1986). For chemical analyses, the pH was determined in $CaCl_2$ solution, according to Raij et al. (1987). Total acidity (H + Al; mmolc/dm³) was determined following methods described in Quaggio and Raij (2001). Phosphorus (H_2PO_4-) was determined by spectrophotometry after extraction via an anion exchange resin (Raij et al., 1987). Potassium (K^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) were determined by flame spectrophotometry (Allen et al., 1974). Soil base saturation was then quantified using the formula BS = [(Ca^{2+} + Mg^{2+} + K^+)/(Ca^{2+} + Mg^{2+} + K^+ + H + Al)] x 100, where BS is the base saturation in percentage. Organic matter was determined after the addition of potassium dichromate-sulphuric acid to the soil (Sims and Harby, 1971).

Data collection

Seedling emergence in each seed hole from SS-experiment was verified monthly after seeding during 12 months (from February 2016 to December 2017). We considered that seedlings emerged when there was an emergence of any vegetative part above ground. To evaluate sapling growth, we measured stem height and base diameter (1 cm above the ground) in October 2016 (initial measure) and October 2017 (final measure) in SS-experiment for all living saplings. Sapling survival was evaluated in June 2019 (41 months after planting). We consider saplings as alive when presenting at least fresh stem parts (undried). During the experimental period we recorded, whenever possible, if emerged seedlings and planted saplings presented morphological responses associated with flooding tolerance, such as hypertrophic lenticels and adventitious roots in the stem base.

Physiological parameters were taken from saplings in Phy-experiment. Carbon assimilation rate (A; µmol m^{-2} s^{-1}) and stomatal conductance (g_s; mol m^{-2} s^{-1}) measurements were obtained on fully expanded leaves from the second or third node of saplings (n = 26–31; one leaf per individual) between 08h00 and 11h00, with a portable system for gas exchange measurements (LcProSD; ADC BioScientific Ltd., Hoddesdon, UK). These measurements

were obtained under environmental temperatures from 25 to 28 ºC and at a photosynthetic photon flux density (PPFD) of 1,400 µmol m^{-2} s^{-1}, sufficient to saturate the leaf photosynthetic apparatus of most savanna plants (Franco and Lüttgue, 2002). Leaf water potential (Ψ_w) was evaluated using a pressure chamber (PSM 1000, Albany, USA) between 04h00 and 06h00 (predawn measurements; Ψ_{pd}) and between 11h00 and 12h30 (midday measurements; Ψ_{md}), in the same plants used for gas exchange measurements. All leaf physiological parameters were obtained in April 2017 (end of the wet season) and September 2017 (end of the dry season).

Data analyses

The monthly average percentage of seedling emergence (SE%) for each line and species was calculated using the equation: SE% = $((\sum niN^{-1}) \times 100))$, where Σn_i is the number of seedlings in relation to the number of seeds (N) placed to germinate in the field. Seedling establishment was defined as the number of surviving seedlings after 12 months divided by the number of seeds sown in each line (Hoffmann et al., 2004). Differences in species seedling establishment were tested using generalized linear mixed models (GLMMs) with a binomial distribution. For this model, the random effect was the block.

To evaluate sapling growth, we calculated the stem-volumetric increment (SVI) of saplings for each species by subtracting the final stem volume (*FSV*) in 2017 from the initial one (*ISV*) in 2016, using the following equation: SVI = $FSV(\frac{\pi}{4} \times (\emptyset_f \times \emptyset_f \times H_f) - ISV(\frac{\pi}{4} \times (\emptyset_i \times \emptyset_i \times H_i)$, where $\emptyset_f$ (cm) is the final diameter, H_f (cm) is the final height, $\emptyset_i$ (cm) is the initial diameter, and H_i (cm) is the initial height. The average SVI value for each line was used in the analysis to compare the sapling growth among species. To calculate the average percentage of sapling survival for each species, we used the equation: SS% = $((\sum niN^{-1}) \times 100))$; where Σn_i is the number of saplings alive in relation to the total number (N) of saplings planted in each line. To compare stem-volumetric increment (SVI) and survival of saplings among species, we performed GLMMs with Gaussian (with *inverse* link function) and binomial distribution, respectively, considering block as the random effect for both models.

For the physiological traits, we performed GLMMs to compare *A*, g_s, Ψ_{md}, Ψ_{pd} among species and seasons, with a Gaussian distribution and *inverse* link function to *A* and g_s, and Gaussian distribution and *identity* link function to Ψ_{md} and Ψ_{pd}. In all models, blocks were treated as random effects. We also investigated the effect of soil properties and groundwater

depth on leaf physiological parameters (A, g_s, Ψ_{md}, Ψ_{pd}) of each species separately, using GLMMs with a Gaussian distribution, *identity* link function and block as a random effect. To test for the effect of environmental variables (soil properties and groundwater depth) on leaf physiology of saplings, environmental data collected in each piezometric well position were attributed to a set of 10 saplings (per species) positioned around that well. As explanatory variables, we chose variables with physical, hydrological and chemical meaning and only those with a correlation coefficient less than R = 0.7, $P < 0.05$ (Supplementary data, Table S2): (I) clay content, representing the soil texture; (II) base saturation (BS%), representing soil fertility; and (III) minimum depth of groundwater (Groundwater depth: GD), representing the hydrological factor.

