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Ecofisiologia da germinação de sementes e de plantas de Styracaceae em diferentes fisionomias de Cerrado

CAMILA KISSMANN

Tese apresentada ao Instituto de Biociências do Campus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Doutora em Ciências Biológicas (Biologia Vegetal).

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À minha família,

pelo amor e incentivo.

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*"***Compreender que há outros pontos de vista é o início da sabedoria***"*

Thomas Campbell

ÍNDICE

1. RESUMO

As espécies *Styrax ferrugineus* Ness & Mart., *S. camporum* Pohl. e *S. pohlii* A. DC. apresentam distintos padrões de ocorrência. *S. ferrugineus* é comumente encontrada no Cerrado *sensu strictu* (*s. str.*), enquanto *S. camporum* apresenta ampla distribuição no Cerrado *sensu latu* (*s.l.*), especialmente nas bordas dos fragmentos e *S. pohlii* é abundante nas florestas ripárias e de galeria. As performances germinativas das sementes e ecofisiológicas das plantas destas três espécies foram avaliadas através de experimentos de enterrio das sementes e plantio recíprocos em fragmentos de Cerrado *s.str*, Cerradão (clareira e sub-bosque) e mata ripária, os quais diferem em disponibilidade hídrica no solo e na atmosfera e luminosidade. Simultaneamente, o crescimento e desempenho fotossintético de *S. pohlii* em resposta ao alagamento do solo foram estudados em casa de vegetação. As sementes das três espécies germinaram em todos os locais experimentais onde foram enterradas. Porém, as plantas jovens de *S. ferrugineus* e *S. camporum* não sobreviveram às condições de baixa irradiância e alta disponibilidade de água no solo, encontradas na floresta ripária. A sobrevivência e o sucesso de *S. pohlii* neste ambiente parece estar relacionado ao rápido crescimento inicial da parte aérea, para obtenção de luz, e à adaptação que ela apresenta em resposta ao alagamento do solo. *S. pohlii* foi capaz de sobreviver a alagamentos sazonais e mostrou rápida recuperação fisiológica após inundação artificial. *S. camporum* apresentou maior eficiência fotossintética, crescimento e biomassa quando as plantas foram cultivadas sob altas irradiâncias, em comparação a ambiente sombreados, o que explica o maior número de indíviduos nas bordas em comparação ao interior dos fragmentos de Cerrado. Os dados de trocas gasosas, fluorescência e crescimento de *S. ferrugineus* e *S. pohlii*, por outro lado, não explicaram o sucesso dessas espécies no Cerrado *s. str.* e floresta ripária, respectivamente, sugerindo que a distribuição destas espécies é governada pela interação de múltiplos fatores.

Palavras chave: biomassa, fotossíntese, germinação, sobrevivência, *Styrax*

2. ABSTRACT

The species *Styrax ferrugineus* Ness & Mart., *S. camporum* Pohl. and *S. pohlii* A. DC. show distinct geographical distribution patterns. *S. ferrugineus* is commonly found in the Cerrado *sensu stricto* (*s. str.*), whereas *S. camporum* is widely distributed in the Cerrado *sensu lato* (*s. l*), especially at the edge of vegetation fragments, and *S. pohlii* is abundant in riparian and gallery forests. Seed germination performances and ecophysiological performances of plants of these three species were assessed through reciprocal burial and planting experiments in a Cerrado *s. str.* fragment, a Cerradão fragment (gap and understory conditions) and in a riparian forest remnant, where water and light resources are differently availabe. Concurrently, growth and photosynthetic parameters of *S. pohlii* potted plants in response to soil flooding was assessed in a greenhouse experiment. Seeds from each of the tree species were able to germinate in every experimental condition where the seeds were buried. Nevertheless, young plants of *S. ferrugineus* and *S. camporum* did not survive the low irradiance and high soil water availability found in the riparian forest. The survival and the success of *S. pohlii* in such environment seems to be related to a faster shoot initial length in order to capture sunlight, and to the adjustment that this species exhibits in reponse to different levels of soil flooding, enabling this species to fast recover from flooding periods. *S. camporum* showed higher photosynthetic performance, growth and biomass production when it was cultivated under high irradiances in comparison to shaded environments. These results explain the higher number of individuals of this species observed at the edge in comparison to the interior of Cerrado fragments. Gas exchange, fluorescence and growth parameters measured on *S. pohlii* and *S. ferrugineus* failed to explain the reasons why these species are observed in forest and savanna environments, respectively, suggesting that their distribution is influenced by multifactorial responses.

Key words: biomass, photosynthesis, germination, survival, *Styrax*

3. INTRODUÇÃO GERAL

O Cerrado é o segundo maior domínio fitogeográfico brasileiro, com uma área original de 2 milhões de Km^2 (Klink & Machado, 2005). Dada a grande extensão do território, o Cerrado abrange uma variação de clima, relevos e solos, o que possibilita a ocorrência de uma ampla gama de formações vegetais, que variam desde formações campestres e savânicas até as formações florestais (Oliveira Filho & Ratter, 2002).

Contudo, o Cerrado é o domínio brasileiro mais ameaçado de extinção devido à ocupação das áreas originais de Cerrado pela agricultura e pecuária (Klink & Machado, 2005), apresentando uma taxa de desmatamento anual de 0,7% na primeira década do século XX (Ministério do Meio Ambiente, 2010). No Estado de São Paulo restam menos de 7% da área original do Cerrado, a qual se encontra espalhada em fragmentos (Durigan et al., 2006). A transformação de áreas contínuas de vegetação nativa em pequenos fragmentos isolados resulta na formação de bordas, com alteração da luminosidade e diferentes microclimas, interferindo na reprodução das espécies.

Assim, conhecer como os fatores abióticos afetam a regeneração natural das plantas nativas do Cerrado é essencial para o planejamento de ações tanto de conservação como de recuperação de áreas degradadas deste domínio fitogeográfico.

Muitos congêneres do Cerrado apresentam padrões distintos de ocorrência, o que se acredita estar relacionados tanto às diferenças de recursos entre as diferentes fisionomias, bem como à capacidade da espécie em adaptar-se às condições do ambiente. Um exemplo destes congêneres são as espécies de *Styrax*. *Styrax ferrugineus* Nees & Mart. é uma espécie típica de Cerrado *sensu stricto* (*s.str*), enquanto que *S. camporum* Pohl tem ampla ocorrência em áreas de Cerrado *sensu lato* (*s.l.*) como o Cerradão, e *S. pohlii* A. DC. é encontrada em fisionomias florestais associadas aos cursos d'água.

Objetivou-se investigar comparativamente o desempenho germinativo de sementes e o crescimento inicial de plantas destes congêneres de *Styrax* e relacionar suas respostas aos padrões observados na distribuição destas espécies em diferentes fisionomias do Cerrado.

Buscou-se responder as seguintes questões:

(i) A ocorrência de *S. ferrugineus*, *S. camporum* e *S. pohlii* nas diferentes fisionomias do Cerrado é limitada pela germinação das suas sementes?

(ii) O excesso de água no solo e a baixa irradiância, encontrados na floresta ripária, limitam a ocorrência de *S. ferrugineus* e *S. camporum* neste ambiente? Por outro lado, a maior ocorrência de *S. camporum* nas bordas dos fragmentos é condicionada pelo melhor aproveitamento da luz?

(iii) A distribuição destes congêneres no Cerrado está relacionada à maior eficiência fotossintética das espécies nos seus respectivos ambientes preferenciais de ocorrência?

(iv) O que explica o sucesso de *S. pohlii* nas florestas ripárias e de galeria?

4. REVISÃO DE LITERATURA

4.1 O Cerrado

O domínio fitogeográfico Cerrado inclui as fisionomias campestres, savânicas e florestais encontradas no Cerrado *s.l.* e ainda outros tipos vegetacionais (Batalha, 2011), que diferem quanto à estrutura e composição florística (Méio et al., 2003; Batalha et al., 2011). No Estado de São Paulo são encontradas formações vegetais de Cerrado e da Mata Atlântica, as quais mostram contrastantes disponibilidades de recursos hídricos, nutricionais e de luz (Haridasan, 2000; Hoffmannn & Franco, 2003).

A ausência de barreiras geográficas entre Cerrado e a Mata Atlântica possibilita a troca de elementos florísticos entre essas formações, porém, estudos fitossociológicos

mostram diferenças na composição das espécies destes hábitats, com ocorrência de espécies endêmicas e espécies comuns a ambas as formações (Méio et al., 2003; Ratter et al., 2003). A ocorrência de formações florestais no Cerrado é resultante de fatores temporais (evolutivos) e espaciais (variações na hidrografia, topografia, profundidade do lençol freático, entre outros) (Ribeiro & Walter 1998).

As alterações climáticas que ocorreram durante o Quaternário promoviam a expansão das florestas úmidas no intervalo entre as glaciações e a retração das mesmas durante as glaciações, dando origem às formações florestais do Cerrado (Pinheiro & Monteiro, 2010). O grande número de espécies congenéricas encontradas nas formações de Cerrado e de matas (Rizzini, 1977) sugere que, ao longo da evolução, a especiação tenha produzido, nas populações de mata, novas entidades adaptáveis ao Cerrado e vice-versa. Assim, acredita-se que após mecanismos adaptativos e de seleção, a vegetação de Cerrado passou a ser competitiva. Vários fatores ambientais são apontados como determinantes das diferentes formações vegetais do Cerrado e serão discutidos a seguir.

4.2 Fatores ambientais e a distribuição das espécies no Cerrado

A distribuição e a manutenção das diferentes fisionomias do Cerrado são determinadas por fatores abióticos, principalmente edáficos, climáticos (chuva e temperatura) (Oliveira Filho & Ratter, 2002) e pirogênicos (Geiger et al., 2011). Fatores edáficos como a proximidade à superfície do lençol freático, a presença de concreções no perfil e a drenagem, são considerados importantes condicionantes da variação florística e estrutural que ocorre entre as diferentes fisionomias (Haridasan, 2000).

As altas temperaturas e deficiência de água e nutrientes dos solos encontradas nas formações savânicas, como o Cerrado *s. str.,* restringem a ocorrência de espécies florestais do Cerrado nessas fisionomias. Por outro lado, nas formações florestais, como o Cerradão por

exemplo, o fator luz deve ter maior importância na seleção de espécies (Hoffmannn & Franco, 2003). Já nas florestas ripárias e de galerias, a ocorrência das espécies parece ser limitada mais pela condição de hipoxia (resultante do alagamento sazonal do solo) à qual as raízes são submetidas do que pela disponibilidade de luz (Teixeira et al., 2011)

Além dos fatores edáficos e climáticos, o fogo também exerce forte influência na seleção de espécies nas diferentes fisionomias do Cerrado. Espécies típicas de formações savânicas mostram-se não apenas tolerantes ao fogo (Hoffmannn et al., 2003), mas também dependentes dele para a reprodução sexuada (Coutinho, 1977). A tolerância destas espécies ao fogo está relacionada à maior espessura da casca e maior investimento em biomassa de raiz (maior disponibilidade de recursos para rebrota após a passagem do fogo), em relação às espécies de mata (Hoffmannn, 2005).

4.3 Ecofisiologia da germinação de sementes e de plantas do Cerrado

O recrutamento vegetal, definido como o processo de entrada de novos indivíduos em uma população ou comunidade (Ribbens et al., 1994), pode ser limitado pela dispersão das sementes (Turnbull, 2000) ou pelos processos críticos pós-dispersão, como sobrevivência e germinação das sementes e sobrevivência das plântulas (Calviño-Cancela, 2002). Assim, ao longo da evolução, as plantas desenvolveram diferentes estratégias adaptativas para sobreviver às adversidades impostas pelo ambiente. Algumas destas estratégias podem estar refletidas nas características morfológicas e/ou fisiológicas das sementes. Muitas espécies de Cerrado, por exemplo, produzem sementes com dormência (Zaidan & Ranal, 2008), o que garante a sua viabilidade no solo até que as condições ambientais sejam favoráveis para a germinação e o posterior estabelecimento da plântula.

Quando as sementes encontram condições favoráveis de luz, água e temperatura ocorre a germinação. Em seguida, o desenvolvimento pós-embrionário inicial da plântula se

faz, a princípio, heterotroficamente à custa das reservas da semente. Porém, uma vez esgotada essas reservas, a planta passa a depender dos recursos do meio (água, luz, nutrientes e gás carbônico) para a sua sobrevivência e crescimento (Larcher et al., 2000). Portanto, a distribuição geográfica das espécies vegetais, que se reproduzem sexuadamente, é influenciada não apenas pela capacidade de dispersão e germinação das sementes, mas também pela capacidade das plantas em assimilar carbono e regular o balanço hídrico.

No Cerrado há uma variação espacial na disponibilidade de recursos, principalmente hídricos e luminosos (Habermann et al., 2011; Kissmann et al., 2012), que são essenciais à fotossíntese. Assim, para ocupar esses ambientes os vegetais apresentam adaptações morfológicas e/ou fisiológicas que asseguram a obtenção e utilização eficiente dos recursos disponíveis nos locais onde eles ocorrem.

Espécies congenéricas de formações savânicas e florestais apresentam uma grande variação na alocação de biomassa. As espécies savânicas alocam mais biomassa nas raízes em detrimento de caules e folhas, o que permite a estas espécies a obtenção de água das camadas mais profundas do solo e, assim, manter um balanço positivo mesmo durante a estação sazonal seca (Hoffmannn & Franco, 2003; Scholz et al., 2002). Por outro lado, espécies de ambientes florestais, onde há intensa competição por luz, apresentam maior alocação de biomassa nos órgãos diretamente envolvidos com a captação de luz, como caules e folhas (Hoffmannn & Franco, 2003).

Entretanto, para algumas espécies, as adaptações para obtenção de luz podem não ser suficientes para garantir a ocorrência destas em formações florestais associadas aos cursos de água, também encontradas no domínio Cerrado. Nestes casos, a distribuição das espécies é determinada, sobretudo, pela tolerância ao alagamento, que tem efeitos drásticos sobre a germinação das sementes, sobrevivência e crescimento das plantas (Kozlowski, 1997).

Portanto, tanto o excesso como a limitação dos fatores ambientais como luz e água, que se mostram altamente variáveis nas diferentes fisionomias de Cerrado, podem representar condições de estresse para espécies não adaptadas a estes ambientes. Assim, estudos com pares congenéricos de espécies de ambientes contrastantes (Hoffmannn & Franco, 2003; Barros et al., 2012) utilizados em experimentos de plantio recíproco, onde os congêneres são postos a crescer nos seus sítios nativos e no sítio de ocorrência preferencial da outra população (Ackerly et al., 2000), podem auxiliar na elucidação dos fatores que governam a distribuição geográfica das espécies.

4.4 Styracaceae

A família Styracaceae compreende aproximadamente 11 gêneros e 160 espécies, sendo *Styrax* L. o gênero mais representativo, com cerca de 130 espécies, as quais se encontram distribuídas principalmente na América do Sul, em ambientes como florestas úmidas, restingas e Cerrado (Fritsch, 2001). No Brasil, a maioria das espécies nativas de *Styrax* ocorre em áreas de Cerrado (Souza & Lorenzi, 2005).

Das três espécies estudadas no presente trabalho, *S. camporum* é a que tem ocorrência mais ampla em relação ao hábitat, sendo encontrada em áreas do Cerrado *s. l.*, transição Cerradão/floresta paludosa, mata mesófila e floresta perenifólia. Contudo, é mais comumente vista nas formações de Cerrado (Nakajima & Monteiro, 1986; Ratter et al., 2000; Durigan et al., 2004; Tannus & Assis, 2004; Kotchetkoff-Henriques et al., 2005; Habermann et al., 2011). A sua ocorrência abrange os Estados de SP, MG, BA, PI, MT, MS, GO e o DF (Nakajima & Monteiro, 1986; Ratter et al., 2000; Durigan et al., 2004; Gomes et al., 2004; Tannus & Assis, 2004; Kotchetkoff-Henriques et al., 2005).

S. ferrugineus é descrita como uma espécie típica de formações savânicas, com tronco tortuoso, casca espessa e folhas pequenas e coriáceas. Os ramos novos são recobertos por

pilosidade ferrugínea. A espécie é bem adaptada ao Cerrado *s. str.*, onde é comumente encontrada, e praticamente não é observada em formações de Cerradão e/ou mata ciliar ou de brejo/paludosa. Ocorre nos Estados do PR, SP, MG, BA, MA, TO, RO, MT, MS, GO e o DF (Goodland, 1970; Nakajima & Monteiro, 1986; Ratter et al., 2000; Durigan et al., 2004; Kotchetkoff-Henriques et al., 2005; Oliveira & Batalha, 2005).

S. pohlii é uma espécie típica e abundante em áreas com maior disponibilidade hídrica (Toniato et al., 1998), como matas ciliar, de galeria, de brejo e paludosa (Durigan et al., 2004; Kotchetkoff-Henriques et al., 2005; Teixeira & Assis, 2005). A sua ocorrência abrange os Estados de SP, MG, BA, MT, MS, GO e o DF (Durigan et al., 2004; Kotchetkoff-Henriques et al., 2005; Teixeira & Assis, 2005).

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Capítulo 1

GERMINATIVE RESPONSES OF SEEDS OF THREE *Styrax* SPECIES HELP UNDERSTAND THEIR DISTRIBUTION IN CERRADO AREAS IN BRAZIL[1]

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Germinative responses of seeds of three *Styrax* species help understand their distribution in Cerrado areas in Brazil

Abstract

We assessed seed germination in laboratory conditions and using such data obtained in the field we discuss the distribution patterns of three species in the Cerrado areas in Brazil. We described these responses for *Styrax pohlii*, *S. camporum* and *S. ferrugineus* seeds tested at 5, 10, 15, 20, 25, 30, 35, 40 and 45°C. We also assessed the percentage germination (%G) of *S. pohlii* seed samples with different seed water concentrations, as the recalcitrant seed behavior of this species is still unclear. Intrigued by its capacity to germinate directly from puddles formed on poorly drained soils of riparian forests, where it typically occurs, we tested the effect of de-pulping fruits on germination of *S. pohlii* seeds under hypoxia and normoxia conditions. Also, we tested whether distinct concentrations of gibberellic acid (GA$_3$) could break *S. ferrugineus* seed dormancy, which is not well understood. No germination occurred at extreme temperatures (5, 40 and 45°C), regardless of the species. The optimal temperature for germination was 20°C for *S. pohlii* and 25°C for *S. camporum*. However, *S. ferrugineus* seeds showed a very low %G, regardless of the temperature tested. For *S. pohlii* seeds, the higher the seed desiccation the lower the %G, and fruit pulp removal showed to be critical for seed germination of this species. *S. pohlii* seeds germinated independently of oxygenation conditions, reinforcing its adaptation to riparian forests. We were not able to demonstrate that GA$_3$, applied at any concentration, consistently break possible physiological seed dormancy in *S. ferrugineus* seeds.

Key words: temperature, desiccation tolerance, hypoxia, gibberellin, Styracaceae

Introduction

Seed germination performances studied only under laboratory conditions are meaningless to understand species distribution patterns in nature. For species from the Cerrado, which is a biodiversity *hot spot* in South America (Klink & Machado 2005), many germination tests under laboratory conditions have been reported (Zaidan & Carreira 2008), but a few, if any, show relationships with results obtained in the field. On the other hand, the plant reproductive success and species distribution are intimately related because of the ability of seeds to germinate and establish plants in natural communities (Kissmann *et al.* 2012).

The germination process is widely influenced by environmental conditions or resources, such as sunlight (Takaki 2001), temperature (Fenner 1991) and water availability (Fenner & Thompson 2005). Nevertheless, seed dormancy, maturity, tolerance to desiccation and age are intrinsically important (Bewley & Black 1994; Daws *et al.* 2004). Requirements for germination are species-specific, but these requirements may also vary between and within populations due to genetic reasons or local conditions under which seeds are selected (for laboratory studies), or where species successfully develop (Guterman 2000).

Unlike orthodox seeds of most savanna species from the Cerrado that tolerate desiccation, recalcitrant seeds or desiccation intolerant seeds are dispersed with high water concentration and active metabolism (Roberts 1973). These species usually occur in humid environments, being palm trees typical examples (Roberto *et al.* 2011), but some species showing seeds with recalcitrant behavior also occur in dry environments (Pammenter & Berjak 2000). Therefore, no desiccation during maturation enables recalcitrant seeds to rapidly germinate after dispersal, as these seeds would not require rehydration like orthodox seeds (Bewley & Black 1994). These facts may be interpreted as an advantage in humid environments because these seeds are able to promptly use the resources. On the other hand, rapid germination may not be considered advantageous in climatic unstable habitats, such as

the savannas. Seeds of species occurring in savannas commonly exhibit dormancy, preventing these seeds from germinating under unfavorable conditions (Zaidan & Carreira 2008).

Styrax L. includes species with distinct distribution patterns in the Cerrado. *S. pohlii* is a forest tree species frequently occurring in swamp forests within Cerrado areas (Teixeira & Assis 2005). *S. camporum* is widely distributed in the Cerrado, and it is greatly favored by the edge of forest fragments (Habermann *et al.* 2011), whereas *S. ferrugineus* is well adapted to savanna-type physiognomies that exist in the Cerrado (Habermann *et al.* 2011). Seed germination performances of these three species obtained in the field indicated that their tegument thicknesses are harmoniously adapted to their habitats and dispersion times, which are highly related to water availability in the soil (Kissmann *et al.* 2012). However, it is unclear whether *S. pohlii* seeds are tolerant to desiccation and how it germinates if fruits are not de-pulped (by animals) or if fruits and seeds fall into puddles on poorly drained soils of swamp forests. In addition, low percentage germination of *S. ferrugineus* seeds is still related to some unknown dormancy.

Therefore, given data from field conditions (Kissmann *et al.* 2012), in this paper we characterized germination responses of seeds of these three species under a range of constant temperatures, under laboratory conditions. We also assessed the germination performances of *S. pohlii* seeds under different seed water concentrations and when submitted to hypoxia and normoxia, and also the effect(s) of de-pulping *S. pohlii* fruits on seed germination. Finally, we evaluated the effect of different concentrations of gibberellic acid (GA_3) on the germination of *S. ferrugineus* seeds. We discuss the distribution patterns of these species based on data obtained through field experiments (literature) and under laboratory conditions.

Material and Methods

Plant material

Mature fruits of *Styrax ferrugineus* Nees & Mart. were harvested from October to December 2011 in a fragment (470 ha; 22°18'S and 47°11'W) of Cerrado *sensu stricto* (*s. str.*), which is a savanna-type physiognomy of the Cerrado *sensu lato* (*s. l.*). Fruits of *S. camporum* Pohl. were harvested from March to April 2011 in a remnant (37 ha; 22°15'S and 47°00'W) of a forest physiognomy called 'Cerradão', and fruits of *S. pohlii* A. DC. from February to March 2011 in a riparian forest fragment (32 ha; 24°00'S and 47°30'W), being all these sites in São Paulo state, Brazil. These harvests were performed after the respective dispersal time of each of the three species.

After harvesting, the fruits were de-pulped through friction against a 1 mm-steel sieve, under tap water, aiming to mimic natural conditions of seed dispersal, either by considering seeds after digestion or as regurgitated seeds (Kissmann *et al.* 2012). Part of the sample of *S. pohlii* fruits was not de-pulped in order to test the effect of the pulp on the seed germination. The seed water concentration of samples of each species was gravimetrically determined (adapted from International Seed Testing Association 2011) soon after the harvests.

Temperature experiments

De-pulped seeds of the three species were immersed in a solution of sodium hypochlorite (1%, v/v) for 1 min to prevent fungal infection during the germination period. Disinfected seeds were placed on filter paper that was wetted with distilled water inside transparent plastic boxes called "gerboxes" (13 cm × 13 cm × 4 cm). Thirty seeds per gerbox (replications) were incubated under constant fluorescent light (80 μmol m^{-2} s^{-1}) in BOD (Biochemical Oxygen Demanding) germination chambers (Eletrolab, São Paulo, Brazil), in which constant temperatures were set to 5, 10, 15, 20, 25, 30, 35, 40 and 45°C.

Seeds that did not germinate at the end of the tests were submitted to the tetrazolium test to assess their viability. For this test, seed halves were placed in a tetrazolium solution (0.5%, v/v) at pH 6.5-7.0, and incubated at 35°C in the dark for four hours (adapted from International Seed Testing Association 2011). Afterward, these seeds were classified into viable or unviable seeds according to the degree of staining.

For each of the three species, it was used a completely randomized experimental design, with six replications. The number of germinated seeds was monitored every other day until 90 days after sowing (DAS) for *S. camporum* and *S. ferrugineus*, and up to 60 DAS for *S. pohlii* seeds. We did not monitor seeds under green light because seeds of these three species are aphotoblastic (personal observation). Root protrusion with 2 mm long was used as evidence of seed germination. The percentage of germinated seeds (%G) and the relative frequency of germination (RF = n_i : Nt, where n_i is the number of germinated seeds between times t_i - 1 and t_i) were calculated according to Labouriau and Agudo (1987).