All analyses were performed in R software version 3.5.1 environment (R Development Core Team, 2018). We verified the adjustment and the presence of outliers for all models through graphical analyses (Quinn and Keough, 2002). To perform all GLMM models, we used the "*lme4*" package (Bates et al., 2015), and "*multcompView*" (Graves et al., 2019) and "*lsmeans*" (Lenth, 2018) package for multiple comparisons. When significant differences were detected for species performances, we performed contrast analysis by "*emmeans*" functions from "*emmeans*" package (Russell, 2019).

RESULTS

Soil characteristics and groundwater depth dynamics

The wet grassland soils are acidic with low nutrients, high organic matter, high aluminium concentrations and a high proportion of clay (Supplementary data, Table S3). In 53.3% of the samples, soil was characterized as medium texture, in 26.7% of the samples was characterized as clayish texture and in 20% of the samples was characterized as sandy texture (Supplementary data, Table S3). Groundwater depth was superficial in most locations sampled, while deeper groundwater measurements and clear seasonality effects were noted only near the savanna edge (position 1) and near the gallery forest edge (position 5) (Fig. 2).

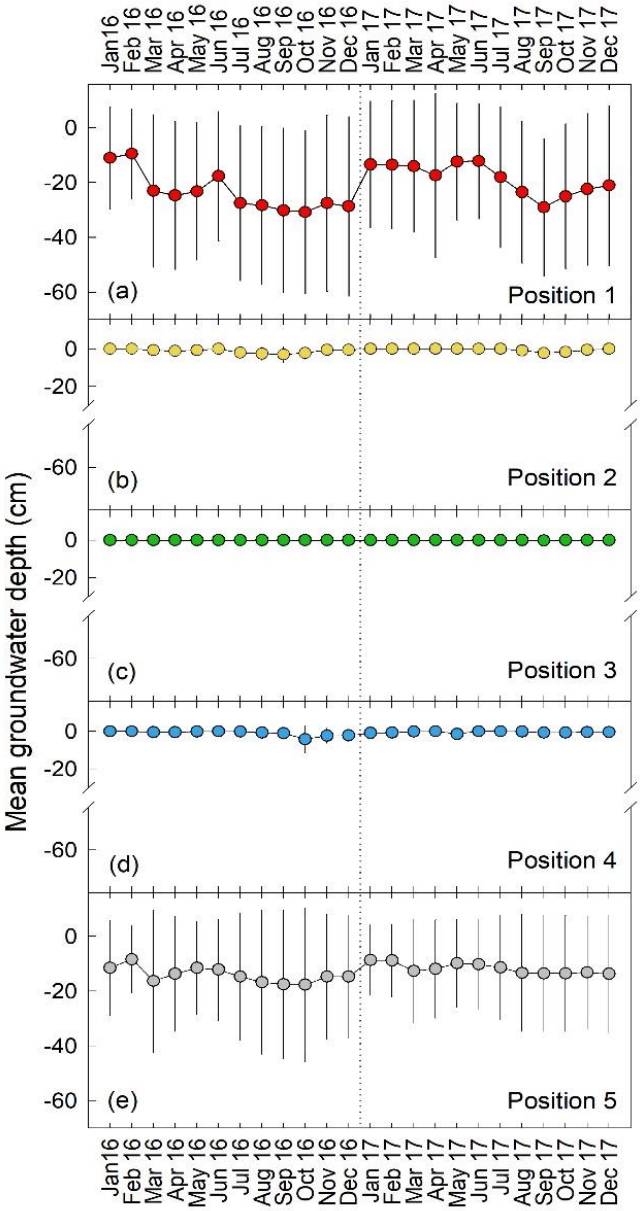


Figure 2. Groundwater dynamics in wet grasslands from southeastern Brazil. The circles represent the mean groundwater depth (± standard deviation) in different positions (a, b, c, d, and e) over wet grasslands where, in ascending order, position 1 represents piezometric wells near the savanna edge and position 5 are piezometric wells near the gallery forest edge.

Seedling emergence and establishment

The gallery forest species was the only one presenting an increase pattern of seedling emergence across the study period (Fig. 3a), while the savanna and generalist species presented a downward trend in seedling emergence (Fig. 3b, c). Seedling establishment was higher for the gallery forest species (28.1%), intermediate for savanna species (17.1%) and lower for generalist species (3.0%) (χ^2 = 261.89, df = 2, P < 0.001) (Fig. 3d). During seedling stage, we only observed the presence of hypertrophic lenticels and adventitious roots in the stem base of *C. brasiliense*, the gallery forest species.

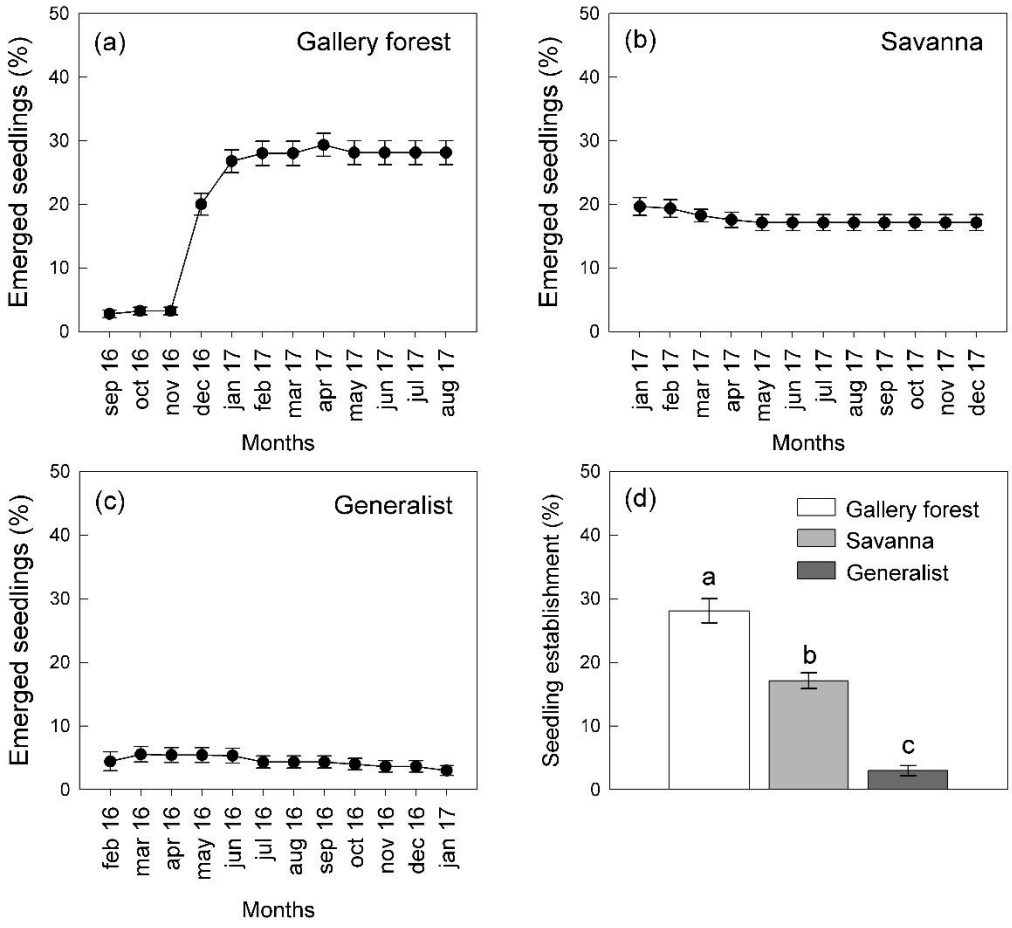


Figure 3. Emerged seedlings per month (a–c) and seedling establishment (d) of tree species sown in wet grasslands from southeastern Brazil. Species are represented in the figures by the environment where they occur: (a) gallery forest (*Calophyllum brasiliense*), (b) savanna (*Hymenaea stigonocarpa*) and (c) generalist, adapted to both habitats (*Tapirira guianensis*). Bars represent means ± standard error (N = 30). Different letters on the top of the bars indicate significant differences between species by contrast analysis (p < 0.05).

Sapling growth and survival

Stem-volumetric increment (SVI) of saplings differed among species (χ^2 = 54.75, df = 2, p < 0.001). Saplings of generalist species showed the highest mean value of SVI and savanna species exhibited negative SVI values (Fig. 4a). Sapling survival was higher for the generalist species, intermediate for gallery forest species and lower for savanna species (χ^2 = 300.04, df = 2, p < 0.001; Fig. 4b). Additionally, we observed hypertrophic lenticels and adventitious roots in the stem base of both gallery forest and generalist species.

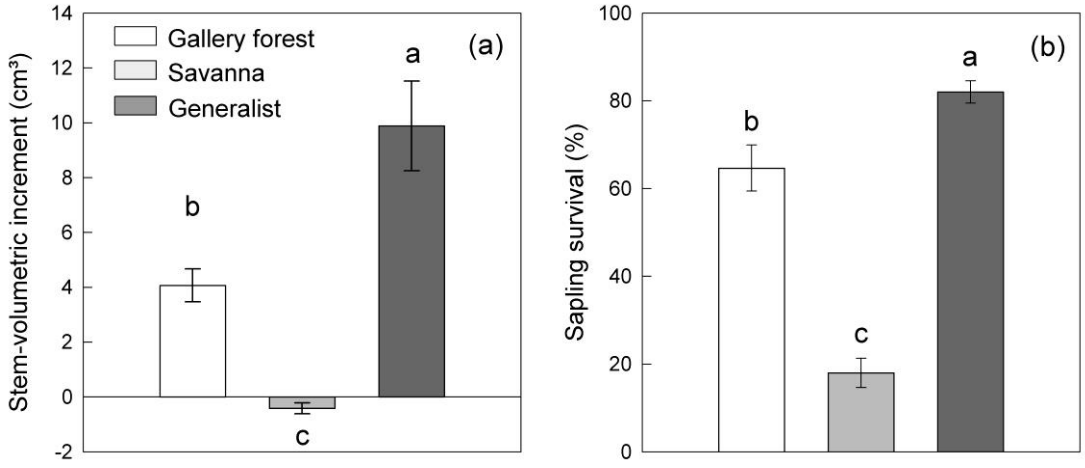


Figure 4. Stem-volumetric increment and survival of tree saplings planted in wet grasslands from southeastern Brazil. Species are represented by the environment where they normally occur: gallery forest (*Calophyllum brasiliense*), savanna (*Hymenaea stigonocarpa*) and generalist, which occur in both habitats (*Tapirira guianensis*). (a) Stem-volumetric increment of saplings over a one-year period. (b) Sapling survival at 41-month after planting. Bars represent means ± standard error (N = 30). Different letters on the top of the bars indicate significant differences between species by contrast analysis (p < 0.05).