For *S. pohlii* and *S. camporum* seeds, a one-way analysis of variance (ANOVA) was performed to test differences in %G assessed at 10, 15, 20, 25, 30 and 35°C, whereas for *S. ferrugineus* the same procedure was used to test %G values measured at 10, 15, 20 and 25°C, because not all species had seeds germinated under the nine temperatures tested. Mean values were compared by Tukey's test ($\alpha = 0.05$), after transforming %G into arcsin of $\sqrt{(G\%/100)}$.

Germination tests of S. pohlii under different seed water concentrations

De-pulped seeds of *S. pohlii* were dried inside a glass desiccator containing silica gel (anhydrous calcium chloride), which was replaced whenever its color started changing from blue to purple. The target seed water concentration was obtained by monitoring the loss of fresh mass of seed samples. The final sample dry mass was calculated according to the following equation: $Mf = M_0 \times [(100 - C_0)/(100 - C_f)]$. In this equation, Mf represents the final

target seed mass (g), C_0 and C_f are the initial and final (target) water concentrations (%), respectively, while M_0 represents the initial sample seed mass (Cromarty *et al.* 1990).

Before and after drying samples for target values, the seed water concentration was gravimetrically determined (adapted from International Seed Testing Association 2011). Water concentration of non-dried seeds (control seeds) was $50 \pm 0.5\%$. After drying samples, water concentrations of the three seed samples were 43 ± 0.1, 33 ± 1.1, and $12 \pm 0.4\%$.

Seed samples (180 seeds) of each of the four treatments were submitted to procedures for seed disinfestation and germination tests as previously described. Six gerboxes containing 30 seeds each from each of the four treatments were put under constant fluorescent light (80 μmol m^{-2} s^{-1}) inside BOD germination chambers (Eletrolab, Brazil), in which the temperature was set to constant 25°C.

A one-way ANOVA was used to test differences in %G between seed samples of each of the four treatments. Mean results were also compared by the Tukey's test ($\alpha = 0.05$).

Germination tests of S. pohlii non de-pulped fruits and hypoxia effects on germination

De-pulped (seeds) and non de-pulped fruits of *S. pohlii* were placed to germinate inside gerboxes, under normoxia and hypoxia conditions. Normoxia conditions was obtained by placing the seeds on wet filter paper inside gerboxes, and hypoxia conditions was obtained by placing seeds on filter paper, and adding distilled water so that 2/3 of each seed was covered.

De-pulping, disinfection and germination procedures were conducted as previously described. Gerboxes were placed under constant fluorescent light (80 mmol m^{-2} s^{-1}) inside BOD germination chambers (Eletrolab, Brazil), in which the temperature was set to constant 25°C. Data were used to calculate the cumulative percentage germination and mean

germination time [$T = (\Sigma(n_i t_i) / \Sigma n_i)$], where n_i is the number of germinated seeds in the time interval from t_i - 1 to t_i, according to Labouriau and Agudo (1987).

A two-way ANOVA was carried out to test the effects of the two-level 'pulp' and 'oxygenation' factors, as well as their interactions on %G and mean germination time (T). The Tukey's test was performed for post hoc comparisons of mean results.

Germination tests of GA$_3$-treated seeds of S. ferrugineus

Seed samples of *S. ferrugineus* were immersed in 0, 50, 100, 150, 300 and 600 mg L^{-1} GA$_3$ (gibberellic acid, Gibco BRL, Grand Island, NY, USA) oxygenated solutions for 24 h. After this procedure, these seeds were submitted to germination tests, as previously described. Gerboxes were placed under constant fluorescent light (80 µmol m^{-2} s^{-1}) inside a germination chamber (Eletrolab, Brazil), in which the temperature was set to constant 25°C.

A one-way ANOVA was conducted to detect significant differences in %G between seed samples treated with the six different GA$_3$ solutions. The Tukey's test ($\alpha = 0.05$) was once more used to compare the mean results.

Results

The initial seed water concentration (after dispersion) was $50 \pm 0.50\%$, $11 \pm 0.01\%$ and $18.8 \pm 0.42\%$ for *S. pohlii*, *S. camporum* and *S. ferrugineus*, respectively.

Regardless of the species, seeds started germinating at 10°C and no germination was observed when seed samples were placed under 5, 40 or 45°C. Besides not germinating under these temperatures, *S. ferrugineus* seeds did not germinate under 30°C or 35°C.

S. pohlii seeds exhibited the same percentage germination (%G) when exposed to 10, 15, 20, 25 and 30°C, whereas seeds of this species under 35°C showed significantly lower %G as compared to the other treatments (Fig. 1). *S. camporum* seeds exhibited great variation

in %G evaluated at different temperatures, however, this species demonstrated to best perform germination at 25°C, but this result did not differ from %G observed for seeds of this species under 20, 30 and 35°C (Fig. 2). In addition, at 25°C *S. camporum* seeds seemed to better distribute germination over time (Fig. 2d). Most seeds of *S. pohlii* and *S. camporum* that did not germinate in temperatures ranging from 10 to 35°C were not viable after 60 and 90 days, respectively, as evidenced by the tetrazolium test.

S. ferrugineus seeds showed conspicuously low %G and these values were similar at 10, 15, 20 and 25°C (Fig. 3). Compared to seeds of *S. pohlii* (best %G = 82 ± 6.5% at 20°C; Fig. 1c) and *S. camporum* (best %G = 64 ± 14 at 25°C; Fig. 2d), *S. ferrugineus* seeds, in general, exhibited less than half of the %G of the other two species, and after 90 days, about 50% of these seeds remained viable.

Seed germination of the three species was well distributed over time and no synchronization/concentration of this response was observed, even at temperatures that returned the highest %G (Figs. 1, 2 and 3). In such temperatures, *S. pohlii* seeds started germinating within 14 days (Fig. 1c), while *S. camporum* and *S. ferrrugineus* seeds started germinating within 20-25 days (Figs. 2d and 3).

The reduction in the seed water concentration negatively affected %G of *S. pohlii* seeds. The higher the seed desiccation the lower the %G, but samples with 12% seed water concentration were still able to germinate (Fig. 4d). Seeds with 43 and 32% water concentration exhibited germination peaks earlier than seeds with 50% water concentration (Figs. 4b,c).

S. pohlii non de-pulped seeds showed great fungal infestation, which led to 100% mortality. On the other hand, the pulp removal resulted in 83% germination, regardless of the oxygenation conditions (Fig. 5). Interestingly, seeds were able to germinate even when

partially covered by a water layer (hypoxia), showing the same germination performance as compared to normoxia conditions (Fig. 5).

$S.$ $ferrugineus$ seeds treated with 100 mg L^{-1} GA_3 showed 17 ± 11% germination, which was 6% higher than the result exhibited by control seeds (%G = 9 ± 5%). On the other hand, besides being extremely variable, GA_3 did not consistently improve %G (Fig. 6).

Discussion

Limiting temperatures for seed germination, or 'cardinal temperatures', define in a species-specific manner the range of temperature under which germination is possible, allowing inferences about the origin of species (Labouriau 1983). Seeds of *S. pohlii* and *S. camporum*, which are typical of forest physiognomies of the Cerrado, germinated in a wide range of temperatures (Figs. 1 and 2) in comparison to seeds of *S. ferrugineus* (Fig. 3), which is a typical savanna species. Considering the optimal germination temperature as the one that promotes the highest %G within the lowest mean germination time (Bewley & Black 1994), our results showed that the optimal temperature for *S. pohlii* was 20°C, and for *S. camporum*, 25°C. The ability of these seeds to germinate under a wide range of temperatures may represent a competitive advantage in Cerrado areas. In fact, seeds of these three species buried in different physiognomies of Cerrado areas showed prompt germination after 60 days (Kissmann *et al.* 2012). However, the germination performance of *S. ferrugineus* seeds is particularly intriguing. Under field conditions (Kissmann *et al.* 2012), 40% of seeds of each of the three species had germinated after 60 days, but the present results showed *S. ferrugineus* seeds with 10% germination, regardless of the temperature (Fig. 3). These observations suggest possible effects of thermal amplitude occurring in soil seed banks, which could break any seed dormancy, as generally suggested by Zaidan and Carreira (2008).

Moreover, in the present report, around 50% of *S. ferrugineus* seeds remained viable, as evidenced by the tetrazolium test, reiterating that these seeds show some type of dormancy.

Seeds are considered as dormant when they fail to germinate under favorable conditions and if germination takes longer than four weeks (Baskin & Baskin 1998). Under the most favorable temperatures (20 and 25°C), *S. ferrugineus* seeds started germinating after 20 days, and lasted until 90 days (Fig. 3). Barbosa *et al.* (1985) reported dormancy in *S. ferrugineus* seeds, but these authors did not specify the dormancy type. Seeds of *S. ferrugineus* with fully developed embryos at the dispersal time (data not shown) and water-permeable seed coat (Kissmann *et al.* 2012) exclude the possibility of morphological or physical dormancy, respectively.

Gibberellin solutions are commonly used to induce germination of physiologically dormant seeds, acting both on the endosperm degradation (Karssen *et al.* 1989) as well as on the expansion of embryo cells (Bewley & Black 1994; Roberto *et al.* 2011). GA_3-treated seeds of *S. ferrugineus* increased %G from 9% (control) to 17% (100 mg L^{-1} GA_3; Fig. 6). Nonetheless, the effects of GA_3 in releasing seeds from physiological dormancy vary greatly according to the deepness of dormancy (deep, intermediate or non-deep; Baskin & Baskin 2004). Therefore, our data confirmed that *S. ferrugineus* seeds are naturally dormant, as generally suggested by the literature (Barbosa *et al.* 1985; Zaidan & Carreira 2008), but were not able to demonstrate that gibberellins overcome such dormancy, which would, then, be an indication of physiological dormancy.

Seeds of each of the three species exhibited well-distributed germination over time (Figs. 1, 2 and 3). The slow and non-synchronized seed germination may represent a strategy, which is suitable for climatic unstable environments or seasonal climates, since this behavior prevents seeds from germinating after any particular situation (Ferreira & Borghetti 2004). Despite the differences in seed coat thicknesses of these species (*S. camporum* >> *S.*

ferrugineus > *S. pohlii*; Kissmann *et al.* 2012), each of these species are harmoniously adapted to its respective habitats, and their fruit/seed dispersion times seem to be adjusted to soil water availability (Kissmann *et al.* 2012). For *S. pohlii* seeds, the different germination performances under the distinct seed water concentrations (Fig. 4) reinforced such dependence on soil water availability. Seeds of this species were dispersed with high water concentration (50%), and exhibited the highest %G under such conditions (Fig. 4a). Therefore, our data confirmed the recalcitrant behavior of *S. pohlii* seeds, but it did not allow classifying these seeds into a specific degree of recalcitrance.

S. pohlii fruits are small drupes (5 mm), and this trait together with high seed water concentration, represent limitations for wind dispersal. Thus, *S. pohlii* seeds are probably dispersed autochorically or zoochorically. Zoochory is crucial for seeds requiring pulp removal to germinate, as we observed for *S. pohlii*. Fruit pulps may limit germination because of germination inhibitors commonly present in the pulp (Yagihashi *et al.* 2000), or because it may offer a moist and nutritious medium for fungal infestation, or even because it may represent a physical barrier for seed germination. We observed that *S. pohlii* pulp provided conditions for fungal infestation, leading to 100% seed mortality.

Fleshy drupes of *S. pohlii* fruits are described as an important diet for birds in forest remnants (Zaca *et al.* 2006). As *S. pohlii* seeds exhibit thin seed coat and fast imbibition (Kissmann *et al.* 2012), as well as high %G after pulp removal, as evidenced by the present study, it seems reasonable to conclude that seeds of this species is zoochorically dispersed.

Because *S. pohlii* seeds are dispersed in the rainy season, when the soil is usually flooded, the capacity of these seeds to germinate in hypoxia conditions (Fig. 5) shows an important advantage of this species in flooded environments. These seed germination responses, together with the fast recovery capacity of gas exchange rates observed in *S. pohlii* seedlings after flooding periods (Kissmann *et al.*, unpublished data), and the high specific leaf

area of *S. pohlii* young plants in swamp forests, which leads to advantageous photosynthetic performances in such shaded environments (Habermann & Bressan 2011) may explain the successful occurrence of this species in swamp forests (Teixeira & Assis 2005).

In the present paper we used important data on seed germination performances of three congeneric *Styrax* species obtained under laboratory conditions and complemented with the literature, which constituted substantial information to discuss the distribution patterns of these congeneric species in the Cerrado areas in Brazil. In conclusion, our results showed that the optimal germination temperature for *S. pohlii* is 20°C and for *S. camporum*, 25°C. The seeds of *S. ferrugineus* have physiological dormancy and the gibberellin was not able to overcome such dormancy in this seeds. *S. pohlli* seeds are recalcitrant, able to germinate under hypoxy conditions, but the fruit pulp removal is essencial for the germination of the seeds.

Acknowledgements

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Figures
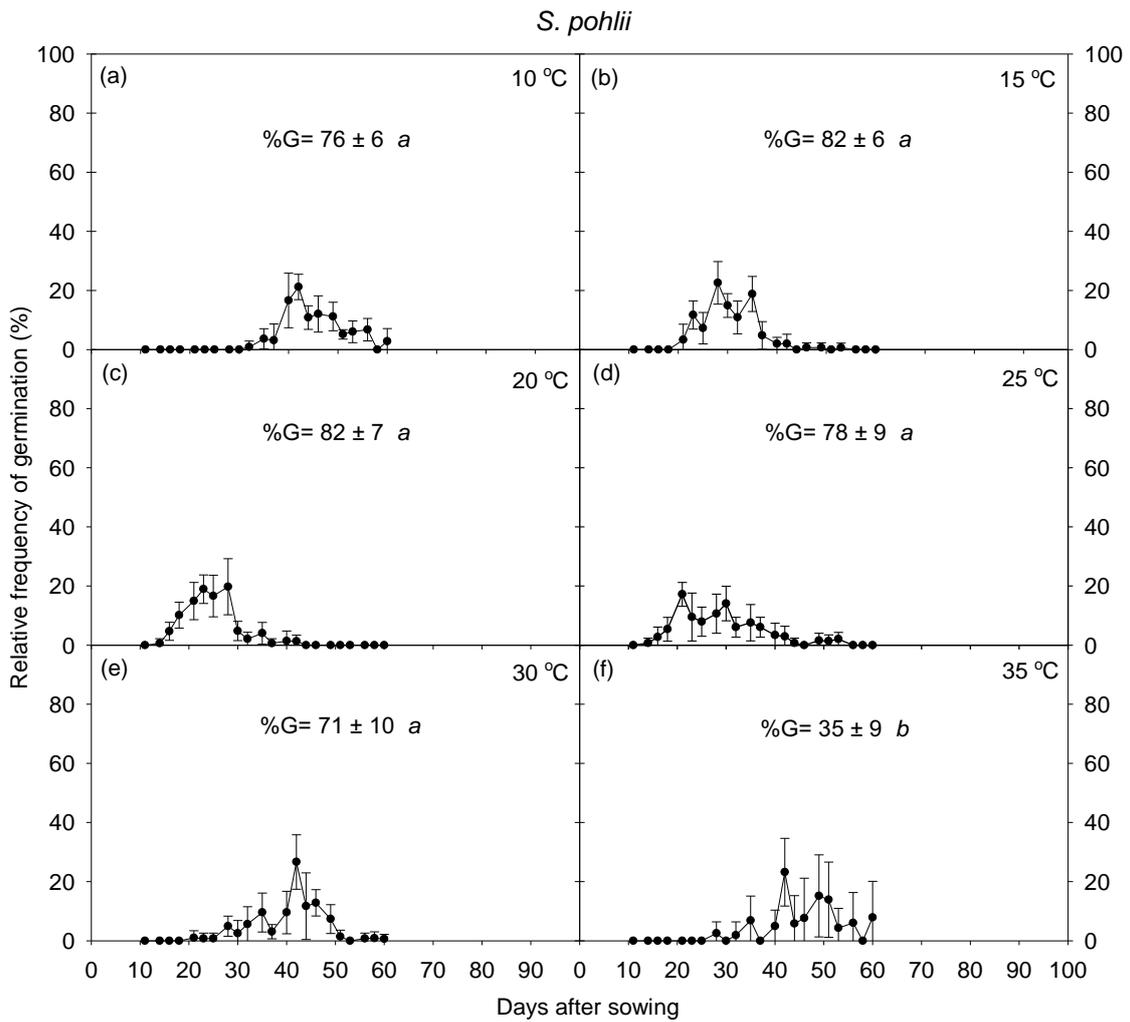
Fig. 1. Relative frequency of germination of *S. pohlii* seeds at 10, 15, 20, 25, 30 and 35 ℃ and under fluorescent light (80 μmol m^{-2} s^{-1}). Dots represent mean values (*n = 6*), and vertical bars are S.D. (%G = percentage germination ± S.D.; Different letters show significant differences – P < 0.05 – in %G between treatments).

Fig. 2. Relative frequency of germination of *S. camporum* seeds at 10, 15, 20, 25, 30 and 35 °C and under fluorescent light (80 μmol m^{-2} s^{-1}). Dots represent mean values (*n = 6*), and vertical bars are S.D. (%G = percentage germination ± S.D.; Different letters show significant differences – P < 0.05 – in %G between treatments).
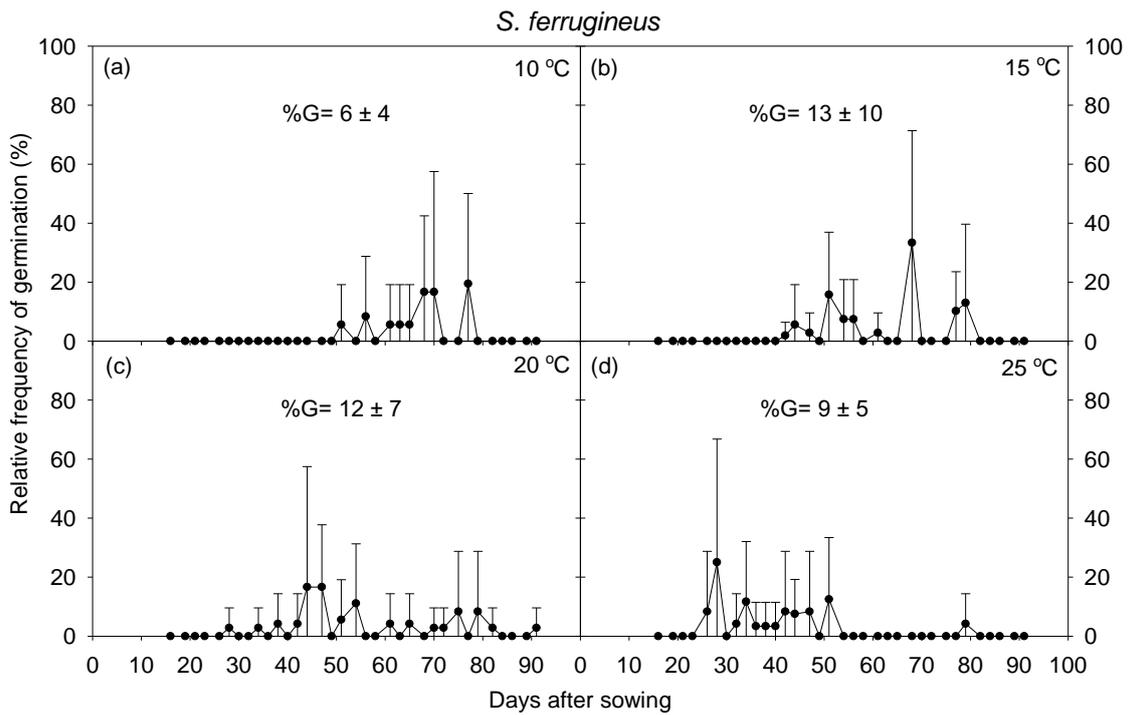
Fig. 3. Relative frequency of germination of *S. ferrugineus* seeds at 10, 15, 20 and 25 ℃ and under fluorescent light (80 μmol m^{-2} s^{-1}). There was no germination at 30 and 35 ℃. Dots represent mean values (*n = 6*), and vertical bars are S.D. (%G = percentage germination ± S.D.; Absence of letters indicates the lack of significant differences – P > 0.05 – in %G between treatments).
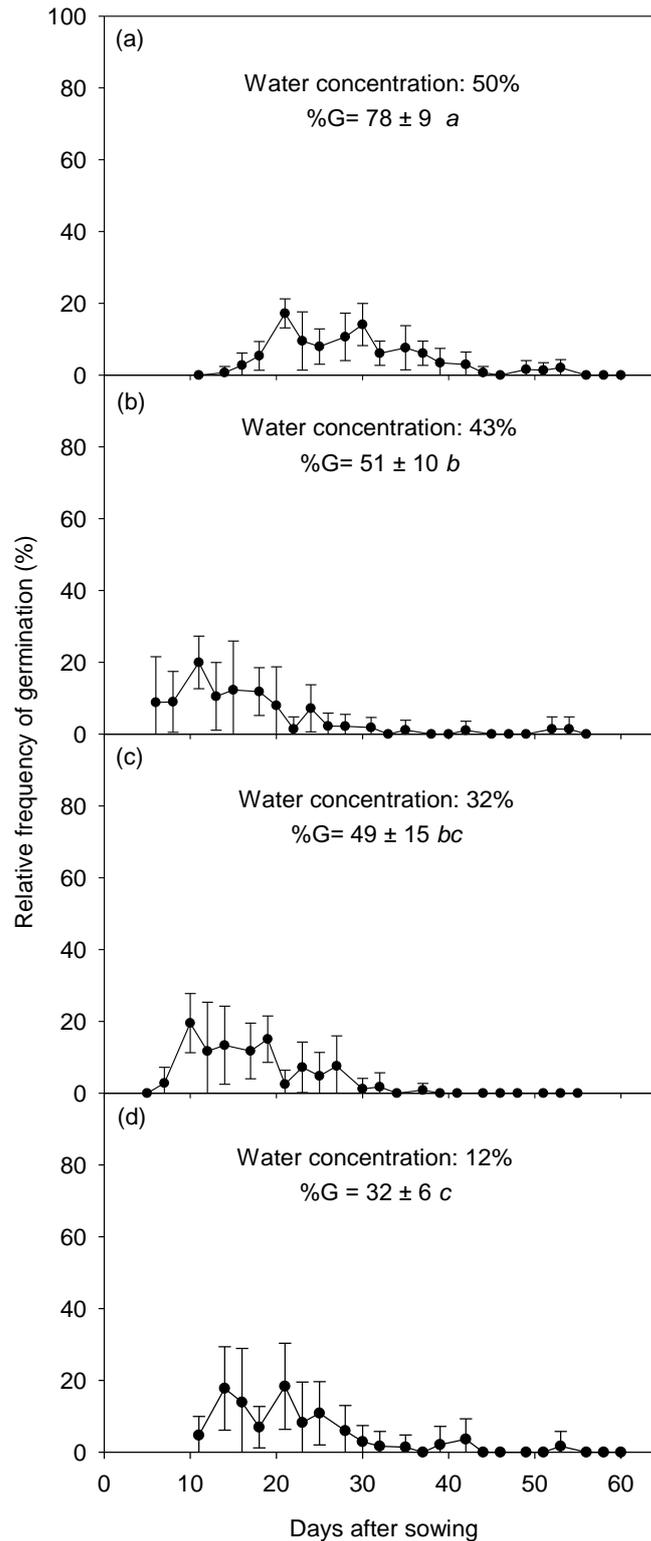
Fig. 4. Relative frequency of germination of *S. pohlii* seeds, at 25 ºC and under fluorescent light (80 μmol m^{-2} s^{-1}), in response to seed water concentrations of 50%, 43%, 32% and 12%. Dots represent mean values (*n = 6*) and vertical bars are S.D. (%G = percentage germination ± S.D.; Different letters show significant differences – P < 0.05 – in %G between treatments).
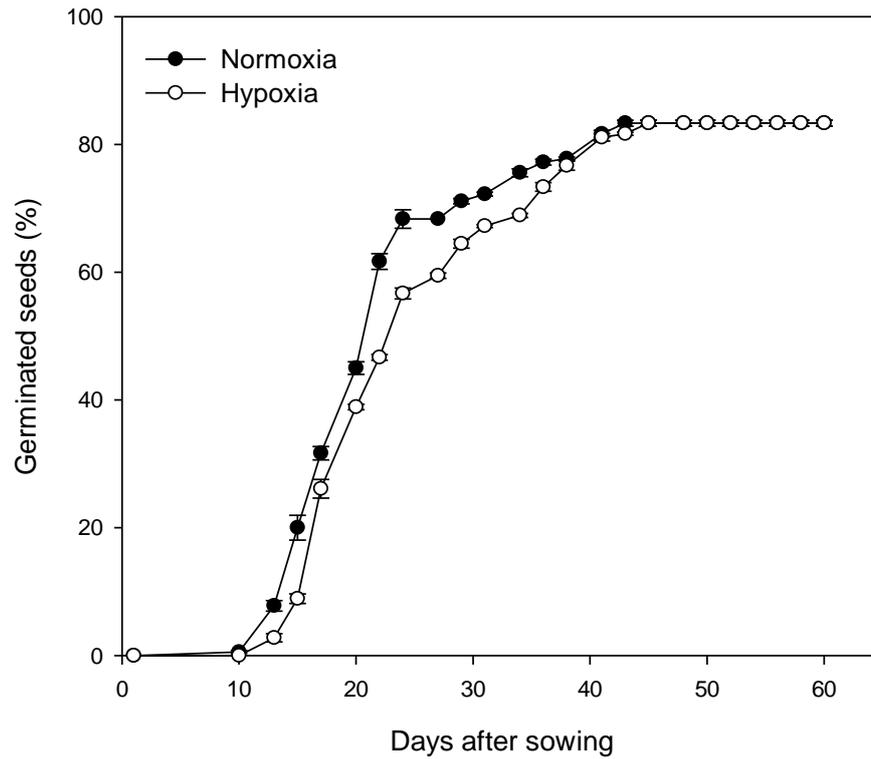
Fig. 5. Cumulative percentage germination of *S. pohlii* seeds, at 25 ℃ and under fluorescent light (80 μmol m^{-2} s^{-1}), submitted to normoxia and hypoxia conditions. Dots represent mean values (*n = 6*) and vertical bars are S.D.
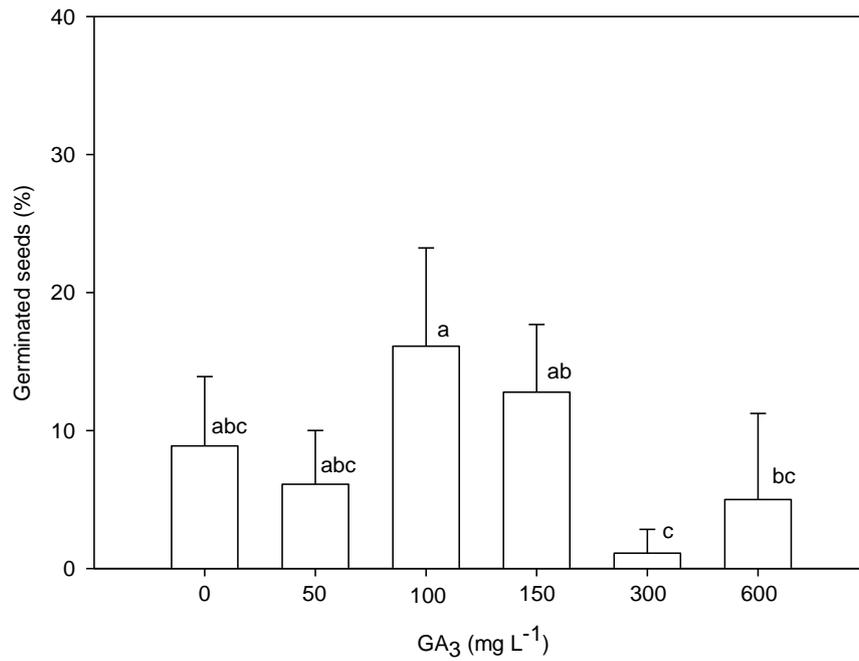
Fig. 6. Percentage germination of *S. ferrugineus* seeds, at 25 ºC and under fluorescent light (80 μmol m^{-2} s^{-1}) in response to GA$_3$ treatments. Columns represent mean values (*n = 6*) and vertical bars are S.D. Distinct letters show significant differences (P < 0.05) between treatments.