Physiological performance and season effect

The carbon assimilation rate (A) was lower to savanna species regardless of season, with no significant differences between the gallery forest and generalist species (χ^2 = 113.1, df = 3, p < 0.001; Fig. 5a); additionally, there was no effect of season in A (p = 0.14). For stomatal conductance (g_s), gallery forest and generalist species presented higher values than those obtained for the savanna species, and for all species, lower values were recorded in the dry season compared to the rainy season (χ^2 = 102.5, df = 3, p < 0.001; Fig. 5b). Leaf water potential at midday (Ψ_{md}) differed only between generalist and savanna species, with lower

values for savanna species (Fig. 5c). Season significantly affected Ψ_{md}, with lower values in the dry season (χ^2 = 14.5, df = 3, p < 0.001; Fig. 5c). During predawn, values were lower for savanna species regardless of season. Season significantly affected Ψ_{pd}, with lower values in the dry season (χ^2 = 174.4, df = 3, p < 0.001; Fig. 5d).

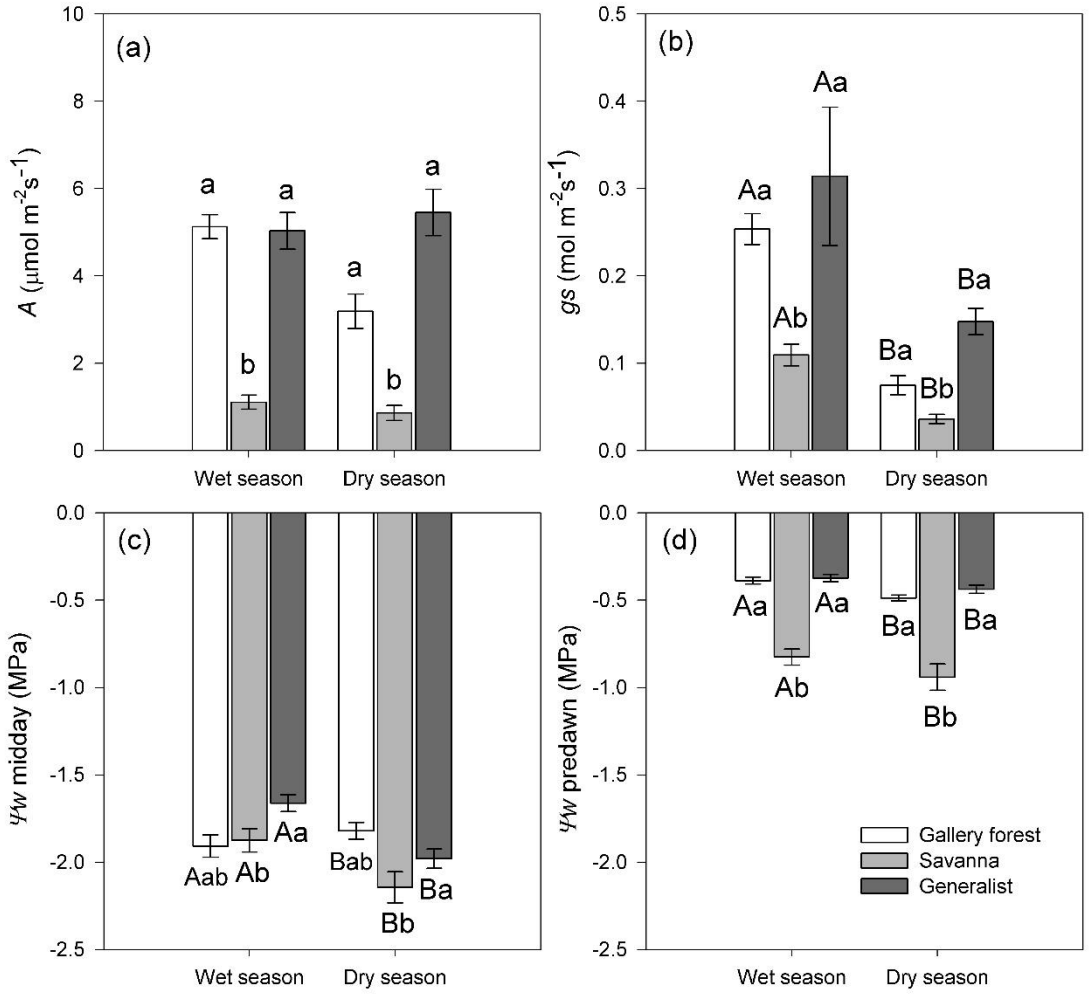


Figure 4. Leaf physiological parameters recorded in the dry and wet seasons in tree saplings planted in wet grasslands from southeastern Brazil. (a) Carbon assimilation rate, *A*; (b) Stomatal conductance, g_s; leaf water potential (Ψw) at (c) Midday and (d) Predawn. Bars represent means (± SE, N = 17–45). Different lowercase letters on top of the bars indicate significant differences between species by contrast analysis (P < 0.05) in each season (wet and dry season). Different capital letters on top of the bars indicate significant differences between seasons. Species are represented by the environment where they normally occur: gallery forest (*Calophyllum brasiliense*), savanna (*Hymenaea stigonocarpa*) and generalist, which occur in both habitats (*Tapirira guianensis*).

Effects of environmental factors on saplings physiological performances

For gallery forest species, g_s was significantly affected by the minimum groundwater depth, both in the dry and wet seasons, with positive relationships. In other words, the greater the minimum groundwater depth, greater the g_s (Table 1). The minimum groundwater depth also positively affected Ψ_{md} in dry season and g_s in wet season in savanna species (Table 1). In addition, for savanna species, we also detected positive effects of base saturation and clay content on Ψ_{pd} in the wet season (Table 1). For the generalist species, A was positively affected by minimum groundwater depth and g_s was positively affected by minimum groundwater depth and clay content, all of them during the wet season (Table 1).