Capítulo 2

GERMINATION PERFORMANCE OF CONGENERIC *Styrax* SPECIES FROM THE CERRADO *SENSU LATO* AREAS AND THEIR DISTRIBUTION PATTERN IN DIFFERENT PHYSIOGNOMIES[2]

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Germination performance of congeneric *Styrax* species from the cerrado sensu lato areas and their distribution pattern in different physiognomies

Abstract

When studying congeneric species, it is of reasonable importance to understand different ecophysiological performances which might determine the distribution of species in habitats with different natural resources. *Styrax ferrugineus* is exclusive and well adapted to the Brazilian Cerrado *sensu stricto* (*s. str.*); *S. camporum* is widely distributed in the Cerrado *sensu lato* (*s. l.*) areas, with young trees being observed at the edge of cerradão and other vegetation fragments; and *S. pohlii* occurs in permanently waterlogged soils of the Cerrado region, such as those of riparian forests. We tested the hypothesis that the higher the soil water content in the physiognomic gradient of the vegetation, the higher is the germination success of *S. pohlii*, but the lower is the germination success of *S. ferrugineus*. We also discuss whether gap conditions inside a cerradão fragment imply a high germination rates of seeds of *S. camporum*. Seeds from each of the three species were buried within nylon bags containing soil from the respective sites. Burial occurred in a Cerrado *s. str.*, in understory and gap conditions of a *Cerradão*, and in the understory of a riparian forest fragment, and lasted for 60, 120, 180 and 240 days, respectively, after the fruit dispersal time of each of the three species. After 60 days, a relationship was found showing that the percentage of germinated seeds diminished, and the percentage of damaged seeds increased as soil water content increased (Cerrado *s. str.* < cerradão gap < cerradão understory << riparian forest). *S. camporum* still showed viable seeds 60 days after burial (DAB), and germinated seeds 120 DAB, indicating that it needed a longer time to germinate, which might be associated to its thicker seed coat, in relation to the other two species. The germination performance of each of the three species was the same in the gap and understory conditions of the cerradão. The

higher concentration of adult *S. camporum* plants at the edge of vegetation fragments is not related to a particular high germination performance and seedling establishment.

Key words: Brazilian savanna, seed longevity, seed bank, seed reserves, Styracaceae

Introduction

The expansion of the Cerrado vegetation (Neotropical savanna of Brazil) occurred concurrently with retraction of mesophytic forests, during the Holocene (Oliveira and Marquis, 2002). Therefore, great exchange of floristic elements occurs between both the Amazonian and the Atlantic forests on the one hand, and the Cerrado vegetation on the other hand, because there are no geographical barriers separating these different vegetation types (Oliveira Filho and Ratter, 2002; Ratter et al., 2003).

Because the Cerrado vegetation is comprised of different physiognomies (grasslands, forests and savannas), which together are referred to as the Cerrado *sensu lato* (*s. l.*), the role of edaphic factors has been intensely debated as a cause leading to such physiognomically different vegetations (Dantas and Batalha, 2011). However, the soil fertility can be also very similar among the Cerrado physiognomies (Haridasan, 2008; Habermann and Bressan, 2011), and it may not be an important determinant of these vegetation aspects (Assis et al., 2011). On the other hand, the vegetation structure in the Cerrado *s. l.* areas is strongly influenced by seasonal fire events (Loiola et al., 2010; Simon et al., 2009) and micro-climatic factors, such as irradiation load at the soil level, air temperature, vapor pressure deficits (VPD) and soil water availability (Habermann et al., 2011).

Cerrado *sensu stricto* (*s. str.*) is a savanna-type physiognomy, with scattered trees and an herbaceous understory, with high irradiation load at the soil level. Cerradão is a forest physiognomy of the Cerrado vegetation, and tall trees (8–15 m) form a semiclosed canopy, limiting sunlight penetration. Enriching the Cerrado vegetation, there are riparian forests, a

moist and closed-canopy environment which follows small rivers. In these physiognomies, the sunlight availability declines, whereas the soil water availability increases as the plant density per area is increased (Habermann et al., 2011).

Seed germination and seedling survival are the most critical stages in a plant community (Kitajima and Fenner, 2000) and are influenced by the soil water availability, which is a determinant of local- and regional-scale distributions of tropical and subtropical trees (Engelbrecht et al., 2007; Navarro et al., 2011).

Morphologically similar congeneric species exist, and these species present distinct patterns of distribution in the Cerrado *s. l.* areas, e.g. in the genus *Styrax*. *Styrax ferrugineus* Ness & Mart. is exclusive and well adapted to the Cerrado *s. str.*; *S. camporum* Pohl. is widely distributed in the Cerrado *s. l.* areas, with young trees being found at the edge of the cerradão, suggesting it to be a shade intolerant species; and *S. pohlii* A. DC. is a forest species, typically occurring in riparian forests (Habermann et al., 2011).

The fleshy drupes of these three species are eaten and dispersed by birds (Lorenzi, 1992), although fruits of *S. ferrugineus* may also be consumed by guans and coatis (Alves-Costa and Eterovick, 2007). *S. pohlii* fruits, for instance, are described as an important diet of birds in forest remnants (Zaca et al., 2006). A general zoochory is, therefore, described for the three species, birds being the most important dispersers (Lorenzi, 1992; Zaca et al., 2006). Considering the current ecological knowledge, it seems reasonable that seeds of the three *Styrax* species have equal chance of germination in the three environments evaluated in the present study (Cerrado *s. str.*, cerradão and riparian forest), since these environments are all inhabited or eventually visited by birds and small fruit consumers.

To understand the success of these *Styrax* species in Cerrado areas, we hypothesized that the higher the soil water content in the physiognomic gradient, the higher is the germination success of *S. pohlii*, but the lower is the germination success of *S. ferrugineus*.

To test this hypothesis we assessed the percentage of germinated, viable and damaged seeds of the three species, which were artificially buried in a Cerrado *s. str*., in understory and gap conditions of a cerradão, and in the understory of a riparian forest fragment. Additionally, we assessed the imbibition curves of these seeds, and their total storage content of starch, lipids, and proteins. Ultra-structural and anatomical seed coat analyses were performed as a framework for the functional studies. Based on the data, we discuss whether the soil water availability in different vegetation types of the Cerrado *s. l.* areas will have any significance for the distribution patterns of the three species, regarding their sexual propagation strategies; and whether the gap conditions of a Cerradão positively influence the germinative performance of seeds of *S. camporum.*

Material and methods

Site description

This study was conducted in three fragments of physiognomically different Cerrado *s. l.* areas: (1) a Cerrado *s. str.* fragment on the São José da Conquista farm (260 ha); geo-coordinates 22°13'S, 47°53'W); (2) a Cerradão fragment (38.7 ha; geo-coordinates 22°15'S, 47°00'W); and (3) a riparian forest remnant (32 ha; geo-coordinates 24°00'S, 47°30'W), in the municipalities of Itirapina, Corumbataí and Rio Claro (Ajapi district), respectively, in southern São Paulo state, Brazil.

Plant material

Mature fruits of *Styrax camporum* and *S. pohlii* were harvested from adult trees in the cerradão, and in the riparian forest fragments, respectively. Fruits of *S. ferrugineus* were harvested in a Cerrado *s. str.* fragment in the Mogi-Guaçú Biological Reserve (22°15'–16'S, 47°08'–12'W) in the municipality of Mogi-Guaçú, in the northeast of São Paulo state, Brazil.

The fruits of the three species are small purple drupes, containing a single seed or, less frequently, a couple of seeds. The seeds of *S. pohlii* show recalcitrant behavior, whereas seeds of *S. camporum* and *S. ferrugineus* are orthodox, although their seed viability cannot be maintained for a long time (personal observation).

Fruit harvests were carried out weekly until the number of seeds (de-pulped fruits) was sufficient for the assays. After harvesting, the fruits were de-pulped in the laboratory, through friction against a 1 mm-steel sieve, under tap water. Inert materials and damaged fruits were discarded. Since the three species are zoochoric, depulping fruits was the strategy used to mimic the natural conditions of seed dispersal, either by considering seeds after digestion or as regurgitated seeds. Seed moisture content was gravimetrically determined (adapted from International Seed Testing Association, 2011), soon after the de-pulping of the seeds.

Imbibition Curves

Imbibition curves of the seeds were determined for each of the three species after their respective fruit dispersal time in the 2009/2010 season. Fruits of *S. camporum* are dispersed during the dry season (April–June) and fruits of *S. ferrugineus* at the beginning of the rainy season (September–November), while fruits of *S. pohlii* are dispersed at the peak of the rainy season (January–March).

After de-pulping and washing the seeds, they were placed on four layers of paper towels inside a tray for 24 h in order to dry naturally under room temperature conditions. The seeds were then immediately placed on filter paper that was wetted with distilled water and placed inside transparent plastic boxes (13 cm $\times$ 13 cm $\times$ 4 cm), and these boxes were covered with their respective transparent plastic lids. Six boxes, which contained 30 seeds each, were regarded as replications. The replications were incubated in a germination chamber (EL 2002, Eletrolab, SP, Brazil) at 25°C, under constant fluorescent light (80 μmol m^{-2} s^{-1}).

The water mass gain per seed was measured by using an analytical balance at 0, 2, 4, 6, 8, 10 h, and subsequently at every 24 h until 1440 h (60 days). Germinated seeds (showing the protrusion of 1 mm of the primary root) were removed from replications. The curves were plotted using fresh mass per seed against time, with subsequent regression analysis.

Seed Burial Experiments

Burial of seeds from each of the three species was performed after their respective fruit dispersal time, in the 2009/2010 season. The seeds of *S. camporum*, *S. ferrugineus*, and *S. pohlii* were buried in July 2009, October 2009, and March 2010, respectively.

Thirty de-pulped fruits of each of the three species were enclosed inside 0.5 L bags made of 1-mm mesh size nylon fabric (Trifil, Guarulhos, SP, Brazil), which contained 0.25 L of the soil from the respective habitat fragments where seeds were buried. Six bags (for each of the three species) containing seeds of *S. camporum*, *S. ferrugineus* or *S. pohlii* were buried in six spots in the Cerrado *s. str.*, in understory and gap conditions of the Cerradão, and in the understory of the riparian forest fragment.

The bags were buried at 5 cm in soil depth in each of the four described environments. Because fruits of these species are mainly dispersed by birds (Lorenzi, 1992), it is expected that seeds are somehow deposited on the soil surface. However, since natural predators or dispersors of seeds (small rodents, beetles, among others) were not excluded from the studied areas, the seeds were buried (hidden from seed predators) at 5 cm in depth. This is assumed to be not un-natural for zoochoric species, because in Cerrado areas 0 (litter) to 5 cm in depth can be considered a mixed layer containing both the transient and the persistent soil seed bank (Pereira-Diniz and Ranal, 2006).

To provide the gap conditions in the Cerradão, small plants (trunks up to 3 cm in diameter) were eliminated, and larger trees got their canopy partially pruned, resulting in a

crown gap with na oval shape, producing an illuminated area of approximately 25 m^2 on the ground. The six gaps were monitored every other month, in order to guarantee the intended conditions (higher sunlight penetration at the soil level in comparison with understory conditions). On unclouded typical days of the rainy (February/2010) and dry (August/2010) seasons, at every site (Cerrado *s. str.*, understory and gap conditions of the Cerradão, and the understory of the riparian forest) VPD and air temperature were measured every 30 min from 09:00 h to 17:00 h using an aspirated psychrometer Model SK-RHG, Sato, Tokyo, Japan). In addition, the photosynthetic photon flux density (PPFD) was similarly obtained using a quantum sensor (9901-013, LI-COR, Lincoln, NE, USA) (Fig. 1). For each of the three species, the six seed samples were recovered at 60, 120, 180, and 240 days after burial (DAB).

After recovering the samples, seeds were taken to the laboratory, where the number of germinated and non-germinated seeds, and damaged seeds were recorded. Damaged seeds exhibited visual changes of appearance, exposing the reserves or the embryos. Intact seeds were considered as non-germinated and were submitted to the tetrazolium test to assess their viability. For this test, seed halves were placed in a tetrazolium solution (0.5%, v/v) at pH 6.5–7.0, and the seeds were incubated at 35 °C in the dark for 4 h (adapted from International Seed Testing Association, 2011). Subsequently, these non-germinated seeds were classified as viable or unviable seeds.

Rainfall and soil water availability

Soil moisture contents were measured monthly, from July 2009 to November 2010. Five fresh soil samples were randomly collected below the leaf litter, at 5 ± 1 cm depth, at each of the four experimental sites, and these samples were immediately weighed using a portable balance (ELB 300, Shimadzu, Tokyo, Japan). Dry soil mass was determined by oven-drying

of the samples at 105 °C to constant mass, and the soil water content was calculated and expressed in g H_2O g soil^{-1}.

During the same period (July 2009–November 2010), monthly rainfall (mm) was monitored by weather stations located at the Centro de Recursos Hídricos e Ecologia Aplicada (USP, São Carlos, SP), which is 18 km distant from the Cerrado *s. str.* fragment; at the Iracema sugarcane farm (Corumbataí, SP), which is located 3 km from the Cerradão fragment; and at the Centro de Análise e Planejamento Ambiental (CEAPLA – Unesp, Rio Claro, SP), which is 12 km distant from the riparian forest fragment.

Ultrastructure of seed coat

Mature fruits of each of the three species that were harvested after their respective fruit dispersal time in the 2009/2010 season were also used for a study of the ultra-structure of seeds.

For light microscopy, the fresh seeds were dehydrated in a series of normal-butyl alcohol (NBA), and embedded in historesin 2-hydroxyethylmetacrylate (Leica Historesin Embedding Kit, Leica Microsystems, Wetzlar, Germany; Feder and O'Brian, 1968). Longitudinal and transverse sections were made with a Reichert-Jung microtome (2040, Leica Microsystems, Wetzlar, Germany) and double-stained with periodic acid, Schiff's reagent (PAS reaction) and with a 0.05% (v/v) solution of toluidine blue (Feder and O'Brian, 1968).

For scanning electron microscopy (SEM), mature seeds were fixed in a 2.5% (v/v) glutaraldehyde solution (with 0.1 M phosphate buffer, at pH 7.3; overnight at 4 °C), dehydrated in a series of ethyl alcohol, dried to the critical point, mounted on aluminum stubs, and coated with gold. Images were examined using a scanning eléctron microscope (SEM 515, Phillips, USA) of 20 kV.

Seed Reserves

The total reserve contents were measured in seeds of each of the three species that were also harvested after their respective fruit dispersal time, in the 2009/2010 season. The samples, taken from six different trees, were comprised of 34 seeds of *S. ferrugineus*, 36 seeds of *S. camporum* and 16 seeds of *S. pohlii*.

Lipids were extracted from uncoated fresh seeds (without their seed tegument), which were ground using a mortar and pestle. The ground fresh seeds were submitted to an organic extraction, using methanol (10 mL) and chloroform (10 mL). After removing the solid component through filter paper, 5 mL of chloroform and 10 mL of deionized water were added to the aqueous filtrate. After centrifugation at 5000 rpm, the lower phase (chloroform) was collected and dried in beakers of known mass, and the quantification of lipids was gravimetrically calculated, according to Bligh and Dyer (1959).

For starch quantification, the uncoated seeds were ground with mortar and pestle and then extracted three times with ethanol 80% (v/v), for the removal of free sugars. The remaining pellet from the previous extraction was then further extracted using 1 mL of a 30% (v/v) perchloric acid solution, for 90 min (with occasional stirring), and subsequently centrifuged at 5000 rpm for 30 min. The supernatant was assayed for starch with the I_2-KI reagent, according to Dirk et al. (1999). For this, 100 μL of the sample were mixed with 900 μL of a 30% (v/v) perchloric acid solution followed by the addition of 1 mL of the I_2-KI reagent, vortexed, and left standing for 10 min. The absorbance was read at 620 nm, using soluble starch (G.R., Merck, Germany) as standard.

The extraction of total proteins was performed according to Alfenas (2006). The fresh uncoated seeds were ground in a sodium phosphate buffer solution (0.05 M, pH 7.0), using mortar and pestle. The ground seeds were centrifuged at 5000 rpm for 5 min, filtered, and the total soluble protein content was determined according to Bradford (1976). For this, 5 mL of

the Bradford reagent was added to 100 µL of the previous sample. The reaction was left standing for two minutes and then the absorbance was read at 595 nm, using bovine serum albumin (G.R., Merck, Germany) as standard.

Results were obtained in triplicate and, since we used uncoated seeds of almost the same size (elliptical shape seeds of 5 ± 2 mm in length and 2 ± 0.5 mm in width), results were expressed in mg of total contents (total lipids, proteins or starch) per seed, even though each species showed different seed moisture contents (*S. pohlii* $\gg$ *S. camporum* > *S. ferrugineus*).

Data Analysis

For the seed burial study, a two-way analysis of variance (ANOVA) was performed to test the effects of the environment fator (four environments) and of the period-of-burial factor (60, 120, 180 and 240 DAB) on the germination performance of the three species. For each of the three species, separately, the Tukey test ($\alpha = 0.05$) was used to compare mean results.

For each of the three seed reserve content (starch, lipids and protein) values, separately, a one-way ANOVA was carried out to test differences between the three species. The Tukey test ($\alpha = 0.05$) was also used to conduct post hoc comparisons.

The imbibition curves and anatomical images were used to discuss the germination performances of each of the three species in the four environments.

Results

In the Cerradão fragment (Fig. 1B and C), the VPD amplitude between rainy and dry season was greater than this same amplitude observed in the Cerrado *s. str.* (Fig. 1A) and in the riparian forest fragments (Fig. 1D). However, VPD and air temperature observed in the understory and in gap conditions of the cerradão were very similar (Fig. 1B and C). The air temperature typically fluctuated during the days of the rainy and dry seasons, regardless of the

studied site (Fig. 1A–D). However, as expected, there was a higher irradiance penetration in the gap (Fig. 1F) compared with the understory condition (Fig. 1G) of the Cerradão, and such irradiance produced by the gap condition was similar to the irradiance measured in the Cerrado *s. str.* (Fig. 1E and F). The understory conditions of both the Cerradão and the riparian forest gave the same low irradiance penetration (Fig. 1G and H).

Mean monthly rainfall was similar for the three vegetation fragments, with a pronounced dry season from May to September 2010. While the values of soil water content in April, May and June 2010 were approximately 0.10 g H_2O g soil^{-1} for the Cerrado *s. str.* and for the gap and understory conditions of the Cerradão, in the riparian forest they amounted to 3-4 g H_2O g soil^{-1}. In general, the riparian forest showed higher values of soil water content in comparison with the other three experimental sites, among which the soil water content values were similar (Fig. 2). This indicates that the water table, rather than the rainfall, is more important to the soil water availability in the riparian forest.

The seed samples that were used to plot the imbibition curves and to study the seed longevity in the soil showed initial water contents of 7.4 ± 0.2, 14.5 ± 0.3 and $52.5 \pm 0.5\%$ for *S. ferrugineus*, *S. camporum* and *S. pohlii*, respectively; and these values were measured at harvest.

Seeds of *S. ferrugineus* and *S. camporum* showed a similar pattern of imbibition. The initial seed water uptake (imbibition phase, or phase I of the imbibition curve) occurred up to 10 h after seeds were put to germinate. After this period, the fresh seed mass stabilized, characterizing the start of the stationary phase of the imbibition curve (phase II) (Fig. 3A–D). Seeds of *S. pohlii* showed a shorter imbibition phase (approximately 2 h), and the stationary phase started after 2 h (Fig. 3E and F).

Sixty days after burial (DAB), seeds of *S. ferrugineus* (Fig. 4A) and *S. camporum* (Fig. 4B) buried in the riparian forest presented a lower ($P < 0.05$) percentage of germinated

seeds (%G) in comparison with the other three experimental sites. Seeds of *S. pohlii* buried in the riparian forest also presented low values of %G, but because of the response variability, this mean result did not differ ($P > 0.05$) from the %G value of *S. pohlii*'s seeds buried in the Cerrado *s. str*. However, %G values of *S. pohlii*'s seeds buried in the riparian forest were significantly lower ($P < 0.05$) than of those seeds buried in the understory and gap conditions of the Cerradão fragment (Fig. 4C).

Compared to the other two species, seeds of *S. camporum* showed higher ($P < 0.05$) percentage of germinated and viable seeds, regardless of the sites where the seeds were buried (Fig. 4).

Seeds of each of the three species that were recovered at 120 DAB showed a significantly lower %G, in relation to when they were recovered at 60 DAB, regardless of the site seeds were buried at (Fig. 4). At 120 DAB, seeds of *S. ferrugineus* (Fig. 4A) and *S. pohlii* (Fig. 4C) showed the same ($P > 0.05$) %G at the four experimental sites where the seeds had been buried, and seeds of *S. camporum* buried in the Cerrado *s. str.* showed higher ($P < 0.05$) %G in comparison with the three experimental sites where the other seeds of *S. camporum* had been buried (Fig. 4B). In general, after 120 DAB, most seeds were damaged, showing deteriorated coats.

Seeds of the three species buried in each of the four experimental sites that were recovered at 180 and 240 DAB were 100% damaged, and no germinated or viable seeds were found.

The seed coat of each of the three species is morphologically similar, and they can be characterized as mesotestal seeds (Fig. 5a–i). The exotesta is formed by thin-walled parenchymatic cells, as seen in *S. pohlii* (Fig. 5c), and the mesotesta is comprised of several layers of thick-walled cells, the brachysclereids (Fig. 5b, c, e, f, h and i). The degree of thickness in this layer is species-specific, being thin in *S. pohlii* (60-150 μm; Fig. 5a and c),

intermediate in *S. ferrugineus* (200-350 μm; Fig. 5d and f) and thick in *S. camporum* (500-700 μm; Fig. 5g and i). The tegmen of each of the three species presents few layers of thin-walled or collapsed parenchymatic cells, as seen in *S. ferrugineus* (Fig. 5f).

The total seed lipid content was higher ($P < 0.05$) in *S. pohlii* seeds in comparison with *S. ferrugineus* seeds, whereas seeds of *S. camporum* showed intermediate values. Starch concentration did not significantly differ among the three species. The total protein content was significantly higher in seeds of *S. pohlii* in comparison with seeds of the other two species (Fig. 6).

Discussion

Understanding the dynamics of soil seed banks is essential to predict both plant recruitment patterns as well as the potential of a species to occupy a certain environment. Several studies in the Cerrado *s. l.* areas have demonstrated that species whose seeds are composing the soil seed banks are not the same species that compose the above-ground vegetation (Durigan et al., 2002; Pereira-Diniz and Ranal, 2006; Salazar et al., 2011; Sassaki et al.,1999). This suggests that seeds of the Cerrado vegetation must not germinate *in situ* and, consequently, seed germination may not be considered as the most important form of reproduction in the Cerrado vegetation. That said, it is also acknowledged that seeds of many Cerrado woody species show some type of dormancy (Zaidan and Carreira, 2008).

Our results showed that the seeds of the three species promptly germinated after their respective dispersal time and subsequente artificial burial (Fig. 4). Additionally, the imbibition curves plotted for each of the three species demonstrated that the seeds did not show any dormancy related to the seed coat because seeds of the three species imbibed within a few hours (Fig. 3). Therefore, theoretically, the germination process does not seem to be a critical phase for the recruitment of any of these species and, apparently, the seeds of the three

species expressed their total germinative capacity after 60 days of burial, regardless of the site in which they were buried.

On the other hand, the longevity of the buried seeds was practically the same for the three species, regardless of the site in which they were buried. The three species exhibited a decrease in seed viability and germination, while showing an increase in the percentage of damaged seeds, as the storage time in the soil increased. Generally, the seeds did not remain alive in the soil for more than 120 days (Fig. 4). Even when emergence is successful, other causes of seed loss from a soil seed bank are seed mortality (as a result of attacks of seed predators and pathogens) and the loss of seed viability (Leishman et al., 2000). The loss of seed viability observed for the three species may be related to their seed reserves, which were found to be predominantly lipids. It is expected that lipid-rich seeds have a low survival rate because of peroxidation reactions, which contribute to the production of free radicals and thus to the loss of seed viability (Corbineau et al., 2002), especially when exposed to high soil moisture (Stewart and Bewley, 1980). This may be the case for the low seed viability and germination observed after 60 days, especially for *S. ferrugineus* (Fig. 4A) and *S. pohlii* (Fig. 4C) in the riparian forest, where the soil water content values were about 20 times higher than the values observed in the other three experimental sites (Fig. 2). However, it is noteworthy that after 60 days, the percentage of viable seeds was significantly greater for *S. camporum* (Fig. 4B) in comparison with *S. ferrugineus* (Fig. 4A) and *S. pohlii* (Fig. 4C). We concluded that this is because of the thicker seed coat of *S. camporum* (Fig. 5g–i) in relation to the other two species (Fig. 5a–f). Thus, the relatively thick seed coats observed for the three species did not offer a barrier for seed water uptake (imbition), but in the Cerrado *s. l.* areas, thick seed coats seem to protect seeds from predators and, consequently, enhance seed longevity in the soil, especially in soil from humid areas.

Therefore, we could not support our hypothesis that the higher the soil water content in the physiognomic gradient, the higher is the germination success of *S. pohlii*, but the lower is the germination success of *S. ferrugineus*. If this hypothesis would have been corroborated, it would, then, have explained the success of *S. pohlii* in the humid and shaded environment of riparian forests (Teixeira and Assis, 2005). Furthermore, our results does not even support that seeds of any of these three species contribute essentially to the soil seed bank in Cerrado areas. Rather, based on the observed evidence that the seeds of *S. pohlii* promptly imbibe and germinate, it is more reasonable to attribute the success of *S. pohlii* to rapid germination right after its dispersion time, when water and high temperatures are not limiting the germination, and also to a rapid vegetative growth strategy used by forest species to reach the forest crown. Although shaded environments negatively affect the photosynthetic rates of Cerrado species (Habermann et al., 2011), for *S. pohlii* shaded environments enhance the specific leaf area, which enhances the photosynthesis of this species, allowing it to grow and reach the forest crown. By contrast, *S. camporum* and *S. ferrugineus* are not able to perform as well in their photosynthesis (Habermann and Bressan, 2011).

Nevertheless, in the Cerrado *s. l.* areas, the major input of seeds occurs at the end of both the dry and wet seasons (Pereira-Diniz and Ranal, 2006). The germination in the wet season allows seedlings to maximize plant growth before the unfavorable (dry) season starts (García-Núñez et al., 2001). Thus, this scenario is in agreement with the response observed for *S. ferrugineus* and *S. pohlii*. Although showing less germinated and viable seeds at the four experimental sites (Fig. 4A and C) than *S. camporum* (Fig. 4B), seeds of *S. ferrugineus* and *S. pohlii* seem to have been able to express their germinative capacity within 60 days, which would correspond, theoretically, to a period immediately after their respective seed dispersal. Thus, seeds of *S. camporum*, which are released in April/May and must withstand a soil water deficit during the dry season, may remain viable longer in the soil than seeds of the

other two species. In fact, seeds of *S. camporum* showed a very thick seed coat and exhibited higher seed longevity than the other two species, and still presented 25% of germinated seeds in the Cerrado *s. str.* 120 days after burial (Fig. 4B).

In contrast to our prediction, the gap conditions of the Cerradão did not positively influence the germination performance of *S. camporum*'s seeds, because its seed germination and viability were similar ($P > 0.05$) in both the gap and understory conditions of the cerradão, at 60 or 120 DAB (Fig. 4B). These contrasting conditions did not produce differences in the soil water availability (Fig. 2B and C), air temperature or even in the VPD (Fig. 1B and C). The gap conditions generated, however, a higher daily irradiance at soil level in comparison with the natural understory conditions. However, air temperature or even high irradiance load at soil level may be irrelevant to the germination performance of seeds covered by soil. Similar to our results, climatic differences between open and shaded sites were not sufficient either to promote diferente germination responses of *Palicourea marcagravii* and *Psychotria hoffmansegiana* seeds in a Cerrado area (Araújo and Cardoso, 2007) or of *Psychotria limonensis* and *Psychotria simiarum* in a tropical rain forest (Paz et al., 1999).

Young plants of *S. camporum* are frequently observed at the edge of Cerrado vegetation fragments, but our hypothesis that such success might be attributed to an increased germinative performance in gap (or edge) conditions was not confirmed. However, young plants of *S. camporum* show greater photosynthetic performance at the edge of vegetation fragments and under full sunlight when compared to shaded environments (Habermann et al., 2011; Habermann and Bressan, 2011). Therefore, based on this knowledge and on the results presented here, we concluded that gap conditions may be advantageous for this species because of the photosynthetic performance and vegetative growth of recently-emerged seedlings, and not because of its seed germinative performance.

Although we could not support our hypotheses, we were able to show that seeds of the three congeneric *Styrax* species are tightly tuned to the environment in which the adult plants of *S. ferrugineus*, *S. camporum* and *S. pohlii* are found. Thus, since seeds of each of the three species are able to promptly germinate in the four experimental conditions studied, the fact of not observing their seedlings in Cerrado physiognomies lead us to suppose that the environmental factors we studied exert stronger effects on later processes of recruitment (growth and survival) of these species, and not on early processes, such as the germinative performance.

Acknowledgements

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Figures
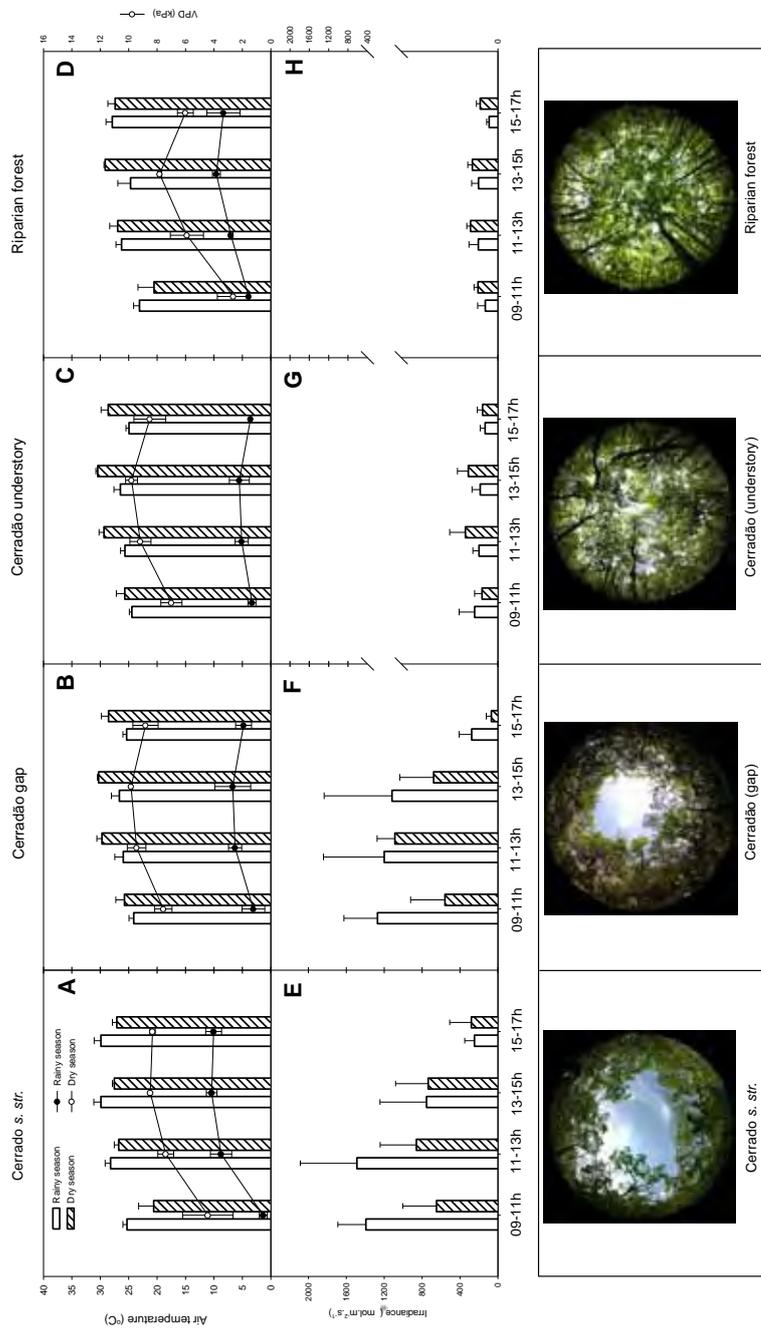
Figure 1. Daily variations of the air temperature (columns), vapor pressure deficit, VPD (lines) (A-D) and irradiance (E-H) measured in a cerrado *s. str.* (A and E), gap (B and F) and understory (C and G) conditions of a cerradão, and an understory condition of a riparian forest fragment, which are located in southeastern São Paulo state, Brazil. Data were collected on unclouded typical days from the rainy (February/2010) and dry (August/2010) seasons. A representative hemispherical photograph of each vegetation canopy is shown. Vertical bars = SD.

The breaks observed in the y-axis of irradiance data in cerradão understory and riparian forest are omitting values from 60 to 400 $\mu mol.m^{-2}.s^{-1}$.
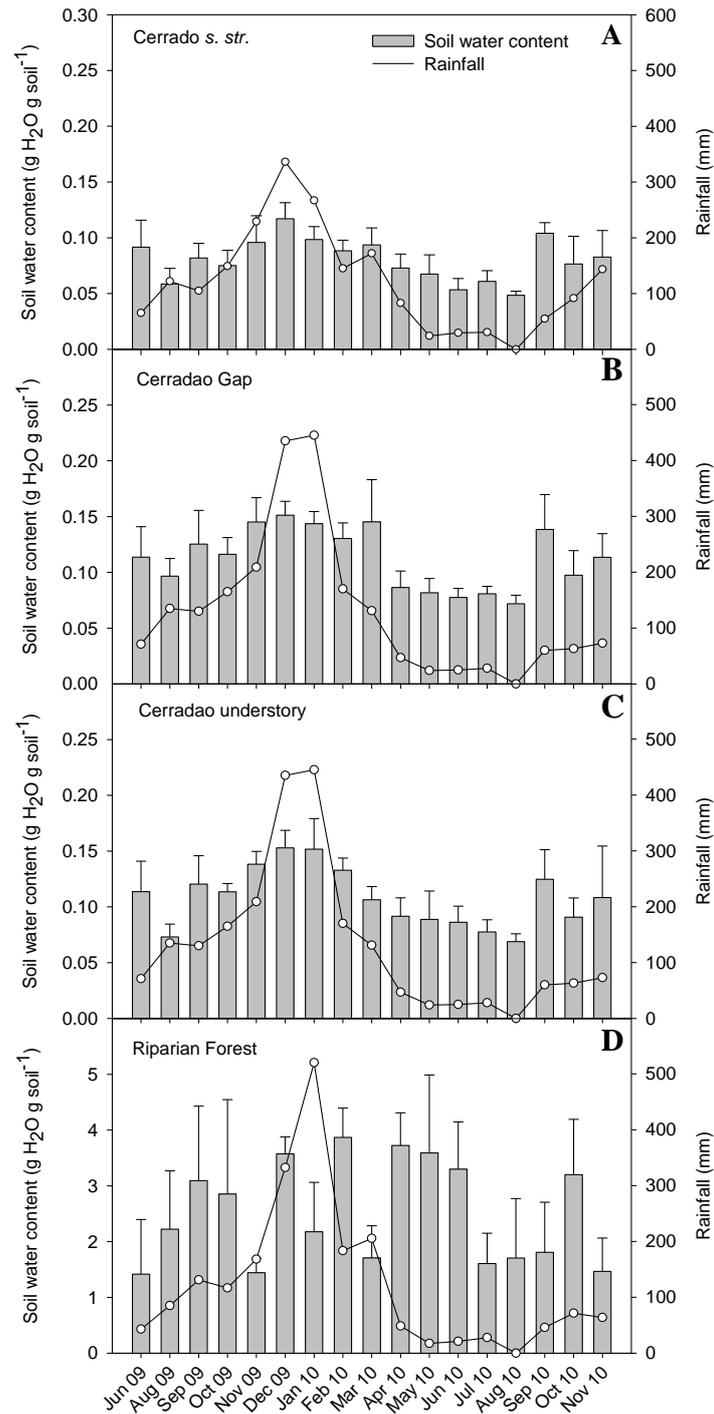
Figure 2. Monthly rainfall (lines) and mean values (*n = 5*) of the soil water content (columns) measured in a cerrado *s. str.* fragment (A), gap (B) and understory (C) conditions of a cerradão, and a riparian forest (D) fragment, located in southeastern São Paulo state, Brazil. Data were collected from June/2009 to November/2010.
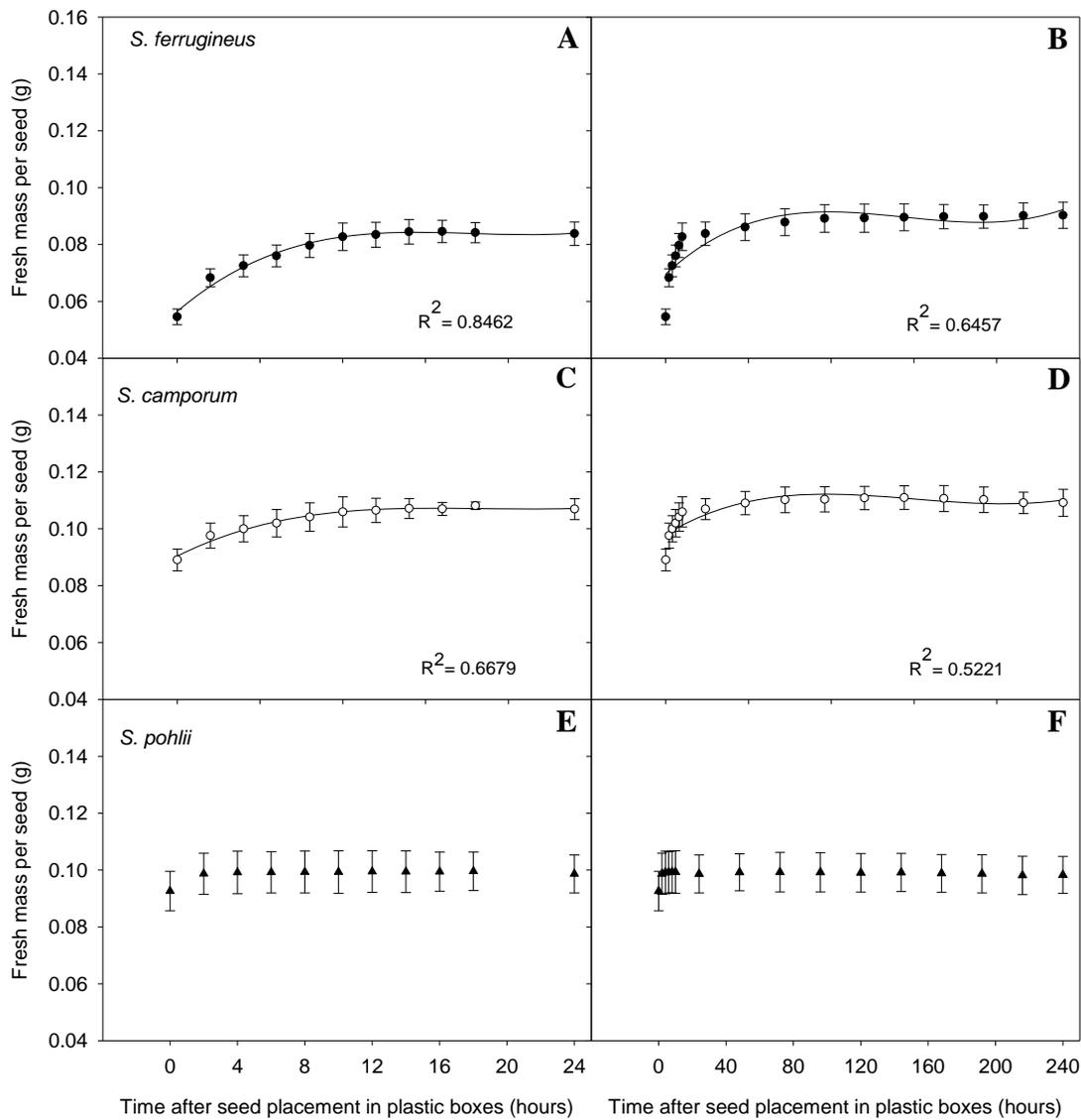
Figure 3. Individual readings (replicates; *n = 6*) of fresh mass per seeds of *Styrax ferrugineus* (A and B), *S. camporum* (C and D), and *S. pohlii* (E and F), incubated at 25°C under fluorescent light (80 μmol m^{-2} s^{-1}) for 24 (A, C and E) and 240 (B, D and F) hours, of an experimental period of 60 days. Data from the total experimental period (1440 h) was not shown due to a lack of detail visualization. Regression equations of imbibition curves are: y= 0.06 + (0.005 x) + (-0.0003 x^2) + (5 x 10^{-6} x^3) for seeds of *S. ferrugineus* imbibed for 24 hours and y= 0.07 + (0.0005 x) + (-4 x 10^{-6} x^2) + (0 x 10^{-9} x^3) for seeds of *S. ferrugineus* imbibed for 240 hours; y= 0.09 + (0.0029 x) + (-2 x 10^{-4} x^2) + (3 x 10^{-5} x^3) for seeds of *S. camporum* imbibed for 24 hours; and y= 0.10 + (0.0003 x) + (-2 x 10^{-6} x^2) + (6 x 10^{-9} x^3) for seeds of *S. camporum* imbibed for 240 hours. Bars = SD. Only regression equations with R^2 > 0.5 are described.
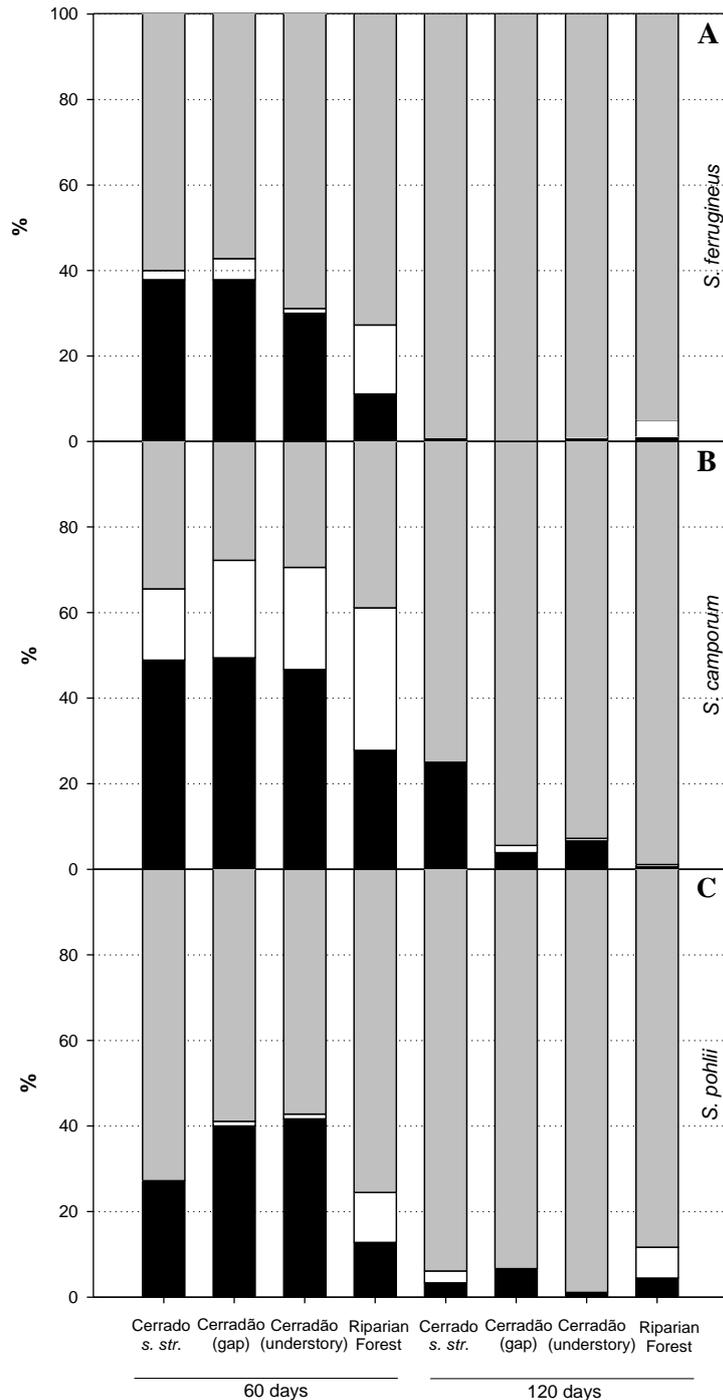
Figure 4. Percentage of germinated (black columns), viable (white columns), and damaged (gray columns) seeds of *Styrax ferrugineus* (A), *S. camporum* (B), and *S. pohlii* (C) buried for 60 and 120 days in a cerrado *sensu stricto* (*s. str.*) fragment, in gap and understory conditions of a cerradão, and in a riparian forest fragment, in southeastern São Paulo state, Brazil.
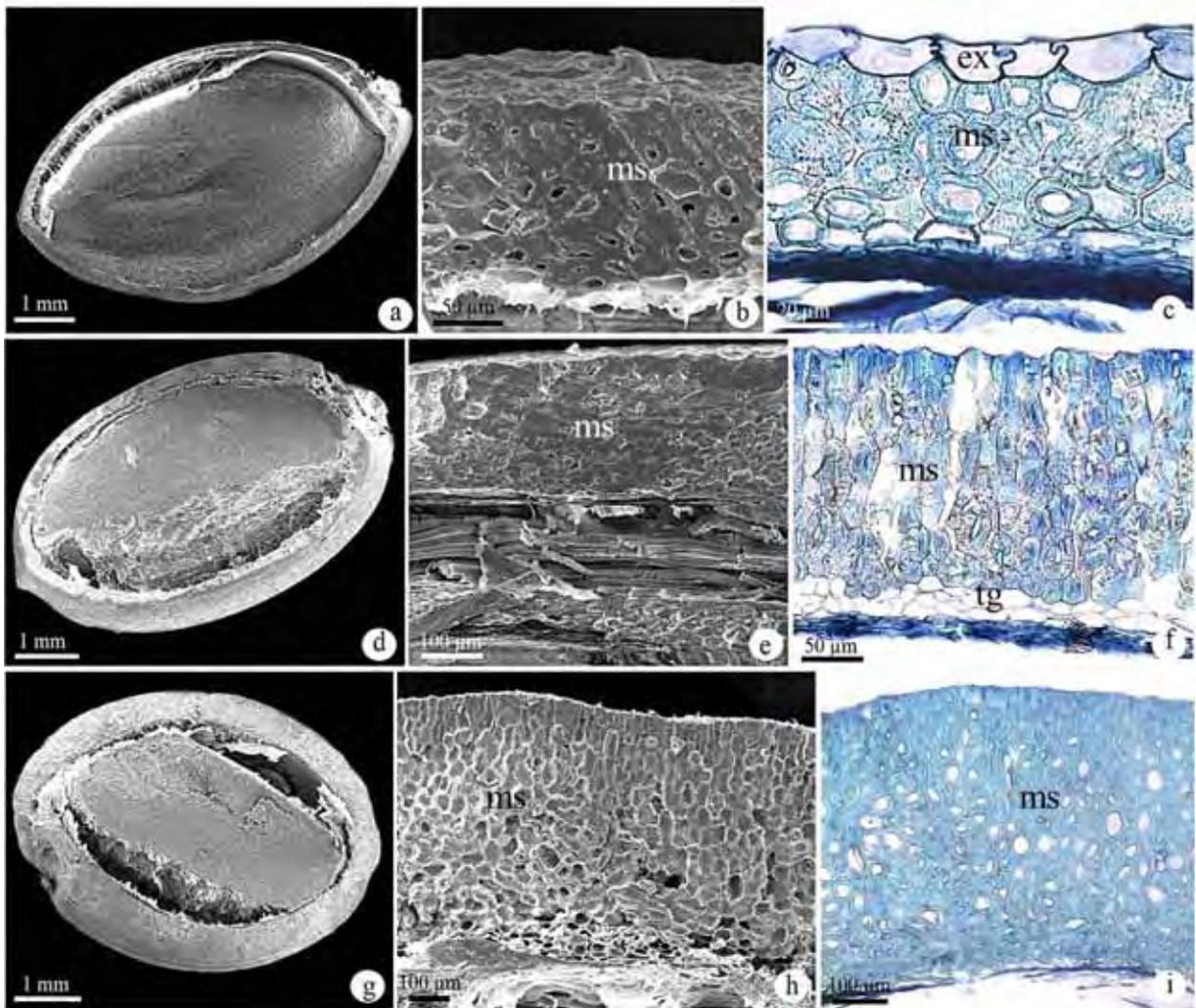
Figure 5. Photoelectronmicrographs (SEM), and photomicrographs of seeds of *Styrax pohlii* (a, b and c), *S. ferrugineus* (d, e and f), and *S. camporum* (g, h and i) species. a-c: *S. pohlii* general view in longitudinal section, LS (a), and seed coat details in transversal sections, TS (b – c); d-f: *S. ferrugineus*, general view in LS (d) and integument details in TS (e - f); g - i: *S. camporum*, general view in LS (g) and integument details in TS (h –i). ex = exotesta; ms = mesotesta; tg = tegmen.
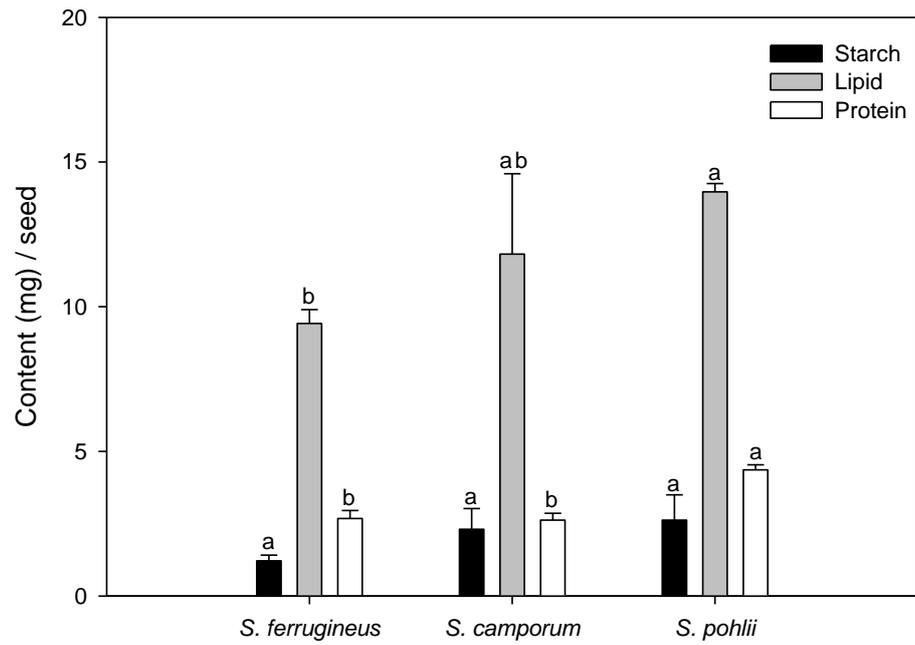
Figure 6. Mean values of total contents of starch, lipid and proteins found in seeds of *Styrax ferrugineus* (*n = 34 seeds*), *S. camporum* (*n = 36 seeds*) and *S. pohlii* (*n = 16 seeds*) species. For each seed content reserve, different letters indicate significant differences (P < 0.05) among species. Vertical bars = SD.