Table 1. Influence of the environmental variables clay content (Clay), base saturation (BS) and minimum groundwater depth (MG) on leaf physiological traits of tree species introduced in wet grasslands from southeastern Brazil. Species are represented by the environment where they normally occur: gallery forest (G. forest; *Calophyllum brasiliense*), savanna (*Hymenaea stigonocarpa*) and generalist, which occur in both habitats (*Tapirira guianensis*). The influence of environmental variables on each parameter was categorized as positive (+) or negative (−).

Species	Parameter	Dry season				Wet season			
		Variable	χ^2	p	AIC	Variable	χ^2	p	AIC
G. forest	A	-	5.0	ns	146.2	-	3.4	ns	202.1
	g_s	MG (+)	7.6	**	-78.9	MG (+)	4.6	*	-45.5
	Ψ_{md}	-	1.9	ns	65.5	-	0.9	ns	71.5
	Ψ_{pd}	-	4.4	ns	-21.5	-	4.1	ns	-7.0
Savanna	A	-	2.9	ns	98.4	-	6.5	ns	142.8
	g_s	-	3.6	ns	-58.1	MG (+)	9.8	**	-89.6
	Ψ_{md}	MG (+)	4.7	*	65.5	-	2.5	ns	88.3
	Ψ_{pd}	-	1.4	ns	44.3	BS (+), Clay (+)	6.3	*	41.0
Generalist	A	-	3.6	ns	176.6	MG (+)	7.9	**	217.7
	g_s	-	3.1	ns	14.6	MG (+), Clay (+)	17.4	***	-48.8
	Ψ_{md}	-	1.9	ns	75.6	-	1.4	ns	69.1
	Ψ_{pd}	-	3.5	ns	5.9	-	5.9	ns	-5.3

A: carbon assimilation rate; g_s: leaf stomatal conductance; Ψ_{md}: leaf water potential at midday; Ψ_{pd}: leaf water potential at predawn; AIC: Akaike information criterion. ns: no significant effect detected; *($p < 0.05$), **($p < 0.01$), ***($p < 0.001$).

DISCUSSION

Here we assessed seedling establishment, growth, survival and physiological performance of three tree species adapted to different conditions in terms of soil waterlogging, searching for a mechanistic explanation for the success or failure of these species in colonizing wet grasslands. Although most of the grassland soils remained waterlogged throughout the year, our results show that waterlogging did not completely prevent seedling establishment; however, the ability to convert seeds into established seedlings greatly varied between tree species. Our results also show that sapling growth and survival are closely related to leaf carbon assimilation rates and leaf water relations of the tree species, being soil waterlogging a major constraint for such physiological parameters. Our data are partially consistent with the general assumption that waterlogging constrains tree colonization in tropical wet grasslands (Xavier et al., 2019); however, depending on plant stage and functional syndromes of species, trees can slowly grow and survive in waterlogged grasslands.

Seedling establishment and sapling growth and survival

Seedling establishment is often the primary demographic bottleneck constraining trees into grasslands (Bond, 2008; Wakeling et al., 2015; Joshi et al., 2020), and soil waterlogging is a strong environmental filter limiting tree establishment in riparian zones (Siebel and Blom, 1998; Oliveira et al., 2015; Oliveira et al., 2019). Since two of the study species converted a reasonable proportion of seeds into established seedlings, our results indicate, at least for these species, that waterlogging did not completely prevent tree-seedling establishment in wet grasslands. Our expectation regarding seedling establishment of the study species was not completely fulfilled. We predicted higher seedling establishment for the gallery forest and generalist species relative to the savanna species, but it was true only for the gallery forest species, which presented the highest seedling establishment rate. The savanna species showed an intermediate performance, while the generalist had the lowest seedling establishment rate.

The different seedling establishment performances among the three study species can be explained by their seed tolerance to flood and other seed features as well. Seeds of the gallery forest species (*C. brasiliense*) can keep their full germination capacity even after 45 days of submerged in water, whereas the generalist (*T. guianensis*) and savanna (*H. stigonocarpa*) species completely lost their germination capacity under the same condition (Table 3, Chapter 1). The higher seed tolerance to flood for *C. brasiliense* is also supported by

previous studies, which demonstrated that their seeds remain viable after a long period under waterlogging conditions (Marques and Joly, 2000) and actively regenerate from seeds in periodically flooded sites in swamp forests (Scarano et al., 1997). In contrast, the higher seedling establishment capacity of savanna species compared to the generalist species is probably related to the large seed mass of the first species compared to the last one (Supplementary data, Table S1), where the large amount of carbohydrate reserves (Tiné et al., 2000) probably favored germination and higher seedling emergence under oxygen deficiency (Ella and Setter, 1999; Pérez-Ramos and Marañón, 2009). Thus, the higher seedling establishment observed for the gallery forest species is related to its higher seed tolerance to flood, which indirectly indicates waterlogging as an environmental filter to seedling establishment for less flood tolerant species in the grasslands.