Capítulo 3

ECOPHYSIOLOGICAL PERFORMANCES AND PLANT GROWTH OF CONGENERIC SPECIES STUDIED IN RECIPROCAL PLANTING IN CONTRASTING PHYSIOGNOMIES OF THE CERRADO AREAS IN BRAZIL[3]

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Ecophysiological performances and plant growth of congeneric species studied in reciprocal planting in contrasting physiognomies of the Cerrado areas in Brazil

Summary

1. *Styrax* L. includes species with intriguing distribution patterns in the Cerrado (Brazilian savanna). *Styrax ferrugineus* is well adapted to the Cerrado *sensu stricto* (*s. str.*), a savanna-type physiognomy. *S. camporum* is widely distributed in the Cerrado *sensu lato* (*s. l*) areas, as well as at the edge of forest physiognomies called Cerradão, and *S. pohlii* frequently occurs in moist and shaded environments, such as riparian forests.

2. Seedlings of these three species were reciprocally planted in a Cerrado *s. str.* fragment, in gap and understory conditions of a Cerradão and in a riparian forest, where the survival rates of each species were assessed six months after planting. The plant growth was measured over a one-year period, whereas leaf gas exchange and chlorophyll fluorescence parameters were measured at the end of the wet and dry seasons.

3. *S. ferrugineus* and *S. camporum* did not survive in the riparian forest, whereas *S. pohlii* was able to grow in every experimental site. *S. ferrugineus* and *S. pohlii* did not show greater growth or photosynthetic performances in their respective natural sites of occurrence when compared to the other three sites. *S. camporum*, however, showed great photosynthetic and growth performances in high-irradiance environments.

4. *Synthesis.* The high photosynthetic and growth performances of *S. camporum*, which benefited from forest gaps, may explain its occurrence as young trees at the edge of vegetation fragments, while as tall trees within those fragments. The reasons why *S. pohlii* and *S. ferrugineus* are frequently observed in forest and savanna environments,

respectively, could not be determined by gas exchange, fluorescence or growth parameters studied in reciprocal planting. These reasons seem to be multifactorial, and morphological traits are of greater significance as indicated by the literature.

Key words: biomass, Brazilian savanna, irradiance, CO_2 assimilation, seasonal drought, Styracacae

Introduction

The Cerrado has a wide variety of physiognomies, comprising open grasslands, savannas (the typical Cerrado *sensu stricto*, *s. str.*), woodlands and even gallery and riparian forests accompanying watercourses (Oliveira-Filho & Ratter 2002). When considered together, these physiognomies are referred to as Cerrado. The Amazonian and Atlantic forests border the Cerrado, and the lack of geographic barriers between these biomes enables the exchange of floristic elements (Oliveira-Filho & Ratter 2002; Ratter, Bridgewater & Ribeiro 2003). Consequently, the flora of the Cerrado *s.l.* areas was strongly influenced by these forests during the Cerrado formation (Oliveira-Filho & Ratter 1995).

However, the floristic composition, richness and density of species greatly differ between the Cerrado physiognomies (Bridgewater, Ratter & Ribeiro 2004; Lenza *et al.* 2011). These differences are possibly determined by unequal soil water availabilities (Assis *et al.* 2011), climatic conditions (Habermann *et al.* 2011a), and fire events (Geiger *et al.* 2011). Soil fertility has already been considered as one of the major determinants of the Cerrado vegetation, but it has been demonstrated that it is very similar among physiognomies (Haridasan 2008; Habermann & Bressan 2011).

The establishment of Cerrado forest species in savannas is constrained by the high air temperature and irradiance, low water availability and fire events (Hoffmann 2000;

Hoffmann, Orthen & Franco 2004). On the other hand, in forest environments the low irradiance is the most critical factor for the success of savanna species (Habermann *et al.* 2011a). In riparian and gallery forests, plant species must also be able to deal with waterlogged soils (Kissmann *et al.* 2012).

Styrax ferrugineus Ness & Mart., *S. camporum* Pohl. and *S. pohlii* A. DC. show distinct distribution patterns in Cerrado *s.l.* areas. *Styrax ferrugineus* occurs exclusively in the Cerrado *s. str.*, which is a savanna-type physiognomy with scattered shrubs and trees, and a herbaceous understory, receiving high irradiation load at the soil level. *Styrax camporum* is widely distributed in Cerrado *s.l.* areas, being observed as tall trees within forest physiognomies termed Cerradão (the augmentative of 'Cerrado', in Portuguese). Young plants of *S. camporum* are noticed at the edge of Cerradão remnants, as it is a shade-intolerant species (Habermann *et al.* 2011a). *Styrax pohlii* is a forest species, occurring in riparian forests, a moist and closed-canopy environment where trees reach 20 m in height.

Sunlight penetration and soil water availabilities are clearly different among these Cerrado physiognomies (Kissmann *et al.* 2012), which may drive ecophysiological performances, as assessed by gas exchange and fluorescence responses (Habermann *et al.* 2011a). Shade-intolerant species, for instance, may show photoinhibition under excessive irradiance (Walters 2005). Thus, ecophysiological performances may indicate the fitness of species to an environment, although plant growth and reproduction may be of higher significance (Ackerly *et al.* 2000). In a previous study (Kissmann *et al.* 2012), however, we demonstrated that the high frequency of *S. camporum* at the edge of forest fragments is not related to germination performance, and we raised the hypothesis that this specific occurrence might be related to photosynthetic performances and/or vegetative growth.

We assessed the growth and photosynthetic performances of young plants of these three congeneric species that were reciprocally planted in Cerrado physiognomies with contrasting irradiance and soil water availabilities. Keeping in mind that *S. pohlii* and *S. ferrugineus* exhibit non-plastic environment-specific traits, such as long initial shoot and root lengths, respectively (Habermann & Bressan 2011), we also hypothesized that *S. pohlii* exhibits great growth performance if planted in the riparian forest, and *S. ferrugineus* in the Cerrado *s. str.* We discuss the occurrence of these species based on physiological parameters.

Material and Methods

Site description

This study was performed in three different Cerrado physiognomies. A Cerrado *s. str.* fragment (260 ha; 22º13S, 47º53W; 730 m of altitude), a Cerradão fragment (38.7 ha; 22º15S, 47º00W; 860 m of altitude), and a riparian forest remnant (32 ha; 24º00S, 47º32W; 660 m of altitude) were chosen in the respective municipalities of Itirapina, Corumbataí and Rio Claro (Ajapi District), southern São Paulo State, Brazil.

In the Cerradão fragment, in addition to the understory conditions, six oval-shaped gaps were opened, providing an illuminated area of approximately 25 m^2 on the ground. For this, trees exhibiting trunks with up to 3 cm in diameter were eliminated, and higher branches of larger trees were pruned not to shade the middle of the artificial gap. These gaps were monitored every other month throughout the study, and managed whenever necessary.

Plant material and experimental description

Seedlings of each of the three *Styrax* species were obtained through germination of seeds that were harvested from adult plants in the same Cerrado physiognomies described above, one year prior to the reciprocal planting. Thus, ten month-old plants (20 ± 5 cm in height) of each of the three species were reciprocally planted in the Cerrado *s. str.* fragment, in gap and understory conditions of the Cerradão fragment, and in the understory of the riparian forest remnant. *S. pohlii* and *S. ferrugineus* seedlings were planted right after their respective fruit dispersion times in the field, so that the former was planted at the end of the wet season (February/2011), and *S. ferrugineus* at the beginning of the wet season (October/2011). *Styrax camporum* seedlings, however, were planted in February/2011, and not between June and September 2011, when its fruits were dispersed, as this procedure would potentially increase the mortality rate during the dry season. As well as assuring that each species could be compared after 12 months, we also wanted to increase the chance of plant survival, since equal experimental times for each species were our main concern. In addition, considering distinct challenges and specific needs for each of the three species in the plant nursery, we were able to use 24 plants of *S. camporum,* 25 of *S. pohlii* and 22 of *S. ferrugineus* in each experimental site, which were the most vigorous ones.

The vapor pressure deficit (VPD) and air temperature of each of the four experimental environments were measured every 30 min from 9:00 to 17:00h using an aspirated psychrometer (SK-RHG, Sato, Tokyo, Japan), on typical unclouded days of the rainy (February) and dry (August) seasons of 2012. The photosynthetic photon flux density (PPFD) of the four experimental sites was also obtained during these same hours and seasons, using a quantum sensor (9901-013, LI-COR, Lincoln, NE, USA).

Monthly rainfall (mm) over the experimental period (Feb/2011 – Oct/2012) was provided by weather stations located at the Centro de Recursos Hídricos e Ecologia Aplicada (USP, São Carlos, SP), which is 18 km away from the Cerrado *s. str.* fragment; at the Iracema farm (Corumbataí, SP), which is located 3 km away from the Cerradão fragment; and at the Centro de Análise e Planejamento Ambiental (CEAPLA - Unesp, Rio Claro, SP), which is 12 km away from the riparian forest remnant.

Growth performance and biomass

Each plant from each of the three species had height (cm), root collar diameter (mm) and leaf number assessed before planting (0 months after planting – MAP). Subsequently, at six, nine and 12 MAP, these parameters were measured in individuals planted in each of the four experimental sites. Plant survival rate was assessed six MAP for each of the three species planted in each of the four sites. After these six months, four randomly chosen plants per each of the four experimental sites had biomass (g) of organs (stems plus petioles, and leaves) gravimetrically assessed, after oven-drying (60°C) the samples until constant mass.

Leaf gas exchange and chlorophyll a fluorescence measurements

Leaf gas exchange and chlorophyll *a* fluorescence were measured by using an open gas exchange system attached to a fluorometer, LI-6400xtr (LI-Cor, Lincoln, NE, USA). Measurements were performed on fully expanded leaves of eight replications (plants) per species in each of the four experimental sites, at the end of the wet (February) and dry (August) seasons of 2012. Therefore, *S. pohlii* and *S. camporum* had gas exchange and fluorescence results obtained at 12 and 18 MAP, whereas *S. ferrugineus* had these parameters measured at six and 12 MAP. These plants were assessed between

9:00 and 11:30h (Prado *et al.* 2004; Feistler & Habermann 2012), because obtaining the most significant photosynthetic response in the midmorning was our main interest.

The PPFD inside the leaf cuvette was provided by an artificial red (90%)-blue (10%) LED light source (6400-02B, LI-Cor, USA) that was set to similar average values measured under natural sunlight conditions at each of the four sites (Fig. 2b). Thus, the PPFD used when measuring plants in the Cerrado *s. str.* was 250 μmol photons m^{-2} s^{-1}, 900 μmol m^{-2} s^{-1} in gap conditions of the Cerradão, and 50 μmol m^{-2} s^{-1} in the understory of the Cerradão and the riparian forest. The VPD in the leaf cuvette was allowed to vary with the external environment, and leaves underwent the same VPD during measurements, which were registered only under stable conditions (CV% ≈ 1%). The CO_2 assimilation (*A*, μmol m^{-2} s^{-1}) and transpiration (*E*, mol m^{-2} s^{-1}) rates, as well as the stomatal conductance (*gs*, mmol m^{-2} s^{-1}) were calculated using the LI–6400xtr software.

For measurements of maximum quantum yield of photosystem II (PSII) [*Fv/Fm* = (Fm-Fo)/Fm], leaves were dark-adapted for 30 min (Bolhàr-Nordenkampf & Öquist 1993) using silver foil. For this parameter, Fm and Fo represent the maximum and minimum fluorescence of dark-adapted leaves, respectively. The effective quantum yield of PSII, or ΦPSII [*ΔF/Fm'* = (Fm'-Fs)/Fm'], apparent electron transport rate [*ETR* = (ΔF/Fm') PPFD 0.5 0.84], and photochemical quenching [qP = (Fm'-Fs)/(Fm'-Fo')] were measured on different light-adapted leaves and for these variables, Fm' and Fs are the maximum and steady state fluorescence values obtained in the light-adapted state, respectively, while Fo' represents the minimum fluorescence after a pulse of weak far-red light, which excites photosystem I, or PSI (Li-Cor 2004; Baker 2008).

Data analysis

Leaf and shoot biomass were obtained from four randomly selected individuals, whereas plant height, root-collar diameter and leaf number were assessed from all alive plants, being both group of parameters obtained from each of the three species in each of the four sites. For each species, a one-way analysis of variance (ANOVA) was performed to test differences between environments at each evaluation time.

The leaf gas exchange (*A*, *gs* and *E*) and chlorophyll fluorescence (Fv/Fm, Φ_{PSII}, ETR and qP) were assessed using eight randomly selected replications per each of the three species planted in each of the four sites. Because no plant of *S. camporum* and *S. ferrugineus* survived in the riparian forest after one year, a two-way ANOVA was performed to test the effects of species (three levels), environments (Cerrado *s. str.*, gap and understory conditions of the Cerradão only) and their interactions, and another one-way ANOVA was carried out, separately, to test differences in photosynthetic performances of *S. pohlii* between each of the four environments. The mean results were compared using Tukey's test (P < 0.05).

Results

Monthly rainfall was quite similar among the three locations, but during some months of the wet seasons it rained more in the Cerradão in comparison to the other two sites. In general, 2012 was wetter than 2011, mainly during the dry season (Fig. 1).

The air temperature was similar among the four experimental sites (25 ± 5°C), being higher in the dry when compared to the wet season for both conditions of the Cerradão; the opposite occurred for the Cerrado *s. str.* (T°C wet > T°C dry season), and similar temperatures were found between both seasons in the riparian forest. The VPD was considerably low in the wet and very high in the dry season, especially in the

Cerrado *s. str.* and in both conditions of the Cerradão (Fig. 2a). Greater irradiance was observed in the Cerrado *s.str.* and in gap conditions of the Cerradão in comparison to the understory of the Cerradão and the riparian forest (Fig. 2b).

Styrax pohlii plants showed high survival rates in every experimental site, whereas *S. camporum* and *S. ferrugineus* showed lower survival in shaded environments, such as the understory of the Cerradão and the riparian forest, when compared to more illuminated environments (Cerrado *s. str.* and gap conditions of the Cerradão). *Styrax ferrugineus* did not survive the wet and shaded environment in the riparian forest fragment (Fig. 3).

After six months in the field, *S. pohlii* and *S. ferrugineus* plants exhibited similar shoot and leaf biomasses regardless of the sites where these plants grew (Fig. 4a,d,c,f). In contrast, *S. camporum* plants that grew in the Cerrado *s. str.* and in gap conditions of the Cerradão showed conspicuous high values of shoot and leaf biomasses when compared to plants of this species that grew in the riparian forest and understory conditions of the Cerradão. However, because of the variability presented by the results of these parameters there were no differences when mean values were compared (Fig. 4b,e).

A great variability in plant height, root-collar diameter and leaf number of *S. pohlii* was observed throughout the study. In general, at 12 MAP these parameters were higher in *S. pohlli* plants that grew in the Cerrado *s.str.* and in the riparian forest (Fig. 5a,d,g). In contrast, *S. camporum* plants exhibited differences in plant height and root-collar diameter only after 12 MAP, when these values were higher in plants that grew in gap compared to understory conditions of the Cerradão, but similar to those values measured in plants that grew in the Cerrado *s.str.* (Fig. 5b,e). At this same time (12 MAP), a significantly larger number of leaves was noticed in *S. camporum* plants that grew in gap conditions of the Cerradão (Fig. 5h). *Styrax ferrugineus* showed similar

values of these three parameters, regardless of the sites where the plants of this species grew (Fig. 5c,f,i).

In general, in the wet season, *A*, *gs* and *E* measured in plants of the three species were higher in the Cerrado *s. str.* and in gap conditions of the Cerradão when compared to understory conditions of the Cerradão. For *A* and *E*, there were significant interactions between the habitat and the species factors (Fig. 6a,e), but this interaction was not observed for *gs* (Fig. 6c) and, therefore, *gs* values between the three species cultivated in each of the three sites (excluding the riparian forest) were statistically similar. In the case of no interaction, *gs* values measured in *S. camporum* and *S. ferrugineus* plants that grew in the gap conditions of the Cerradão were higher than *gs* values obtained in these respective plant species cultivated in understory conditions of the Cerradão; also, *gs* values observed in each of these two species cultivated in the Cerrado *s. str.* were intermediate, being similar to *gs* values of each of these species in the other two sites.

The most conspicuous result was the significantly higher gas exchange performance of *S. camporum* in gap conditions of the Cerradão, when compared to the other two species planted on this site. For *S. pohlii*, *gs* and *E* values were significantly higher (P > 0.05) in the riparian forest when compared to the other three experimental sites, where plants presented the same *gs* and *E* values (Fig. 6c,e). On the other hand, *S. pohlii A* values were higher (P < 0.05) in the gap compared to the understory conditions of the Cerradão, and there were intermediate *A* values (P > 0.05) that were detected in *S. pohlii* plants grown in the Cerrado *s. str.* and in the riparian forest (Fig. 6a).

The general responses of high *A*, *gs* and *E* observed in the wet season for plants that grew in the Cerrado *s. str.* and in gap conditions of the Cerradão could not be repeated in the dry season. For *gs* and *E*, there were significant interactions between the habitat and species factors (Fig. 6d,f), but this interaction was not observed for *A* values

(Fig. 6b) and, therefore, *A* values were similar between the three species regardless of the experimental sites where the plants grew. In this case, *A* values measured in *S. camporum* and *S. ferrugineus* plants that grew in gap conditions of the Cerradão were higher ($P < 0.05$) than those values obtained from these respective plant species that were cultivated in understory conditions of the Cerradão and in the Cerrado *s. str.*, where plants of these respective species had the same ($P > 0.05$) *A* values. For *S. pohlii*, carbon assimilation and transpiration rates were the same ($P > 0.05$) regardless of the experimental sites where plants of this species grew (Fig. 6b,f). However, even in the dry season, in gap conditions of the Cerradão, *S. camporum* showed higher values of *A*, *gs* and *E* when compared to *S. pohlii*, and this gas exchange performance was intermediate for *S. ferrugineus*, which had similar values of these parameters in relation to the other two species (Fig. 6b,d,f). *Styrax pohlii* had the same *gs* response pattern as noted in the wet season, i.e, plants cultivated in the riparian forest showed higher *gs* when compared to the other three sites (Fig. 6d).

In both seasons, Fv/Fm was similar between plant species in each of the three sites, and each species also had the same Fv/Fm when comparing between the three sites, or the four sites if considering *S. pohlii* plants (Fig. 7a,b).

For wet and dry seasons, plants cultivated in understory conditions of the Cerradão exhibited higher ($P < 0.05$) ΦPSII and qP in comparison to plants that grew in the Cerrado *s. str.*, where plants showed still greater ($P < 0.05$) values of these two parameters in relation to plants grown in gap conditions of the Cerradão (Fig. 7c,d,e,f). Consequently, for both seasons, ETR was higher ($P < 0.05$) in plants cultivated in gap conditions of the Cerradão when compared to plants cultivated in the Cerrado *s. str.*, where plants exhibited still greater ($P < 0.05$) ETR values in relation to plants that grew in understory conditions of the Cerradão (Fig. 7g,h).

In gap conditions of the Cerradão, *S. camporum* plants showed the highest ETR values among the three species, especially in the wet season (Fig. 7g,h). In addition, during the dry season, *S. pohlii* plants showed ΦPSII and qP values that were similar ($P > 0.05$) between plants that grew in the riparian forest and understory conditions of the Cerradão (Fig. 7d,f). But in the wet season, *S. pohlii* plants cultivated in understory conditions of the Cerradão showed higher ($P < 0.05$) ΦPSII and qP in comparison to plants of this species grown in the riparian forest (Fig. 7c,e).

Discussion

The most striking results of the present study arose in the survival rates (Fig. 3), and in the aboveground biomasses (Fig. 4), which were evaluated six MAP, and the latter seemed to hold cause-and-effect relationships with gas exchange results (Fig. 6). We demonstrated that the irradiance was considerably lower in the understory of the Cerradão and the riparian forest when compared to the other two sites (Fig. 2). However, previous studies (Habermann *et al.* 2011a; Kissmann *et al.* 2012) conducted exactly in the same places in 2010 and 2011 showed that the water content in the soil of the riparian forest fragment is more than 20 times greater than in the soil of the other two sites. Therefore, low irradiance and high soil water content are important constraints on the occurrence of *S. ferrugineus* and *S. camporum* in riparian forests. Due to the high organic composition of the soil in the riparian forest fragment (Habermann & Bressan 2011), we observed that *S. ferrugineus* seedlings were not able to fix their roots onto that soil, whereas *S. camporum* plants survived in that environment until nine MAP, and *S. pohlii* until the end of the study. In fact, most of the Cerrado species do not tolerate waterlogged soils (Amorim & Batalha 2006) or shaded environments (Hoffmann, Orthen & Franco 2004).

Styrax pohlii exhibits shade-adapted traits, such as long shoot and short root initial lengths, increased leaf area (LA) and specific leaf area (SLA), and consequently, high mass-based CO_2 assimilation rates, A_{mass} (Habermann & Bressan 2011). These traits have already been described for many Cerrado woody species (Franco *et al.* 2005) and general relationships between savanna and forest species of the Cerrado *s. l.*, in which such traits are reinforced for forest species (Scholz *et al.* 2008), suggest that these traits must have enabled *S. pohlii* plants of the present study to survive shaded conditions in the riparian forest. One should also note that *S. pohlii* was able to benefit from the wet soil, as *gs* was markedly higher in plants of this species cultivated in the riparian forest in relation to *S. pohlii* plants cultivated in the other three sites (Fig. 6c). Even when seedlings of these three species could be compared in a riparian forest, a few months after planting (Habermann *et al.* 2011a), *S. pohlii* showed higher *gs* values when compared to the other two species. Furthermore, we have data demonstrating that *S. pohlii* plants are able to rapidly recover gas exchange rates after flooding periods.

On the other hand, such high *gs* values observed in *S. pohlii* plants cultivated in the riparian forest fragment (Fig. 6c) did not result in similarly high *A* values (Fig. 6a) and, consequently, none of the plant development parameters (Fig. 5a,d,g) or plant biomass results (Fig. 4a,d) reflected any advantage for *S. pohlii* in the riparian forest over the other three experimental sites. The same can be described for *S. ferrugineus*. Although significantly higher than *S. pohlii* but similar to *S. camporum*, *A* values obtained from *S. ferrugineus* plants cultivated in the Cerrado *s. str.* were not astonishingly superior to the other two species in that environment (Fig. 6a). Similarly, none of the plant development parameters (Fig. 5c,f,i) or plant biomass results (Fig. 4c,f) reflected any advantage for *S. ferrugineus* in the Cerrado *s. str.* over the other three sites. These gas exchange results are in agreement with Habermann *et al.* (2011a), who

conducted short-period reciprocal planting in the field using the same three species. In addition, these data demonstrated that our hypotheses that *S. pohlii* and *S. ferrugineus* exhibit great initial growth performances if planted respectively in the riparian forest and in the Cerrado *s. str.* could not be confirmed. For us, the reasons why *S. ferrugineus* is a typical and well-adapted shrub to the Cerrado *s. str.* (Nakajima & Monteiro 1987), and *S. pohlii* is a very recurrent tree in riparian and gallery forests (Teixeira *et al.* 2011) of the Cerrado *s. l.* areas seem to be multifactorial, as morphological traits are of greater significance (Machado *et al.* 2005; Habermann & Bressan 2011).

Forest species, such as *S. pohlii*, allocate more resources to capture light, such as long stems with low wood density and high LA and SLA (Scholz *et al.* 2008; Habermann & Bressan 2011), whereas savanna species, such as *S. ferrugineus*, allocate more resources in the roots for capturing water (Hoffmann & Franco 2003; Franco *et al.* 2005; Habermann & Bressann 2011). Therefore, the low investment of *S. ferrugineus* in aboveground biomass (Fig. 4c,f) was not surprising, and long and deep roots of this species would be useless in riparian forests. *Styrax pohlii*, however, showed similar plant height in the Cerrado *s. str.* and the riparian forest (Fig. 5a). Thus, theoretically, *S. pohlii* would be able to invade savanna areas, but one should keep in mind that this species would not survive the Cerrado dry season with such short roots (Habermann & Bressan 2011) and without corked-bark protection (Machado *et al.* 2005; Habermann *et al.* 2011a) against fire events (Hoffmann, Orthen & Nascimento 2003; Geiger *et al.* 2011).

Nonetheless, *S. camporum* plants showed conspicuously great gas exchange rates when growing in high-irradiance environments in comparison to shaded environments, in the wet (Fig. 6a,c,e) and dry (Fig. 6b,d,f) seasons. Such performances were also reflected in ETR and ΦPSII values in plants of this species that grew in gap conditions of the Cerradão in the wet season (Fig. 7c,g) and also in qP and ETR values measured in the dry

season (Fig. 7f,h). *Styrax camporum* plants growing in gap conditions of the Cerradão were able to grow more (Fig. 5b) and produce more leaves (Fig. 5h) than the other two species. Moreover, although not significantly different, due to the variability of results, it was clear that this species gained more shoot (Fig. 4b) and leaf (Fig. 4e) biomasses in high-irradiance environments in relation to shaded ones. These results substantiate our hypothesis that the high frequency of young trees of *S. camporum* at the edge of forest fragments is strongly related to the photosynthetic light use efficiency and consequences for growth performances, rather than related to *S. camporum* seed germination performance, which was similar between gap and understory conditions of the Cerradão (Kissmann *et al.* 2012). In fact, some studies (Habermann *et al.* 2008; Habermann *et al.* 2011b; Feistler & Habermann 2012) demonstrate that the vertical static leaves that are observed in *S. camporum* plants sustain increased whole plant sunlight interception efficiency, as a photoprotective role for these leaves could not be confirmed (Feistler & Habermann 2012). In addition, our results definitely classify *S. camporum* as sun plants. While *S. camporum* takes advantage of high-irradiance conditions to vegetate and form canopies, *S. ferrugineus* grows into the ground (Habermann & Bressan 2011). Acclimation to high-irradiance environments provides a competitive advantage for species in heterogeneous light environments (Walters 2005), such as those found across the Cerrado *s.l.* areas. This may explain the occurrence of *S. camporum* in a wide range of Cerrado areas (Nakajima & Monteiro 1987).

Conclusion

Our results showed that *S. camporum* is able to benefit from occasional gaps opened in forest physiognomies of the Cerrado *s. l.*, and also explain its occurrence as young trees at the edge of vegetation fragments, and as adult tall trees within those

fragments. In addition, the reasons why *S. pohlii* and *S. ferrugineus* are frequently observed in forest and savanna environments, respectively, could not be determined by gas exchange, fluorescence or growth parameters studied using reciprocal planting methods. These reasons seem to be multifactorial.

Acknowledgements

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Figures
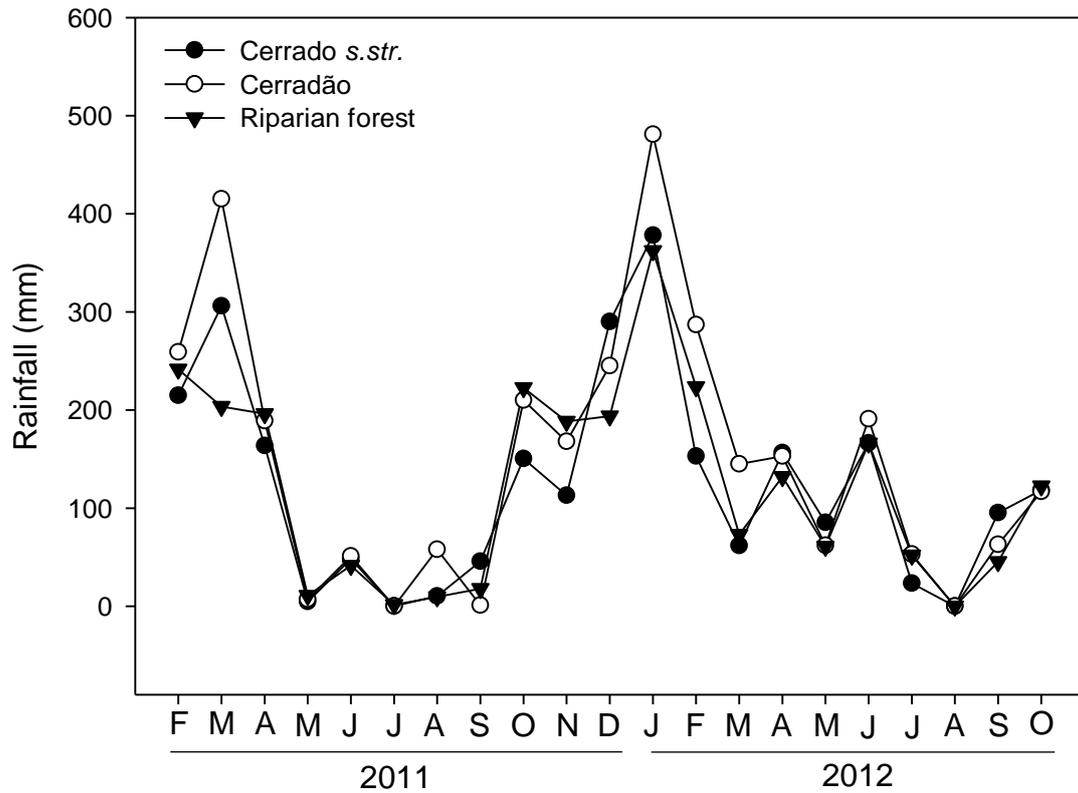
Fig 1. Monthly rainfall (February/2011 – October/2012) measured in a Cerrado *s. str.* (Itirapina municipality), a Cerradão (Corumbataí municipality) and a riparian forest (Ajapi district, Rio Claro municipality), southern São Paulo state, Brazil.
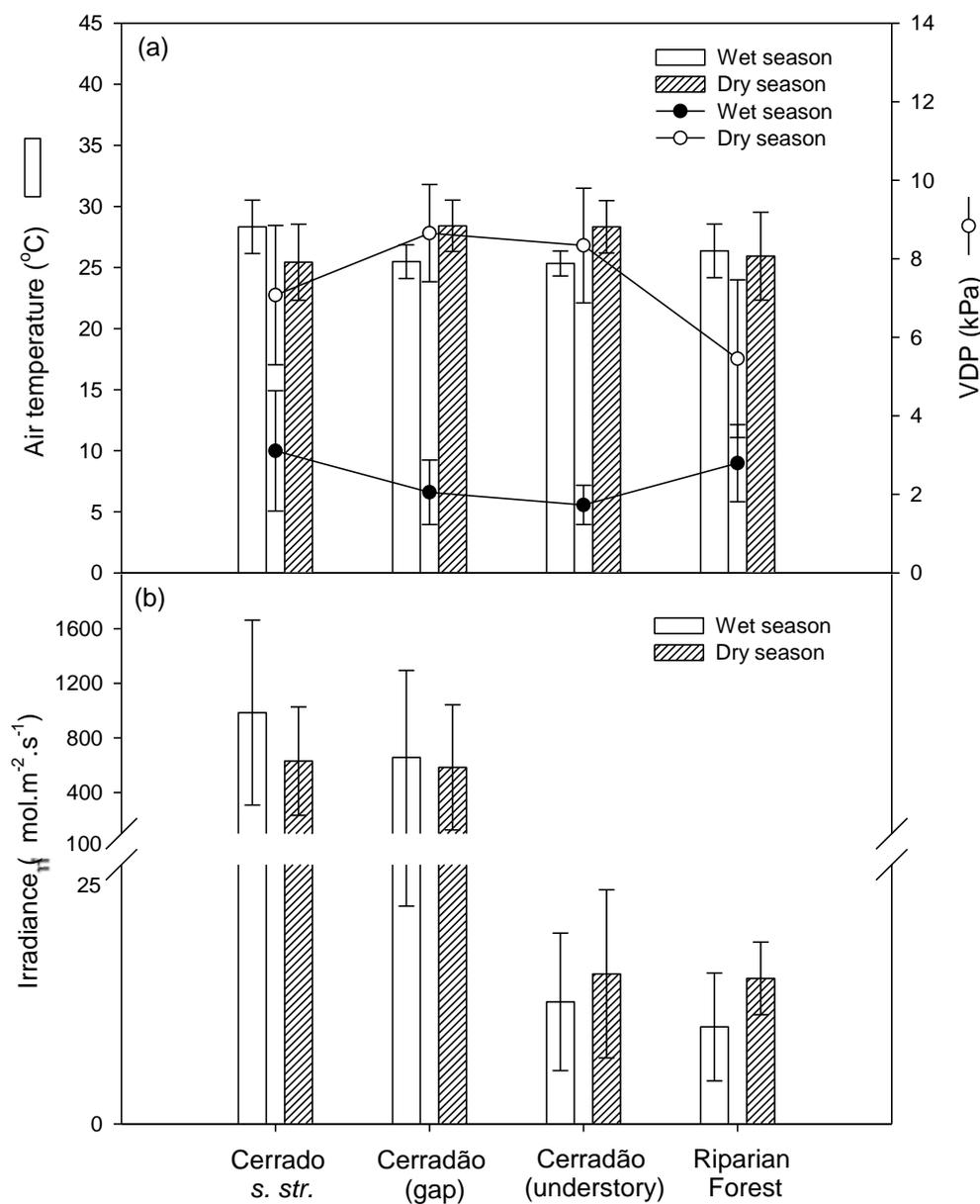
Fig. 2. Mean values (09:00 - 17:00h) of air temperature and vapor pressure deficit (VPD) (a), and irradiance (b) measured in a Cerrado *sensu stricto* (*s. str.*) fragment, gap and understory conditions of a Cerradão, and a riparian forest fragments, southern São Paulo state, Brazil. Vertical bars = SD.
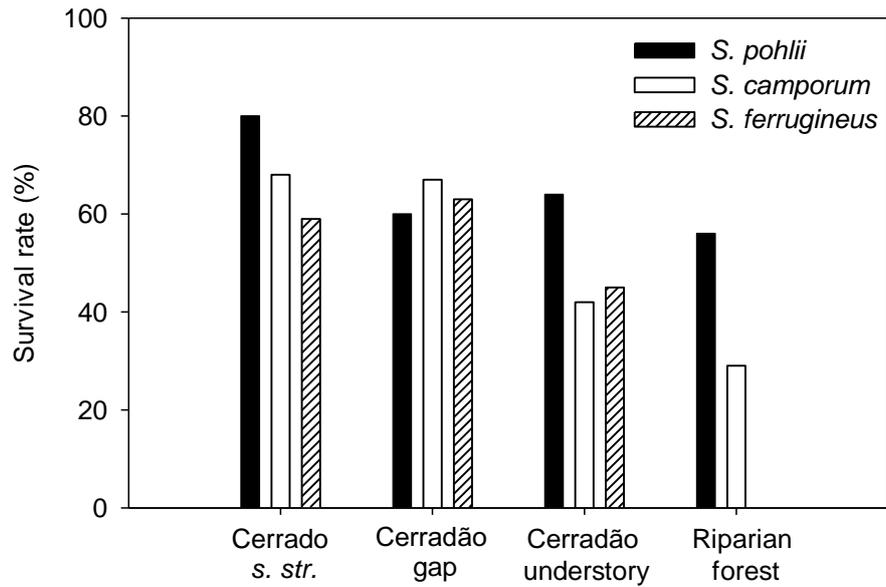
Fig. 3. Survival rate (%) of *S. pohlii* (*n* = 25 plants), *S. camporum* (*n* = 24 plants) and *S. ferrugineus* (*n* = 22 plants) six months after planting in a Cerrado *sensu stricto* (*s. str.*) fragment, in gap and understory conditions of a Cerradão, and in a riparian forest fragment, southern São Paulo state, Brazil.
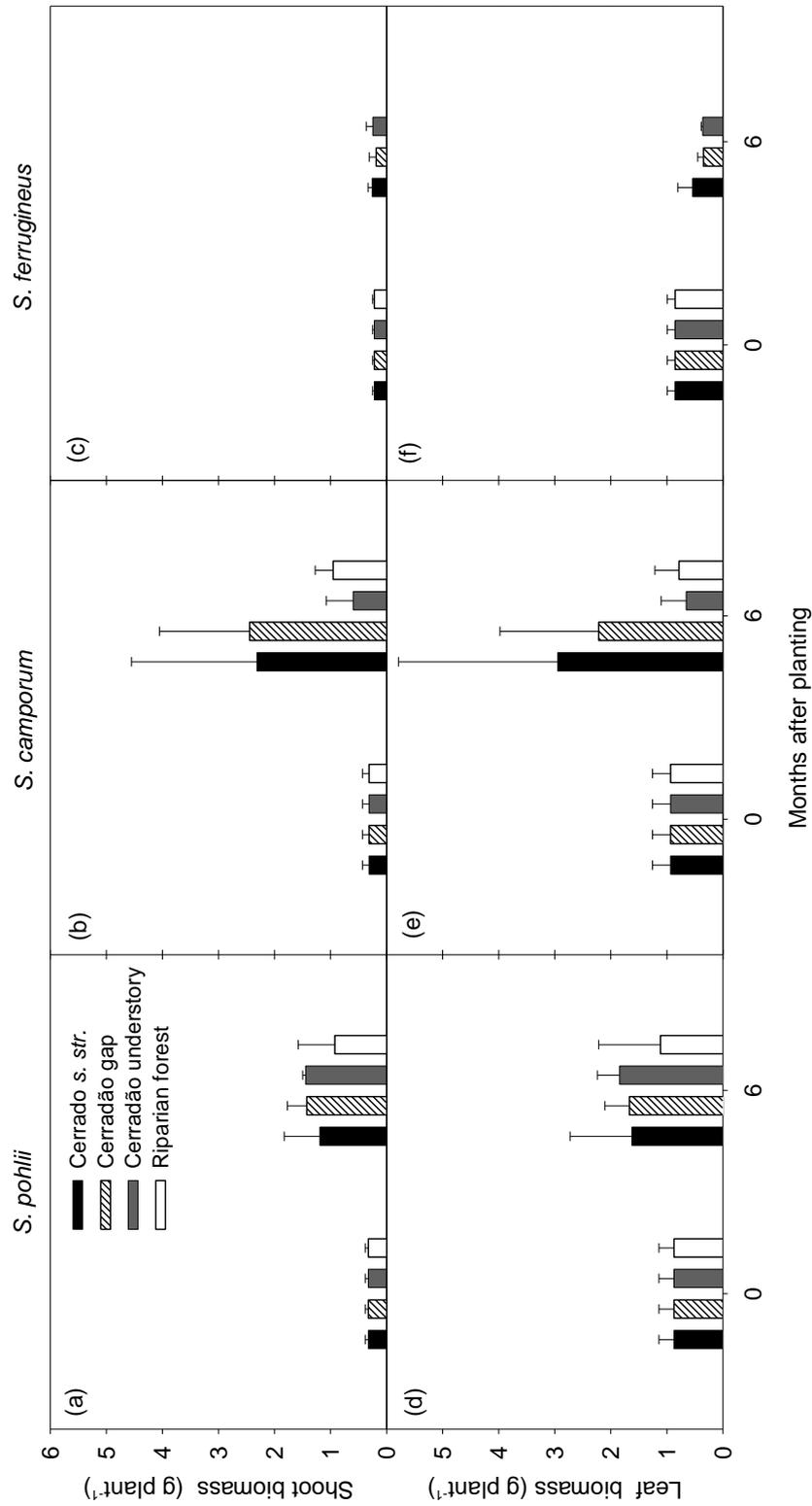
Fig. 4. Mean values ($n = 4$ plants) of shoot (a, b, c) and leaf (d, e, f) biomass of *S. pohlii*, *S. camporum* and *S. ferrugineus* planted in a Cerrado *sensu stricto* (*s. str.*) fragment, in gap and understory conditions of a Cerradão, and in a riparian forest fragment, southern São Paulo state, Brazil. The absence of letters indicates a lack of significant differences ($P < 0.05$) between sites for each species. Vertical bars = SD.
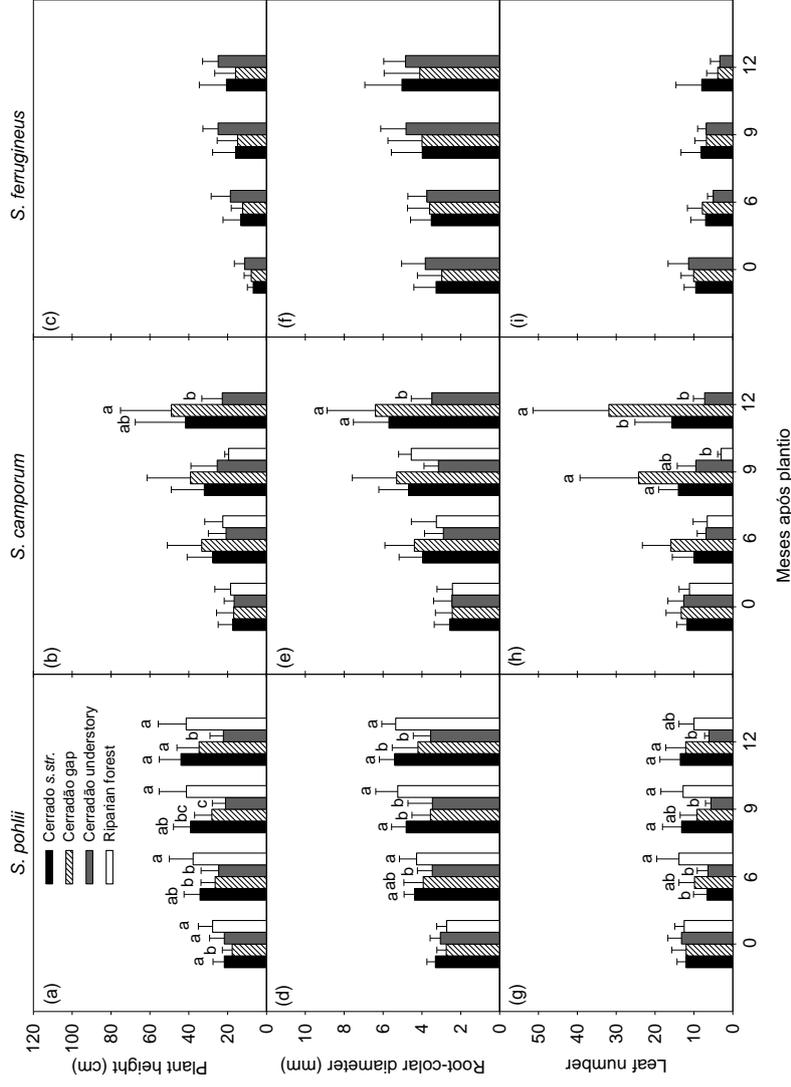
Fig. 5. Mean values of plant height (a,b,c), root-collar diameter (d,e,f) and leaf number (g,h,i) of *S. pohlii*, *S. camporum* and *S. ferrugineus* planted in a Cerrado *sensu stricto* (*s. str.*) fragment, in gap and understory conditions of a Cerradão, and in a riparian forest fragment, southern São Paulo state, Brazil. The same letters or absence of letters indicates a lack of significant differences (P < 0.05) between sites for each species. Vertical bars = SD.
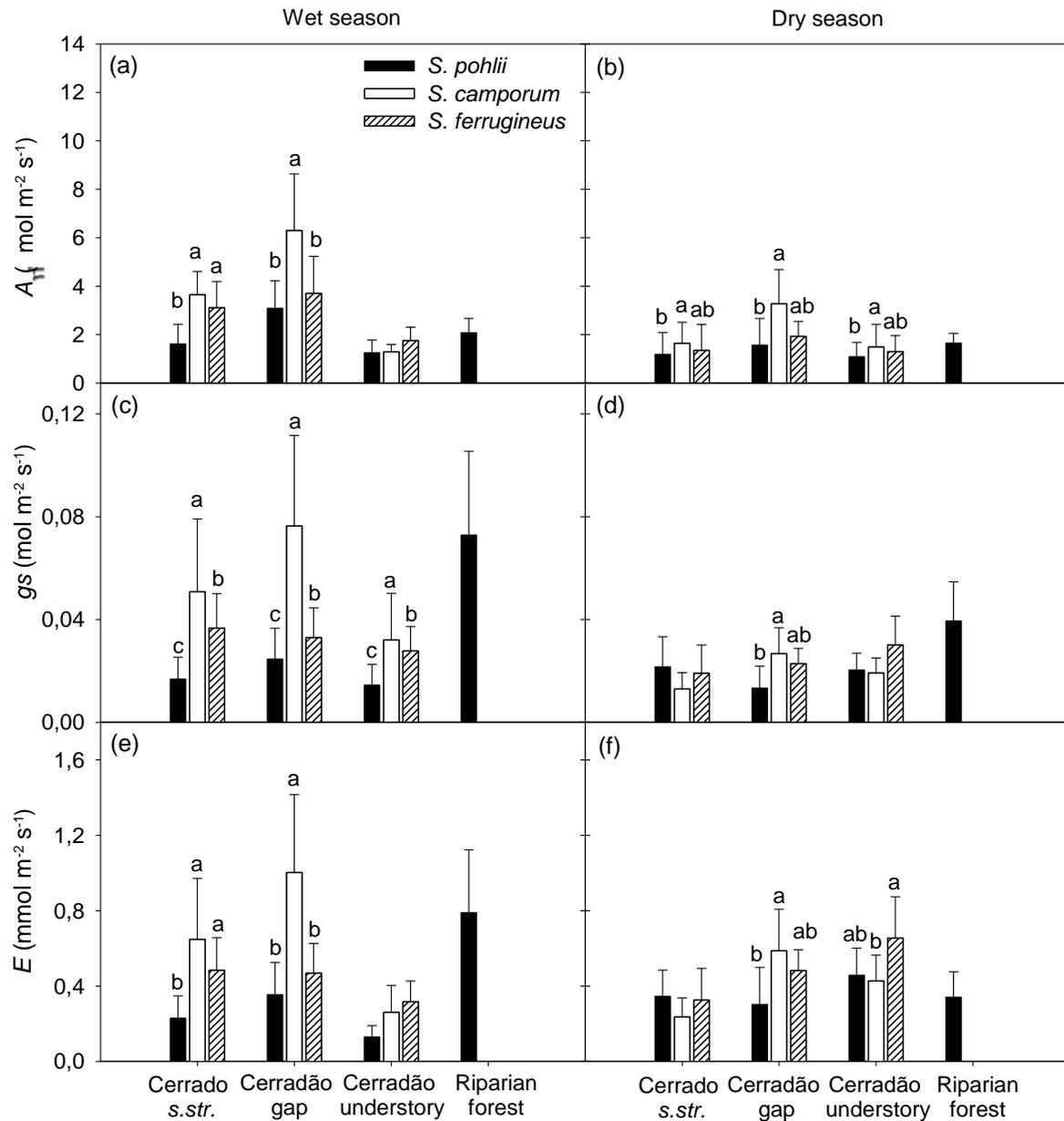
Fig. 6. CO_2 assimilation rates (a and b), stomatal conductances (c and d) and transpiration rates (e and f) of *S. pohlii*, *S. camporum* and *S. ferrugineus* planted in a Cerrado *sensu stricto* (*s. str.*) fragment, in gap and understory conditions of a Cerradão, and in a riparian forest fragment, southern São Paulo state, Brazil. The same letters or absence of letters indicates a lack of significant differences (P < 0.05) among species at the same site. Vertical bars = SD.
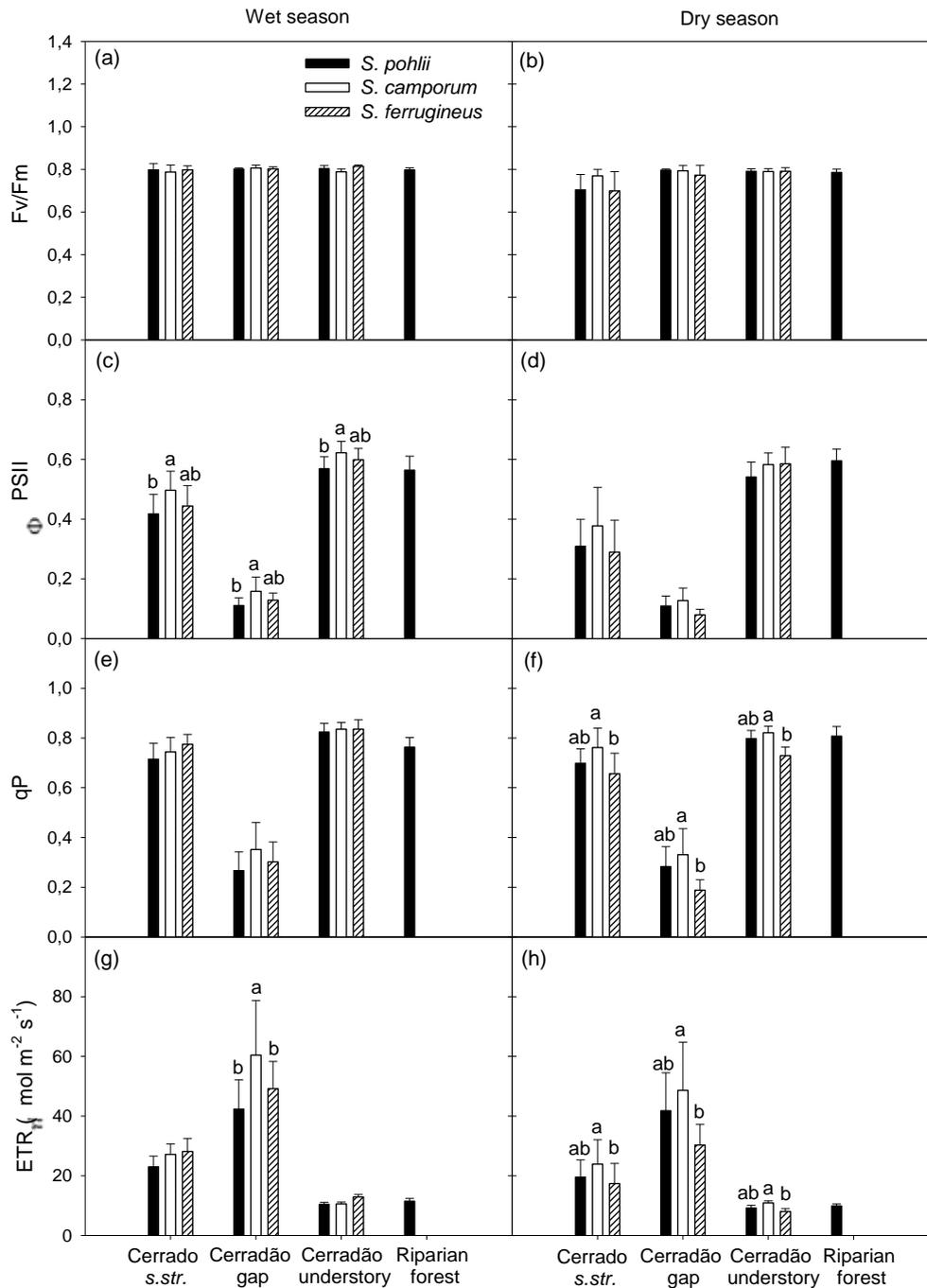
Fig. 7. Maximum (a and b) and effective (c and d) quantum yield of photosystem II (PSII), photochemical quenching (e and f) and electron transport rates (g and h) of *S. pohlii*, *S. camporum* and *S. ferrugineus* planted in a Cerrado *sensu stricto* (*s. str.*) fragment, in gap and understory conditions of a Cerradão, and in a riparian forest fragment, southern São Paulo state, Brazil. The same letters or absence of letters indicates a lack of significant differences ($P < 0.05$) among species at the same site. Vertical bars = SD.