For sapling growth and survival, our expectations match our results, with better performances at this stage for the generalist and gallery forest species than for the savanna species. In contrast with the lowest performance in the early stage, the generalist species presented the best performance in the sapling stage compared to the other two species, with higher SVI and survival of saplings. These results suggest that tree establishment of the generalist species in wet grasslands is strongly limited by low seedling establishment rather than sapling survivorship. Indeed, we observed some established young trees of *T. guianensis* spontaneously colonizing wet grasslands around the experimental sites during the study period, and they were more abundant in less waterlogged terrain. These observations suggest that *T. guianensis* needs better soil aeration conditions for seedling establishment, which is a rare condition in the grasslands studied, while older plants can better withstand waterlogging conditions (Siebel and Blom, 1998). Otherwise, the savanna species seems to be more constrained during the sapling stage compared to the other species, since their saplings did not grow and showed the lowest survival rate. The cumulative effect of low seedling establishment and the inability to grow and poorly survive as sapling are the major constraining factors to tree establishment of *H. stigonocarpa* in wet grasslands. These results are in agreement with the statement that savanna species with restricted distribution to well-drained soils grow poorly and cannot survive in waterlogged soils (Oliveira et al., 2015). The gallery forest species showed intermediate performance in SVI and survival of saplings compared to the other species, demonstrating that *C. brasiliense* has adaptive responses to waterlogging both in early and late plant stages (Marques and Joly, 2000; Oliveira and Joly, 2010). These findings explain the natural occurrence and relative dominance of this species in waterlogged soils of gallery and peat-swampy forests along the Neotropical region (Scarano

et al., 1997; Teixeira et al., 2009) and highlight the greater potential to establish in the waterlogged soils of grasslands, compared to the study savanna and the generalist species.

Physiological performance of saplings

As expected, we found that saplings growth and survival reflected the ability of species to maintain positive carbon assimilation rates and regulate leaf water status over the year. Both the gallery forest and the generalist species showed higher carbon assimilation and better leaf rehydration capacity, which reflected in their significantly higher growth and survival rates compared to the savanna species. In contrast, the savanna species did not grow and poorly survived, presenting then the lowest CO_2 assimilation rates, greater stomatal conductance restriction and higher leaf dehydration. The better growth, survivorship and physiological performances of the gallery forest and generalist saplings may be related to their ability of acclimation in response to waterlogging. They possessed hypertrophic lenticels and adventitious roots in the stem base of saplings, which likely contributed to alleviation of waterlogging stress and allowed the maintenance of growth rates during the study period (Andrade et al., 1999; Mielke et al., 2003; Kolb and Joly, 2009; Oliveira and Joly, 2010; Kissmann et al., 2014).

Despite the permanently wet soils, the dry season was more stressful for all species, resulting in a stomatal restriction of leaf gas exchange and a stronger control of the leaf water status. However, these restrictions were more evident for the savanna species than for the generalist and gallery forest species. In fact, adult trees of gallery forest species, when invading typical savanna environments present a high capacity to regulate their stomatal conductance and leaf water potential to survive under high evaporative demand and high irradiances of the savanna environment (Rossatto et al., 2013). Despite being capable of regulating physiological performance under seasonal drought in well-drained soils (Franco, 1998; Prado et al., 2004), the savanna species studied may be under high stress, being unable to regulate leaf water status (Parent et al., 2011), given the interaction between high vapour pressure demand of the dry season and waterlogging of the wet grasslands. This interaction may pose serious limitations, leading this species to be filtered during sapling stage in such wet grasslands.

We detected significant effects of some soil variables on leaf physiology; however, as expected, groundwater depth was the general factor hampering physiological processes of saplings. Shallow groundwater depth – that generates soil-waterlogging – decreased A for the generalist species and g_s for all study species. Low carbon assimilation and stomatal

conductance after waterlogging have been widely reported for many tree species, including those tolerant to flood (Kozlowski, 1997). Under waterlogged conditions, decreasing *A* is caused by reductions in g_s because lower stomatal conductance restricts the access to CO_2 needed for carbon assimilation (Mielke et al., 2003; Parent et al., 2011; Kissmann et al., 2014). Low g_s values, generally observed in waterlogged plants may be attributed to a decrease in leaf hydration (Pezeshki, 2001) or to non-hydraulic factors, such as hormonal signaling from waterlogged roots to shoots (Zhang and Zhang, 1994; Else et al., 2009). The savanna species was the only one that had leaf water potential decreased by shallow groundwater depth. This result highlights the inability of the savanna species to maintain its water homeostasis, which reinforces its low tolerance to soil waterlogging (Parent et al., 2011).

Clay content and base saturation (BS) affected some physiological parameters, however the effects of these variables deserve careful interpretation, as each variable may have acted synergistically with the other. Leaf water potential at predawn (Ψ_{pd}) increased with higher BS and clay content for the savanna saplings. We believe that BS effect actually is an indirect consequence of maximum groundwater depth, since these variables are positively high correlated with each other (R = 0.81, Table S2). In this way, a less waterlogged soil condition can favour root growth in plants sensitive to flooding (Oliveira et al., 2015), leading to better water uptake and leaf water potential recovery. Additionally, we found soils with higher sand content occurred associated with deeper groundwater depths (Table S2), which could explain the synergetic effect of clay content on Ψ_{pd}, as greater clay content favour retention of soil moisture (Assis et al., 2011), allowing a better leaf rehydration in the savanna saplings during the night. Higher clay content may also be responsible for increased stomatal conductance (g_s) in the generalist saplings; however, this effect may have occurred synergistically with the higher minimum groundwater depth. We also expect that higher clay content increase g_s specifically in more sandy soils, favouring enhanced stomatal opening in such well-drained soil conditions.