Capítulo 4

MORPHOPHYSIOLOGICAL MECHANISMS MAY EXPLAIN THE OCCURRENCE OF *Styrax pohlii* IN RIPARIAN AND GALLERY FORESTS OF CERRADO AREAS IN BRAZIL[4]

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Morphophysiological mechanisms may explain the occurrence of *Styrax pohlii* in riparian and gallery forests of Cerrado areas in Brazil

Abstract

Photosynthetic parameters and growth were assessed in *Styrax pohlii* plants kept under flooding and unflooded conditions to better understand the success of this species in riparian and gallery forests. We checked whether plants develop adventitious roots with aerenchyma, and if they are physiologically capable of fast recovery from the flooding period. Potted plants were totally (water level at 3 cm above the soil surface) or partially flooded (water level at 15 cm below the soil surface) for 100 days, and subsequently recovered under soil field capacity conditions for 50 days. Another group of plants were maintained under soil field capacity conditions for 150 days. Leaf gas exchange and chlorophyll fluorescence were weekly measured until the end of the 150-day experimental period. Growth parameters were measured at 1, 50 and 100 days after flooding (DAF), and after the 50-day recovery period. Biomass of organs was measured at 0, 50 and 100 DAF. Adventitious roots started emerging from the collar-root at 15 DAF, but no aerenchyma was observed. Flooding conditions reduced growth and biomass, which was strongly related to reductions observed in CO_2 assimilation (A) and stomatal conductance (gs) at 25 DAF. Photochemical performances were not as affected by flooding conditions as gas exchange variables, which took longer to recover in comparison to photochemical parameters. Photosynthesis was not biochemically affected, as the A/Ci curve components remained unchanged. *S. pohlii* plants do not rely on adventitious roots with aerenchyma to survive seasonal flooding in the field, and this species is physiologically capable of fast recovery from flooding periods, suggesting an important

adjustment to flooding, which may explain its success in riparian and gallery forests within Cerrado areas.

Key words: Biomass, leaf gas exchange, photochemical performances, Styracaceae, waterlogged environments

1. Introduction

The Brazilian savanna, or the Cerrado vegetation, is comprised of a mosaic of physiognomies that vary from open fields to closed woodlands. But unlike the typical Cerrado vegetation, known as Cerrado *sensu stricto (s. str.)*, where shrubs and small trees are subjected to a lengthy (five months or more) and severe dry season, riparian and gallery forests present high soil water availability throughout the year (Kissmann et al., 2012). This arises because, in Cerrado areas, riparian forests occur along medium (10-20 m in width) and large (> 20 m in width) rivers, and gallery forests occur along small (< 10 m in width) rivers and streams (Oliveira-Filho and Ratter, 2002). This condition provides a suitable habitat for a large number of typical moist forest species (Oliveira-Filho and Ratter, 2002). Therefore, depending on microhabitats formed by the uneven topography along and perpendicular to watercourses, this high moisture in the soil facilitates the occurrence of species with different soil water requirements (Toniato et al., 1998; Marques et al., 2003; Teixeira and Assis, 2005).

In addition, the floristic composition of the Cerrado is strongly influenced by the surrounding vegetation, such as the Amazonian and Atlantic forest domains (Oliveira-Filho and Ratter, 1995; Toniato et al., 1998), and the lack of geographical barriers separating these environments allows the exchange of floristic elements between these forests (Oliveira-Filho and Ratter, 2002). Nevertheless, the success of forest species in savanna areas is constrained by droughts (Hao et al., 2008) and fires events (Geiger et al., 2011).

On the other hand, plants in riparian and gallery forests often experience limited oxygen availability due to soil floods, which can be seasonal or permanent, according to climatic and/or edaphic conditions. Oxygen deficiency derived from flooding conditions affects root respiration and can challenge the occurrence of flood-intolerant species (Lobo and Joly, 2000). During evolution, different species have developed a variety of adaptive mechanisms, which may include morphological, anatomical and/or physiological changes, that have enabled them to occupy flood-prone areas (Kozlowski, 1997).

Although gaps (canopy openness) have a fundamental role in the floristic composition and species distribution in many forest types, soil drainage is pointed as the main factor responsible for species distribution in swamp forests (Teixeira et al., 2011). Swamp habitats (with poor drained soils) exhibit low diversity of plant species in comparison to non-swamp (with well-drained soils) habitats (Marques et al., 2003; Teixeira and Assis, 2005). In the former, only few species occur, and their successes seem to rely on a variety of adaptions to tolerate stresses caused by floods and, consequently, to endure oxygen deprivation (Lobo and Joly, 2000).

Styrax pohlii is a tree species (8–15 m in height) widely distributed in forests that experience high soil water availability, such as riparian and gallery forests (Teixeira and Assis, 2005; Teixeira et al., 2008, 2011). Although this species exhibits high frequency and density of individuals in forests occurring on poorly drained soils, it also appears in areas with well-drained soils, suggesting an important adjustment to flooding conditions, enabling the individuals to deal with different soil moisture situations (Toniato et al., 1998; Marques et al., 2003). As a forest species, *S. pohlii* demonstrates adjustments to capture sunlight, allowing it to survive in the understory before reaching the forest canopy (Habermann et al., 2011; Habermann and Bressan, 2011).

To understand the success of *S. pohlii* in riparian and gallery forests, we submitted *S. pohlii* potted plants to a 100-day period under hypoxia, with subsequent recovery. As flooding condition does not seem to be a survival constraint for *S. pohlii* in the field, we tested the hypothesis that this species is able to develop adventitious roots with aerenchyma, besides being physiologically capable of fast recovery from a flooding period. To test this prediction, we assessed instantaneously measured traits (photosynthetic performances) and integrated physiological performances over time (growth) under three levels of hypoxia: partially- and totally-flooded, and unflooded conditions. The anatomical study of adventitious roots was performed as a framework for functional studies. Therefore, we discuss whether flooding conditions are of any importance for the distribution of *S. pohlii* in waterlogged environments.

2. Material and methods

2.1 Plant materials

Mature fruits of *S. pohlii* were harvested from adult trees in a riparian forest fragment in Ajapi district, Rio Claro municipality (24°00' S, 75°30' W), southern São Paulo state, Brazil. De-pulped fruits (seeds) were sown in vermiculite beds inside gerboxes, and were incubated in a germination chamber (EL 2002, Eletrolab, São Paulo, Brasil) at 25°C, under constant fluorescent light (80 μmol m^{-2} s^{-1}). Seedlings (3 cm in height) were transplanted to 2L plastic bags perforated on the bottom, filled with a commercial substrate comprised of ground pinus bark and vermiculite (Tropstrato florestal$^{®}$, Vida Verde), which is used in forest nurseries. Plants were cultivated in greenhouse conditions, under daily irrigation, and after six months individuals were transplanted to 5L plastic bags containing the same substrate, and grown for five months, until treatments were applied.

2.2 Experimental description and study site

Forty five plants (22 ± 8 cm in height) were separated into three groups: 1) totally flooded plants - water level at 3 cm above the soil surface; 2) partially flooded plants - water level at 15 cm below the soil surface; and 3) unflooded plants - watered daily (control). Flooding conditions were applied by placing plants that were in plastic bags into expanded polystyrene (Isopor®) boxes (47 x 32 x 40 cm; 35L capacity). For each treatment, five boxes were used, with three plants per box, resulting in 15 replications (each plant in a 5L plastic bag) per treatment. The boxes were maintained on benches, and were randomly distributed inside the greenhouse.

The water used for maintaining flooding levels in the boxes was stored in 100L containers 30 days prior to the experiment. This procedure helped maintain low concentrations of dissolved oxygen (DO) in the water. The water level in the boxes were checked weekly, and whenever necessary water was supplemented, in a smooth, slow and gentle manner, in order to avoid bubbles and/or increase in DO concentrations. The concentration of DO in the boxes was assessed weekly, using the Winkler (iodometric) method (Golterman et al., 1978). DO concentrations for both partially- and totally flooded conditions stayed at 4.6 ± 0.3 mg L^{-1} throughout the study.

Plants from treatments 1 and 2 were flooded for 100 days. Subsequently, these plants were recovered under soil field capacity conditions for 50 days.

During the experiment, flooded and unflooded plants were subjected to the same sunlight and temperature conditions. The study was carried out from March to August 2012 and for this period, the average mean temperature between 9:00 and 17:00h inside the greenhouse ranged from 17.1 ± 2.3 to 29.6 ± 2.4 °C. The photosynthetic photon flux density (PPFD) in the greenhouse was 830.6 ± 331 μmol m^{-2} s^{-1}. Air vapor pressure deficit (VPD) was measured weekly from 09:00 to 17:00 h, using an aspirated psychrometer (Model SK-RHG, Sato, Tokyo, Japan), and values ranged from 2.85 ± 1.1 to 5.36 ± 1.7 kPa.

2.3 Photosynthetic parameters

Leaf gas exchange and chlorophyll *a* fluorescence were measured once a week, between 08:00 and 11:30h, along 150 days of experiment, using an open gas exchange system, LI-6400xtr (LI-Cor, Lincoln, NE, USA). Measurements were obtained from completely expanded leaves of eight out of 15 replications. The CO_2 assimilation (A, μmol m^{-2} s^{-1}) and transpiration (E, mol m^{-2} s^{-1}) rates, stomatal conductance (gs, mmol m^{-2} s^{-1}) and intercellular CO_2 concentration (Ci, μmol mol^{-1}) were calculated by the LI–6400xtr data analysis program. Dark respiration (Rd) was also measured and corresponded to A readings obtained under null PPFD, while registering fluorescence in dark-adapted leaves. The PPFD was set to 1,500 μmol m^{-2} s^{-1}, value which was provided with an artificial red–blue LED light source (6400–02B, LI–Cor, USA) held on the top of the leaf chamber fluorometer, LCF 6400-40 (LI-Cor, USA). On each day, VPD in the leaf chamber was allowed to vary with the external environment, and leaves of each treatment underwent the same air humidity during measurements, which was performed under stable conditions (CV% $\approx$ 1%).

The response of A to intercellular CO_2 (*A/Ci* curve) was obtained on the same days gas exchanges were measured, under the same PPFD conditions in the leaf chamber. The CO_2 concentrations inside the leaf chamber (reference CO_2) were modulated in the following sequence to produce *A/Ci* curves: 300, 200, 100, 50, 390, 600, 800 and 1000 µmol CO_2 mol^{-1} air, according to Habermann et al. (2003).

For measurements of maximum quantum yield of photosystem II (PSII) [*Fv/Fm* = (Fm-Fo)/Fm], leaves were dark-adapted for 30 min (Bolhàr-Nordenkampf and Öquist, 1993) using silver foils. For this parameter, Fm and Fo represent the maximum and minimum fluorescence of dark-adapted leaves, respectively. The effective quantum yield of PSII, or ΦPSII [*ΔF/Fm'* = (Fm'-Fs)/Fm'], apparent electron transport rate [*ETR* = (ΔF/Fm') PPFD 0.5 0.84], and photochemical quenching [qP = (Fm'-Fs)/(Fm'-Fo')] were measured on different

light-adapted leaves and, for these variables, Fm' and Fs are the maximum and steady state fluorescences in the light-adapted state, respectively, while Fo' represents the minimum fluorescence after a pulse of weak far-red light, which excites photosystem I, or PSI (Li-Cor, 2004; Baker, 2008).

2.4 Leaf water potential

On the 100th day of experiment the leaf water potential at predawn (Ψ_{pd}) and midday (Ψ_{md}; under maximum VPD) were measured by the pressure chamber method (Turner, 1981), using a DIK-7000 (Daiki Rika Kogyo, Tokyo, Japan) chamber. In addition, aiming to compare Ψ_w values between different water stress disturbances, we used a complementary water deficit study (Veiga, 2011), in which *S. pohlii* plants (10-12 months of age, 50 cm in height) cultivated in 50L pots containing Cerrado soil also had Ψ_{pd} and Ψ_{md} measured, after 53 days under drought conditions.

2.5 Growth parameters

Stem length (cm), root-collar diameter (mm), number of leaves and leaf area (LA, cm^2 $plant^{-1}$) were measured on every plant on the 1st, 50th and 100th day after flooding (DAF), and after the 50-days recovery period. The LA was estimated by a nondestructive method in which the area of leaf blade was obtained by multiplying the leaf length (cm) by the leaf width (cm) by an index (pure number), which is specific for *S. pohlii* leaves, as described by Souza and Habermann (2013).

Destructive analysis was executed to measure root, shoot (stems and petioles), leaf, and total biomasses (g), using three plants per treatment, and it was performed at 0, 50, and 100 DAF. For each plant, organs were separated and oven-dried at 80ºC until constant mass.

2.6 Anatomical structure of adventitious roots

At 100 DAF, adventitious root segments (5 cm long) were collected from three totally flooded plants, since partially flooded plants have not formed such organs. The segments were fixed in FAA 50 (37% formaldehyde, glacial acetic acid, 50% ethanol; 1:1:18 v:v:v) and preserved in 70% alcohol (Johansen, 1940). The anatomical study was based on cross sections from middle portions of adventitious root samples. Sections were made by hand with a razor blade, stained with basic Fuchsin and Astra Blue solutions (Roeser, 1972), mounted in glycerin gelatin and observed under light microscope (DMLB, Leica Microsystems, Wetzlar, Germany).

2.7 Data analysis

The experiment was carried out in a completely randomized design with three treatments (totally- and partially flooded, and unflooded plants). Stem length, root-collar diameter, number of leaves and LA were obtained from 15 plants at 0 and 50 DAF, 12 plants at 100 DAF, and nine plants at 150 days of experiment (after 50 days under recovery), whereas total and organ biomasses were obtained from three randomly selected plants on each evaluation date. Photosynthetic parameters were obtained from eight randomly selected plants for each treatment.

Growth performances and biomasses were analyzed at 0, 50, 100 and 150 DAF, and leaf water potential at 100 DAF, whereas leaf gas exchange and chlorophyll fluorescence were tested once a week using a one-way analysis of variance (ANOVA) to test differences among the three treatments. Mean values of every parameter were compared by Tukey test (α = 0.05).

Anatomical analyses of adventitious roots were used as an attempt to observe the presence of aerenchyma in adventitious roots, although images were not shown in this paper.

3. Results

3.1 Growth and biomass measurements

After 50 days of study, flooded and unflooded plants had the same plant height, leaf number and LA (Fig. 1A, C, D). However, prolonged flooding conditions (100 days) caused negative impacts on growth of flooded plants because unflooded plants demonstrated higher plant height, leaf number and LA compared to totally flooded plants (Figs. 1A, C, D). At 100 DAF, partially flooded plants presented significantly intermediate values of leaf number (Fig. 1C) and insignificant intermediate values (values that did not differ from the other two treatments) of plant height and LA (Fig. 1A, D). Root-collar diameter proved not to be affected by flooding conditions and, consequently, by the recovery period (Fig. 1C).

After the 50-days recovery period, unflooded plants remained as the treatment with the highest plant height, leaf number and LA (Fig. 1A, C, D).

Plant height and root-collar diameter kept increasing along the 150 days, although within limitations imposed by each treatment, but leaf initiation (leaf number) and LA were strongly and negatively affected by flooding conditions, especially in totally flooded plants (Fig. 1).

Only after 100 DAF had plants exhibited significant differences in biomass. Leaf, shoot, and total biomasses were higher in unflooded compared to flooded plants (Fig. 2A, B, D), and partially flooded plants showed insignificantly intermediate values of root biomass between unflooded and totally flooded plants (Fig. 2C). Shoot, root, and total biomasses were statistically similar between partially- and totally flooded plants (Fig. 2B, C, D).

The reduction of leaf biomass in partially- and totally flooded plants (Fig. 2A) was largely a result of the reduction in leaf number (Fig. 1C) and leaf area (Fig. 1D), and such reduction in the leaf number of plants that were submitted to flooding was mainly associated

with low leaf initiation rather than with the anticipation of leaf abscission, albeit some leaf abscission was observed for these plants.

3.2 Gas exchange and chlorophyll fluorescence

Flooding conditions have caused significant reductions in CO_2 assimilation (A, Fig. 3A) and transpiration rates (E, Fig. 3C), stomatal conductance (gs, Fig. 3B), and intercellular CO_2 concentrations (Ci, Fig. 3D) since 25 DAF. However, it was clear that partially flooded plants were not as significantly affected as totally flooded plants, which showed the lowest gas exchange rates throughout the study (Fig. 3). None of these gas exchange parameters had recovered right after the beginning of the recovery period, at 100 DAF. In general, A, E, gs and Ci took around 17-20 days to completely recover from flooding conditions.

The effective quantum yield of PSII (ΦPSII, Fig. 4B) and electron transport rates (ETR, Fig. 4C) have been significantly reduced by flooding conditions since 35 DAF, especially in totally flooded plants. Differently from gas exchange rates, these photochemical parameters were recovered 24 h after the beginning of the recovery period (Fig. 4B, C). The maximum quantum yield of PSII (Fv/Fm) and the photochemical quenching (qP) were not affected by flooding conditions along the study (Fig. 4A, D).

No differences in the dark respiration rate (Rd) among partially- and totally flooded, and unflooded plants were noticed throughout the experiment (Fig. 4E). Similarly, no differences in A between flooded and unflooded plants were observed when varying Ci values (A/Ci curves) during 100 days under flooding conditions (Fig. 5). The initial linear slope, representing the apparent carboxylation efficiency, returned similar values for the three plant groups, as well as R^2 equivalent to 0.99 ± 0.001.

Rates of CO_2 assimilation and transpiration, and Ci were highly dependent on gs, since these parameters showed great correlations with gs values throughout the experiment (data not shown).

3.3 Leaf water potential

Soil flooding significantly affected predawn (Ψ_{pd}) and midday (Ψ_{md}) leaf water potential at 100 DAF. Both Ψ_{pd} and Ψ_{md} were significantly lower in totally flooded plants in comparison to unflooded plants; however, partially flooded plants exhibited insignificant intermediate values (Fig.6A). On the other hand, these values were less than half of the Ψ_{pd} and Ψ_{md} values exhibited by *S. pohlii* plants that remained 53 days under drought conditions in the complementary study (Fig. 6B).

3.4 Anatomical traits of adventitious root

Adventitious roots were observed only in totally flooded plants, and these roots started emerging from the collar-root at 15 DAF. Anatomical analysis of adventitious roots showed absence of aerenchyma (images not shown).

4. Discussion

Although totally flooded plants were able to survive for 100 days developing adventitious roots, these organs did not exhibit aerenchyma. These results clear up part of our predictions, and this is a strong indication that *S. pohlii* survives seasonal flooding in the field without developing aerenchyma in roots. Despite this survival capacity, growth, tissue hydration (Ψw), and photosynthetic performances differed greatly among treatments.