Although our small number of studied species makes broad generalizations difficult, we provide novelty insights about tree establishment process based on the responses of typical Cerrado tree species with distinct functional syndromes regarding flood tolerance. Taken together, our results suggest that once a larger size is reached, more flood-tolerant trees can grow and even slowly colonize waterlogged grasslands in the absence of fire (Arieira et al., 2018). Waterlogging constraints on tree establishment in wet grasslands probably work in combination with fire disturbances (Sarmiento, 1984) to restrict colonization by flood-tolerant

species from gallery forest. Reduced sapling photosynthesis in more severely waterlogged areas probably delay plant growth and increases plant vulnerability to fire (Coop and Givnish, 2008; Hoffmann et al., 2012). Therefore, it is unlikely that waterlogging alone constrains trees in colonizing tropical wet grasslands (Silva, 2015). Variation in waterlogging may directly govern the shift from a seasonal savanna to wet grassland community (Leite et al., 2018; Xavier et al., 2019), while variation in waterlogging and soil properties probably interact with environmental disturbances, such as fire and the rare frost events, to control the wet grassland-gallery forest boundary (Bond, 2008; Hoffmann et al., 2012; Dantas et al., 2013; Hoffmann et al., 2019).

We consider that both the natural hydrologic regime and fire dynamics must be maintained to conserve the rich wet grassland communities, which are composed of herbaceous plants specialized in different waterlogging regimes (Tannus and Assis, 2004; Munhoz and Felfili, 2006; 2008; Xavier et al., 2019). Projections indicate that climate changes would cause a decrease in rainfalls and an increase in droughts during future periods for the Cerrado region (Rodrigues et al., 2020), thereby, we can indirectly infer that if climate change modifies the hydrological pulses or natural disturbance regimes (e.g. fire and frosts), which characterizes the riparian ecosystems in the Cerrado, the complex and relatively stable mosaic forest – wet grassland – savanna will be strongly affected with predictable negative consequences on biodiversity and water-related ecosystem services.

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SUPPLEMENTARY DATA

Table S1. Germination tests performed to verify the viability of seed lots used in the field experiment for each species (5 replicates with 20 seeds in each).

	Species		
	Calophyllum brasiliense	*Hymenaea stigonocarpa*	*Tapirira guianensis*
Test condition	Germination chamber	Green house	Germination chamber
Light condition	12-h photoperiod of fluorescent light	50% shading	12-h photoperiod of fluorescent light
Temperature (ºC)	Alternating temperature of 20 – 30 ± 1	Day mean temperature of 29 ± 1	Constant temperature of 25 ± 1
Substrate	Filter paper	Sand	Filter paper
Seed dry mass (g)*	1.04 ± 0.37	3.11 ± 0.68	0.06 ± 0.01
Mean germination (%)	90.0	93.0	91.0

*Average seed dry mass ± standard deviation; obtained after drying seeds for 48 h at 80 ºC and individual weighing of the seeds with a 0.1 mg precision balance. (*C. brasiliense* n = 100; *H. stigonocarpa* n = 95; *T. guianensis* n = 100).

Table S2. Pearson correlation coefficients (R values) between variables representing the hydrological factors (I, II, III), soil fertility (IV) and soil texture (V, VI) of wet grasslands in the sites studied (Assis State Forest, Assis municipality, state of São Paulo, Brazil). *Significant at $\alpha = 0.05$.

Variables	I	II	III	IV	V	VI
Maximum groundwater depth (I)	-	-	-	-	-	-
Minimum groundwater depth (II)	0.87*	-	-	-	-	-
Range of groundwater depth (III)	0.91*	0.57*	-	-	-	-
Base saturation (V%) (IV)	0.81*	0.61*	0.82*	-	-	-
Clay content (V)	-0.46*	-0.33*	-0.49*	-0.58*	-	-
Sand content (VI)	0.42*	0.29*	0.44*	-0.55*	-0.98*	-

Table S3. Texture and chemical properties of surface soil layer (0-20 cm depth) in each plot of the experimental sites where tree species were introduced in wet grasslands from southeastern Brazil. P: phosphorous (mg/dm³), K: potassium (mmolc/dm³), Ca: calcium (mmolc/dm³), Mg: magnesium (mmolc/dm³), O.M.: organic matter (g/dm³), Al: aluminum (mmolc/dm^3), H+Al: total acidity, V%: base saturation, Sand (g/kg), Clay (g/kg).

Sample	Site	P	K	Ca	Mg	O.M.	pH	Al	H+Al	V%	Sand	Clay	Texture
1	1	5	0.5	5	1	63	4	35	140	4.4	591	318	Medium
2	1	3	0.7	4	1	19	4.1	8	45	11.2	855	118	Sandy
3	1	3	0.7	6	1	22	4	16	70	9.9	794	172	Medium
4	1	5	0.7	4	1	35	4.1	17	88	6.1	704	237	Medium
5	1	6	0.7	3	1	36	4	24	102	4.4	703	243	Medium
6	2	11	1.6	9	1	103	4.1	32	156	6.9	514	301	Medium
7	2	11	1.3	8	1	279	3.8	124	276	3.6	135	564	clayish
8	2	15	1.2	9	3	373	3.6	239	326	3.9	176	457	clayish
9	2	19	2.3	8	3	366	3.6	172	256	4.9	98	543	clayish
10	2	18	2.3	16	4	226	3.7	83	170	11.6	182	467	clayish
11	3	5	0.6	4	1	22	3.9	19	68	7.6	845	123	Sandy
12	3	7	0.6	4	1	38	4.1	16	86	6.1	779	172	Medium
13	3	4	0.7	4	1	34	4	17	72	7.3	828	123	Sandy
14	3	4	0.5	4	1	33	4	23	96	5.4	792	148	Medium
15	3	5	0.6	4	1	33	3.9	33	110	4.8	769	148	Medium
Mean	-	8.7	1.0	6.1	1.5	112.1	3.9	57.2	137.4	6.5	584.3	275.6	-

CONCLUSÕES GERAIS

O presente estudo indica que as respostas germinativas às variações edáficas do campo úmido diferem entre espécies funcionalmente distintas do Cerrado. Espécies típicas da floresta de galeria e espécies generalistas tendem a apresentar maior tolerância ao alagamento em suas sementes, sendo essas capazes de manter a capacidade germinativa após submersas na água por períodos variáveis. Entretanto, o nível de tolerância e a capacidade de germinar em condições de alagamento dependem da espécie e do tempo de exposição ao estresse.

Também as respostas morfofisiológicas de plantas em solos alagados do campo úmido diferem entre espécies funcionalmente distintas de árvores. Os resultados obtidos em casa de vegetação demonstraram que o alagamento (lâmina d'água acima do solo) não interferiu nos parâmetros fisiológicos avaliados para a espécie exclusiva da floresta de galeria (*C. brasiliense*), e, além disso, esta espécie apresentou maior acúmulo de biomassa em solos com elevada saturação hídrica (solos encharcados), evidenciando não apenas tolerância ao alagamento como também preferência por solos mais úmidos. Para a espécie generalista (*T. guianensis*) o encharcamento do solo reduziu a condutância estomática (g_s), mas sem consequências para a taxa de assimilação de carbono (*A*), sendo que ambos os parâmetros foram reduzidos pelo alagamento do solo, porém, sem prejuízos para o acúmulo de biomassa das plantas. A espécie savânica (*H. stigonocarpa*) apresentou acentuada redução de g_s e *A* em ambos os tratamentos, com solo encharcado e alagado, e a condição de alagamento provocou redução da taxa máxima de transporte de elétrons (ETR_{max}), rendimento quântico máximo do fotossistema II (F_v/F_m) e massa das raízes. O desenvolvimento de estruturas relacionadas ao melhor arejamento de tecidos submersos, como hipertrofia de lenticelas e raízes adventícias na base do caule foi observado apenas em plantas das espécies *C. brasiliense* e *T. guianensis*, o que explica, em parte, o melhor desempenho fisiológico dessas espécies nas condições de saturação hídrica do solo avaliadas.

Os resultados obtidos em campo, indicam que espécies arbóreas que ocorrem na floresta de galeria (*C. brasiliense* e *T. guianensis*) apresentam maior capacidade de estabelecimento de plantas sobre os campos úmidos. Essas espécies exibiram maiores taxas de estabelecimento de plântulas (*C. brasiliense*) e maiores taxas de crescimento e sobrevivência de mudas (*T. guianensis*), quando comparadas à espécie de savana (*H. stigonocarpa*). Essa vantagem das espécies que ocorrem na mata de galeria é devida à maior capacidade de manter taxas de assimilação de carbono suficientes para o crescimento, bem como melhor regulação do status hídrico das plantas sob condição de alagamento dos solos. Portanto, os dados

sugerem que espécies arbóreas são capazes de se estabelecer em áreas de campos úmidos, especialmente no caso de espécies cujas sementes apresentam alta tolerância ao alagamento, como é o caso de *C. brasiliense*. O alagamento do solo gerado pelo lençol freático superficial foi o principal fator edáfico limitante para a assimilação de carbono e balanço hídrico de plantas arbóreas no campo úmido, impondo, portanto, restrições ao crescimento e estabelecimento das plantas. Entretanto, essas limitações parecem ser mais pronunciadas em espécies da savana. Dessa forma, é provável que o alagamento dos solos não seja o único fator limitante para o estabelecimento de espécies arbóreas provenientes da floresta de galeria, especialmente quando se trata de espécies tolerantes ao alagamento. Neste caso, acreditamos que distúrbios são importantes fatores ecológicos, desempenhando papel fundamental e complementar para restringir o estabelecimento de espécies tolerantes ao alagamento em campos úmidos. Distúrbios como fogo, pastoreio e geadas, por exemplo, são fatores importantes para a manutenção e resiliência de fisionomias abertas do Cerrado.

Os resultados obtidos na presente tese têm importantes implicações para a conservação e manejo de campos úmidos no Cerrado. A princípio, o lençol freático superficial, que gera condições de alta saturação hídrica e alagamento dos solos, atua como um importante filtro ecológico para espécies arbóreas presentes no “pool” local de espécies. O encharcamento do solo reduz as chances de estabelecimento de árvores através de limitações impostas primeiramente à germinação de sementes e emergência de plântulas, seguida de limitações ao desenvolvimento das plantas por restrições aos processos fotossintéticos, provocadas pela deficiência de oxigênio no solo para as raízes. Portanto, qualquer fator que interfira na dinâmica do lençol freático, tornando os solos mais secos, pode facilitar o processo de estabelecimento e a invasão de campos úmidos por espécies arbóreas.

A redução do regime de chuvas e secas intensas em decorrência de mudanças climáticas, por exemplo, podem alterar os padrões hidrológicos dos campos úmidos, tornando-os mais secos, uma vez que o lençol freático nestas áreas depende da infiltração de água pluvial no relevo adjacente. Nesse sentido, o adensamento e aumento da biomassa lenhosa de fitofisionomias adjacentes aos campos – como as vegetações savânicas – também pode alterar o regime hídrico dos campos úmidos, pois o adensamento arbóreo das vegetações abertas reduz a quantidade de água da chuva que chega até o solo.