The major constraint imposed to plants under flooding conditions is oxygen (O_2) deprivation, which affects gas exchange rates, water relations, nutritional and hormonal balance, carbon partitioning, with markedly effects on plant growth and survival (Pezeshki, 2001). Therefore, the constraints that flooding conditions imposes on roots have marked effects on aboveground organs, such as stems and leaves. Although biomass gain was affected by flooding conditions only after 100 DAF, since flooded plants were able to maintain low but constant growth as illustrated by plant height (Fig. 1A) and root-collar diameter (Fig. 1B), leaf initiation and LA seemed to be affected by flooding conditions since the beginning of the study because totally flooded plants did not increase ($P > 0.05$) the leaf number and LA from 0 to 100 DAF (Fig. 1C, D). Decrease in plant height and the suppression of leaf formation and expansion are common responses to flooding conditions and often result in massive decrease in plant biomass (Kozlowski, 1997).

As plant survival is dependent on the maintenance of a positive carbon balance, it is also suggested that the reduction in plant growth and development caused by flooding conditions represents plant responses to save energy, maintaining metabolism at minimum rates and costs (Wiedenroth, 1993). Some of our results show that dark respiration (Rd), which is indeed related to energy, was not affected by flooding conditions. On the other hand, leaf gas exchange was considerably affected by flooding conditions, without, however, reaching minimum rates. The conspicuous decreases in *A*, *E* and *Ci* (Fig. 3A, C, D) observed a few days after flooding in totally flooded plants encounter explanation in the concomitant reduction of *gs* (Fig. 3B). Moreover, we observed that *A*, *E* and *Ci* returned great correlation coefficients in the relationships between these variables and *gs* values (data not shown).

Low *gs* values soon after flooding have been widely reported for many species, either flood-tolerant or non-tolerant species (Lopez and Kursar, 1999; Mielke et al., 2003; Medina et al., 2009; Oliveira and Joly, 2010). The rapid decrease of *gs* may be particularly important for

plant survival under flooding because it prevents excessive transpiration in conditions in which the water uptake capacity is being impaired. However, in most flood-tolerant species the initial decrease of *gs* is frequently followed by recovery (stomatal reopening) (Kozlowski, 1997), as was observed for *Erythrina speciosa*, a species found in moist habitats subjected to seasonal flooding (Medina et al., 2009).

Stomatal reopening while plants are still experiencing flooding seems to be related to some type of physiological tolerance, similar to morphological adaptations, such as the development of adventitious roots with aerenchyma and lenticels on stems (Medina et al., 2009). However, not all species considered flood-tolerant exhibit this stomatal reopening response when still under flooding conditions (Oliveira and Joly, 2010). We did not observe stomatal reopening during the 100-day flooding period; *gs* (low) values were recovered only 17-20 days after plants were put under soil field capacity conditions, when the other gas exchange variables were also recovered (Fig.3).

Effective quantum yield of PSII (ΦPSII) and electron transport rates (ETR) were not as affected by flooding conditions as gas exchange variables. Low values of ΦPSII and ETR in totally flooded plants were noted only 10 days after low values of *A*, *E*, *Ci* and *gs* were registered for totally flooded plants. Decreases in these photochemical performances remained within ranges of 30-40% reductions (Fig. 4B, C), whereas gas exchange values measured in totally flooded plants fell to 50% of the values observed for unflooded plants (Fig. 3). Moreover, Fv/Fm and qP remained unchanged throughout the experiment (Fig. 4A, D). These results show that the photosynthetic apparatus was not potentially affected, and that photochemical performances returned to values exhibited by unflooded plants 24h after plants were put under soil field capacity conditions. Thus, it is more reasonable to explain the drop in *A* caused by flooding conditions by stomatal factors, rather than by reductions in photochemical performances.

Low gs values generally observed in flooded plants are not necessarily associated with leaf dehydration. For many species under flooding conditions low gs occurs without reductions in Ψw (Kozlowski, 1997). In the present study, lower Ψ_{pd} and Ψ_{md} observed in totally-flooded compared to unflooded plants suggest that low gs may have been caused by leaf dehydration (Fig. 6A). When comparing these values with those of *S. pohlii* plants under water deficit in the complementary study, it is clear that drought represents a more harmful stress for this species than soil flooding (Fig. 6B). Partially- and totally-flooded plants had low *A* not because of a malfunctioning of the photochemical apparatus nor due to damages to carboxylation capacity, which remained unchanged for the three treatments (Fig. 5), but rather because low gs of *S. pohlii* flooded plants restricted access to CO_2, an event which was important, even when facing a reduced stress condition as compared to drought.

Despite the above-mentioned physiological damages during the 100-day flooding period, there was also relatively rapid recovery of physiological responses. Thus, could *S. pohlii* plants be considered tolerant to flooding, explaining the occurrence of this species in riparian and swamp forests (Teixeira and Assis, 2005; Teixeira et al., 2011). It is difficult to establish a definition of flood tolerance because morphological, anatomical and physiological adaptive responses may occur, and also because these responses vary according to flooding duration and frequency (Casanova and Brock, 2000; Lobo and Joly, 2000). Interestingly, flood tolerance seems to be common among species that face little or no flooding in their habitats, and a great number of species that present some degree of flood tolerance fail to become established in inundated habitats. These observations suggest that species diversity in habitats subjected to seasonal flooding are not related only with flood tolerance but also with tolerance to post-flooding conditions, which involves drought periods (Lopez and Kursar, 1999, 2003). In addition, species with high flood tolerance usually have low drought tolerance (Luo et al., 2008).

In fact, young plants of *Styrax ferrugineus*, a congeneric species that is well adapted to the Cerrado *s. str.*, shows longer roots in relation to *S. pohlii,* which has great shoot growth. These traits are considered to be environment-specific and are important to explain the success of these species in savanna and forest habitats, respectively (Habermann and Bressan, 2011). A reciprocal transplanting study also showed that *S. ferrugineus* is not able to tolerate flooding and does not survive in habitats with moist soil, such as riparian forests, while *S. pohlii* can survive in the Cerrado *s. str.*, at least during the wet season (Habermann et al., 2011). These studies point out that *S. pohlii* has some advantage in habitats with different soil water availability.

Our results showed 100% survival of flooded plants during the 100-day flooding period. Plants were able to keep growing, as evidenced by increases in plant height and root-collar diameter. Although not exhibiting aerenchyma in adventitious roots, flooded plants did not undergo critical photochemical or biochemical impairments to photosynthetic performances, which, therefore, cannot be blamed for reduced gas exchange rates observed during the flooding period. Low *gs* was the most important damage caused by flooding. However, gas exchange responses were recovered 17-20 days after plants were transferred to soil field capacity conditions. These results strongly support our hypothesis that *S. pohlii* plants are physiologically capable of fast recovery from flooding periods. Considering that this species is plastic to distinct sunlight conditions in order to grow and survive in forest environments (Habermann and Bressan, 2011), it is reasonable to suggest that *S. pohlii* also relies on physiological adjustment to flooding to be successful in riparian and swamp forests within Cerrado areas.

5. Acknowledgments

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Figures
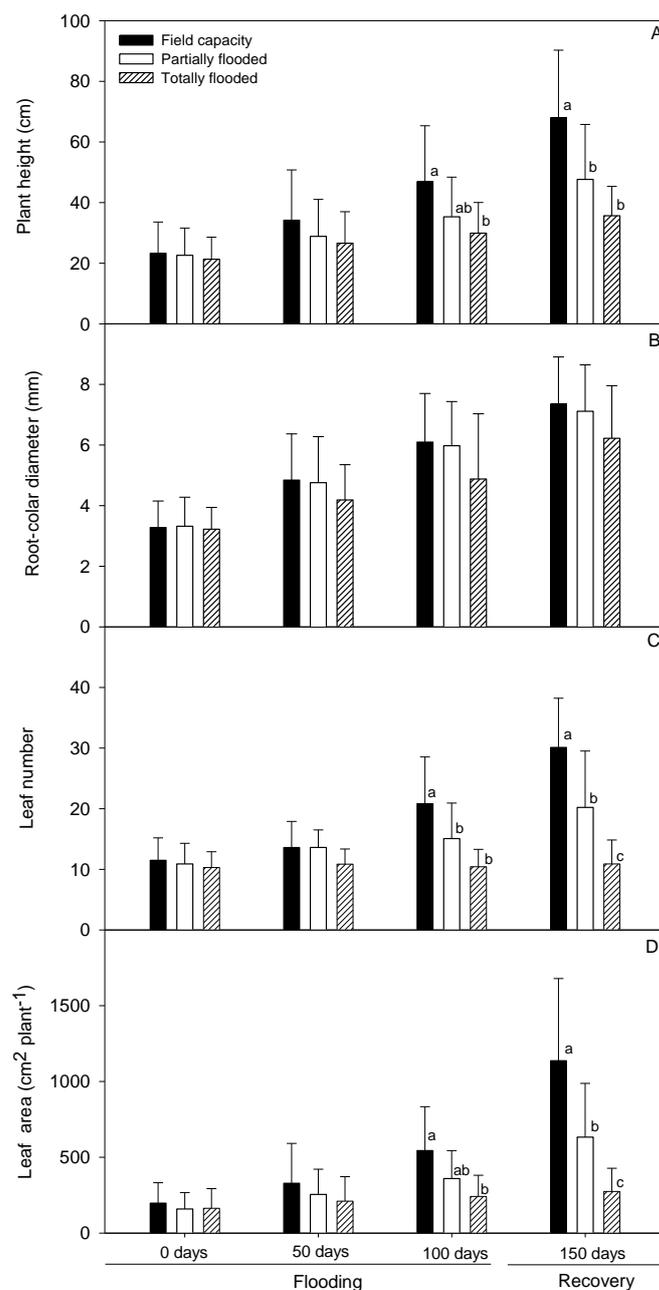
Fig. 1. Mean values (n = 15 plants at 0 and 50 DAF; 12 plants at 100 DAF, and nine plants at 150 days of experiment) of plant height (A), root-collar diameter (B), leaf number (C) and leaf area (D) of *S. pohlii* plants maintained under field capacity and flooding conditions during 100 days, followed by recovery under soil field capacity conditions. For each evaluation date, absence of letters indicates lack of significant differences between treatments and distinct letters indicate significant differences ($P < 0.05$) (vertical bars = SD).
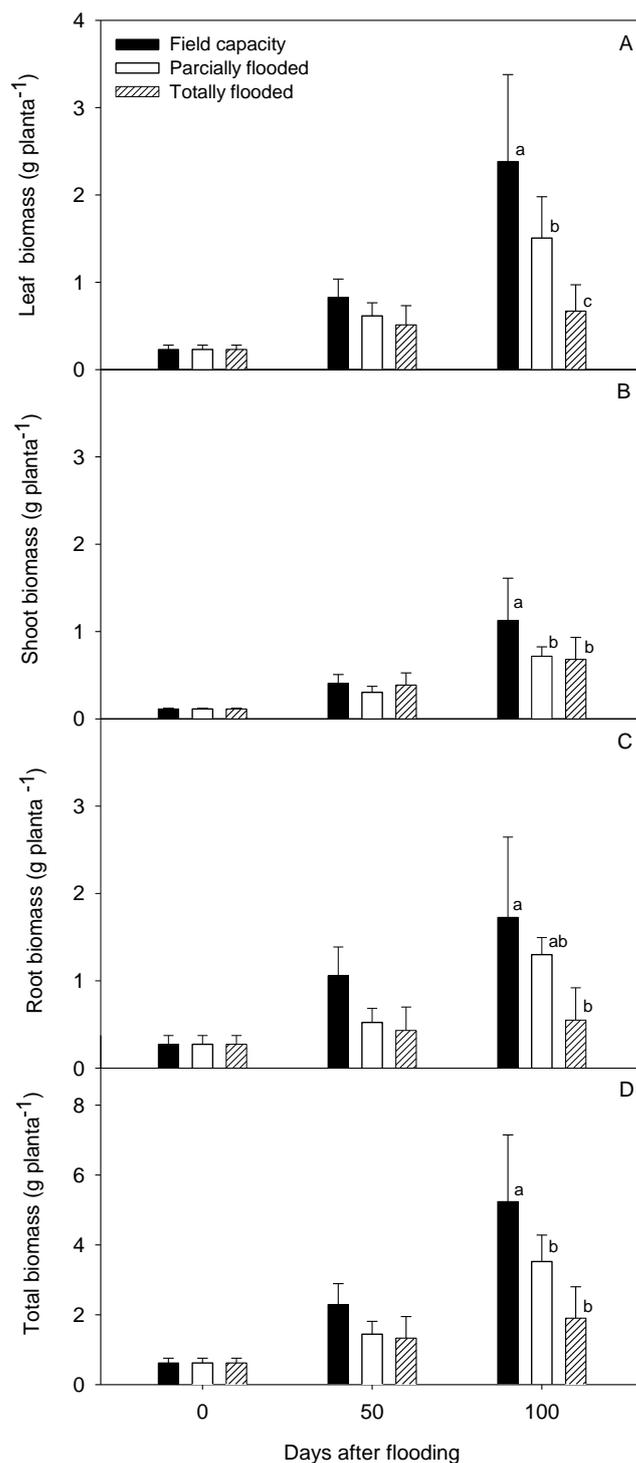
Fig. 2. Mean values ($n = 3$ plants) of leaf (A), shoot (B), root (C) and total biomasses (D) of *S. pohlii* plants maintained under field capacity and flooding conditions. For each evaluation date, absence of letters indicates lack of significant differences between treatments and distinct letters indicate significant differences ($P < 0.05$) (vertical bars = SD).
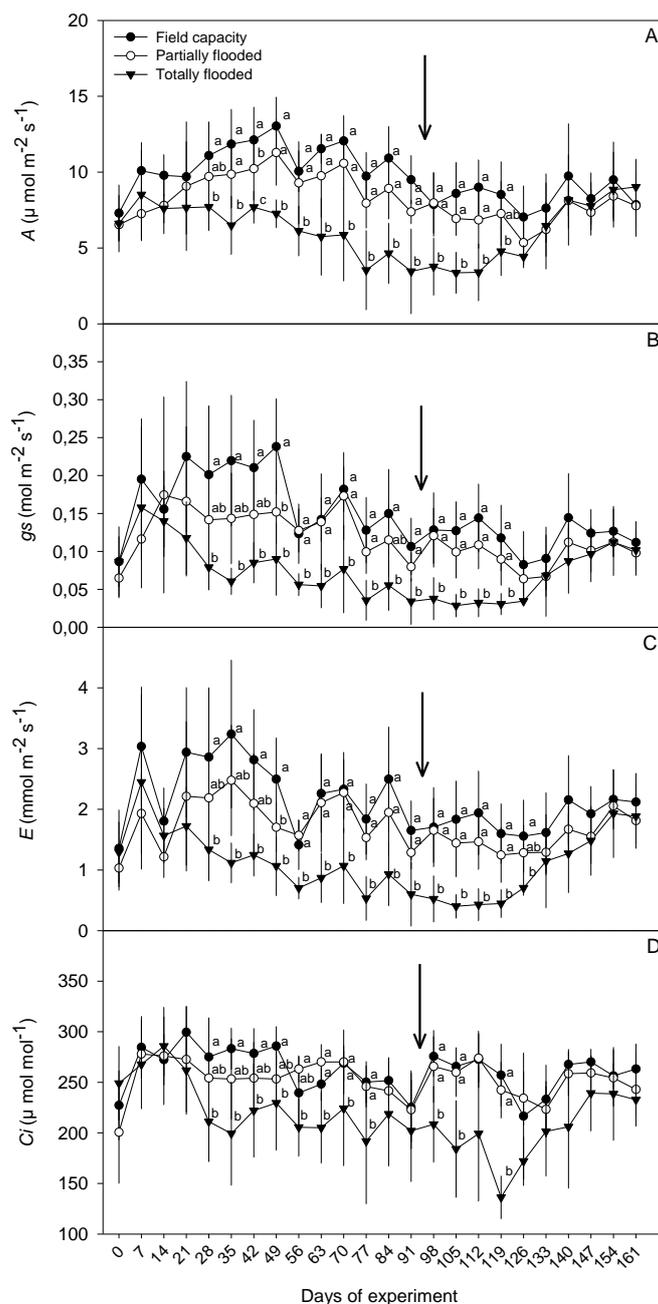
Fig. 3. Mean values ($n = 8$ plants) of CO_2 assimilation rate (A), stomatal conductance (B), transpiration rates (C) and intercellular CO_2 concentration (D) in leaves of *S. pohlii* plants maintained under field capacity and flooding conditions during 100 days, followed by recovery under soil field capacity conditions. For each evaluation date, absence of letters indicates lack of significant differences between treatments and distinct letters indicate significant differences ($P < 0.05$). Arrows indicate recovery (at 100 day after flooding). (Vertical bars = SD).
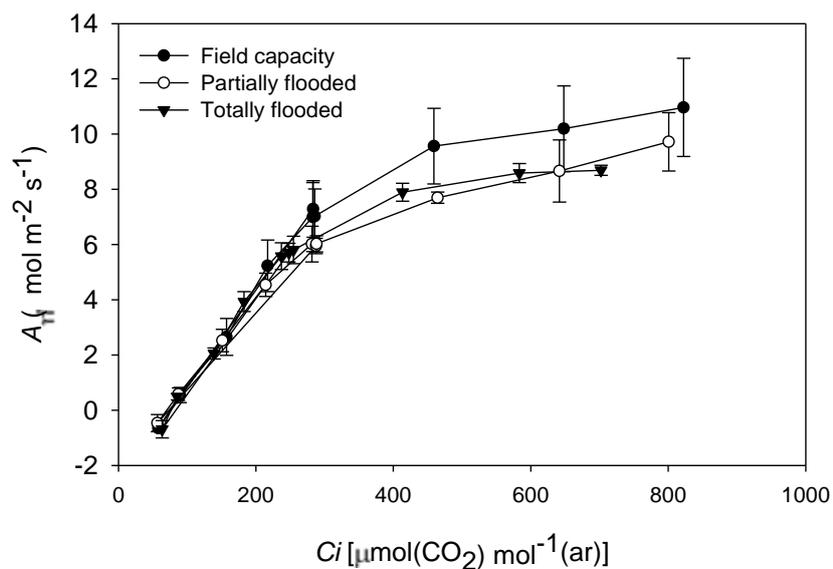
Fig. 5. Mean values ($n = 3$ plants) of CO_2 assimilation rates (A) in response to increasing intercellular CO_2 concentrations (Ci) (A/Ci curves) in leaves of *S. pohlii* plants maintained under field capacity and flooding conditions during 100 days. Absence of letters indicates lack of significant differences between treatments ($P < 0.05$) (vertical bars = SD).
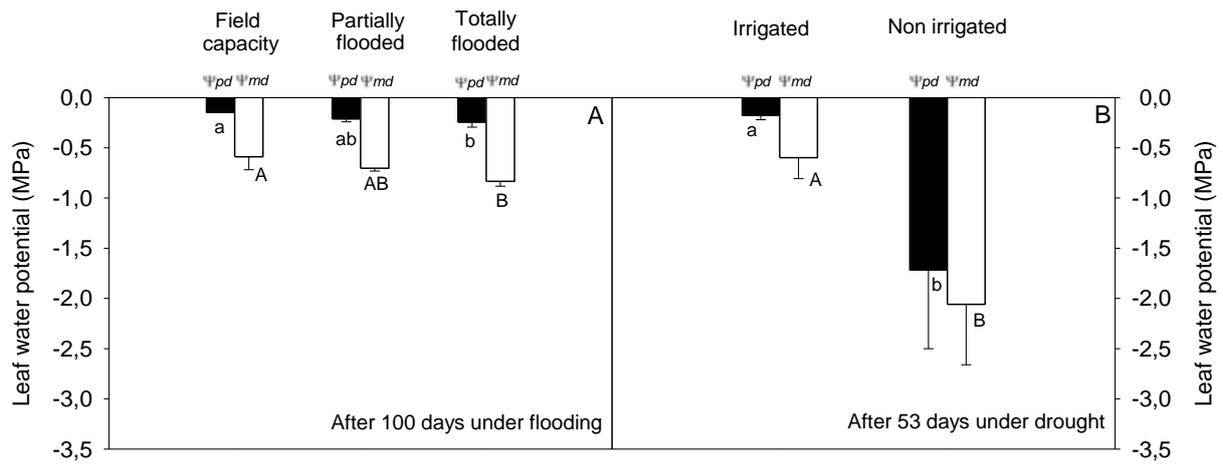
Fig. 6. Leaf water potential at predawn (Ψ_{pd}) and midday (Ψ_{md}) of *S. pohlii* plants submitted to flooding ($n = 3$ plants) (A) and drought conditions ($n = 5$) (B) in greenhouse conditions. For each variable (Ψ_{pd} or Ψ_{md}), absence of letters indicates lack of significant differences between treatments and distinct letters indicate significant differences ($P < 0.05$) (vertical bars = SD).

5. CONSIDERAÇÕES FINAIS

Os aspectos ecofisiológicos das sementes e de plantas de *Styrax pohlii*, *S. camporum* e *S. ferrugineus* evidenciados neste trabalho ajudaram a compreender o distinto padrão de ocorrência dessas espécies no Cerrado.

As variações observadas na espessura do tegumento e nas respostas germinativas das sementes destas três espécies refletem intrínsicas relações com o período de dispersão dos frutos e o ambiente de ocorrência preferencial destas espécies. *S. pohlii,* que ocorre em áreas com grande disponibilidade hídrica no solo e tem os seus frutos dispersos durante a estação chuvosa, produz sementes com respostas fisiológicas responsivas à dessecação e com tegumento mais delgado em relação às outras espécies, que ocorrem em ambientes com menor disponibilidade hídrica e que tem os frutos dispersos fora do pico da estação chuvosa.

Além disso, a germinação das sementes de *S. pohlii* e *S. camporum*, que são encontradas nas fisionomias florestais do Cerrado, ocorre em ampla faixa de temperaturas constantes, enquanto que as sementes de *S. ferrugineus,* que é uma espécie típica de fisionomias savânicas, germinam sob uma faixa mais estreita de temperaturas e apresentam dormência.

Entretanto, as sementes das três espécies germinaram em solos do seu local de ocorrência preferencial e também em solos de outras fisionomias de Cerrado, os quais apresentam grande variação na disponibilidade hídrica. Esse resultado descarta a presunção de que a ausência destas espécies em outras fisionomias que não aquela de ocorrência preferencial da espécie, é devida a restrições impostas pelo ambiente para a germinação das sementes.

Por outro lado, os dados de sobrevivência e crescimento inicial de mudas das três espécies confirmaram a forte influência dos fatores ambientais, sobretudo luz e disponibilidade hídrica do solo, na distribuição destas espécies no Cerrado. *S. ferrugineus* e *S. camporum* não toleram ambientes sombrios e com alto conteúdo de água nos solos, como as

florestas ripárias e de galerias. Enquanto *S. ferrugineus* é abundante no Cerrado *s. str.* e, portanto, adaptada a altas irradiâncias, *S. camporum* ocorre em fisionomias florestais do Cerrado, principalmente no Cerradão. Contudo, os nossos resultados mostraram que a eficiência fotossintética de *S. camporum* é maior quando as plantas são cultivadas sob altas irradiâncias em relação aos locais sombreados. Isso explica a maior ocorrência de árvores jovens de *S. camporum* nas bordas dos fragmentos de Cerradão em relação ao interior destes, onde são observados mais indivíduos adultos do que jovens. Esses resultados sugerem que a espécie é capaz de se beneficiar do surgimento de clareiras para a sua regeneração e que pode avançar os limites dos fragmentos de Cerrado, caso estes não sejam limitados por atividades antrópicas.

Os dados de trocas gasosas, fluorescência e crescimento não foram suficientes para explicar o sucesso de *S. ferrugineus* e *S. pohlii* nos seus respectivos locais de ocorrência preferencial. A distribuição destas espécies parece ser influenciada por múltiplos fatores, e não apenas pelo desempenho ecofisiológico da espécie.

O sucesso de *S. pohlii* nas fisionomias com alta disponibilidade hídrica, é assegurado pelo rápido crescimento inicial da parte aérea das mudas, que permite a espécie competir por luz, e pelo ajuste fsiológico que a espécie apresenta em resposta ao alagamento do solo. Apesar de não aprentar raízes adventícias com aerênquima, *S. pohlii* é capaz de sobreviver a alagamentos sazonais do solo e recuperar rapidamente seu desempenho fotossintético após o alagamento. Além disso, o fato de as sementes de *S. pohlii* serem capazes de germinar em condições de hipoxia confere uma vantagem ecológica para a espécie, cujos frutos podem, ocasionalmente, ser depositados em solos mal drenados.

Já o sucesso de *S. ferrugineus* no Cerrado *s. str.*, embora não tenha sido explicado com os resultados obtidos e apresentados nesta Tese, parece estar relacionado ao longo e profundo sistema radicular das plantas desta espécie, que garantem a sobrevivência na estação seca.

Além disso, o tronco com casca mais espessa que os outros dois pares congenéricos parece ser uma significativa proteção contra a inclemência do fogo, que é frequente no Cerrado *s. str.* (pelo menos no Brasil Central).