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PROGRAMA DE PÓS-GRADUAÇÃO EM BIODIVERSIDADE E CONSERVAÇÃO

TRADE-OFFS UNDERLYING THE VARIABILITY IN LEAF MINIMUM CONDUCTANCE ACROSS CERRADO SPECIES: CAUSES, CONSEQUENCES, AND IMPLICATIONS FOR CARBON ASSIMILATION AND WATER USE AND CONSERVATION

Autor: Renan Silva Machado
Orientador: Dr. Paulo Eduardo de Menezes Silva

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Outubro – 2019

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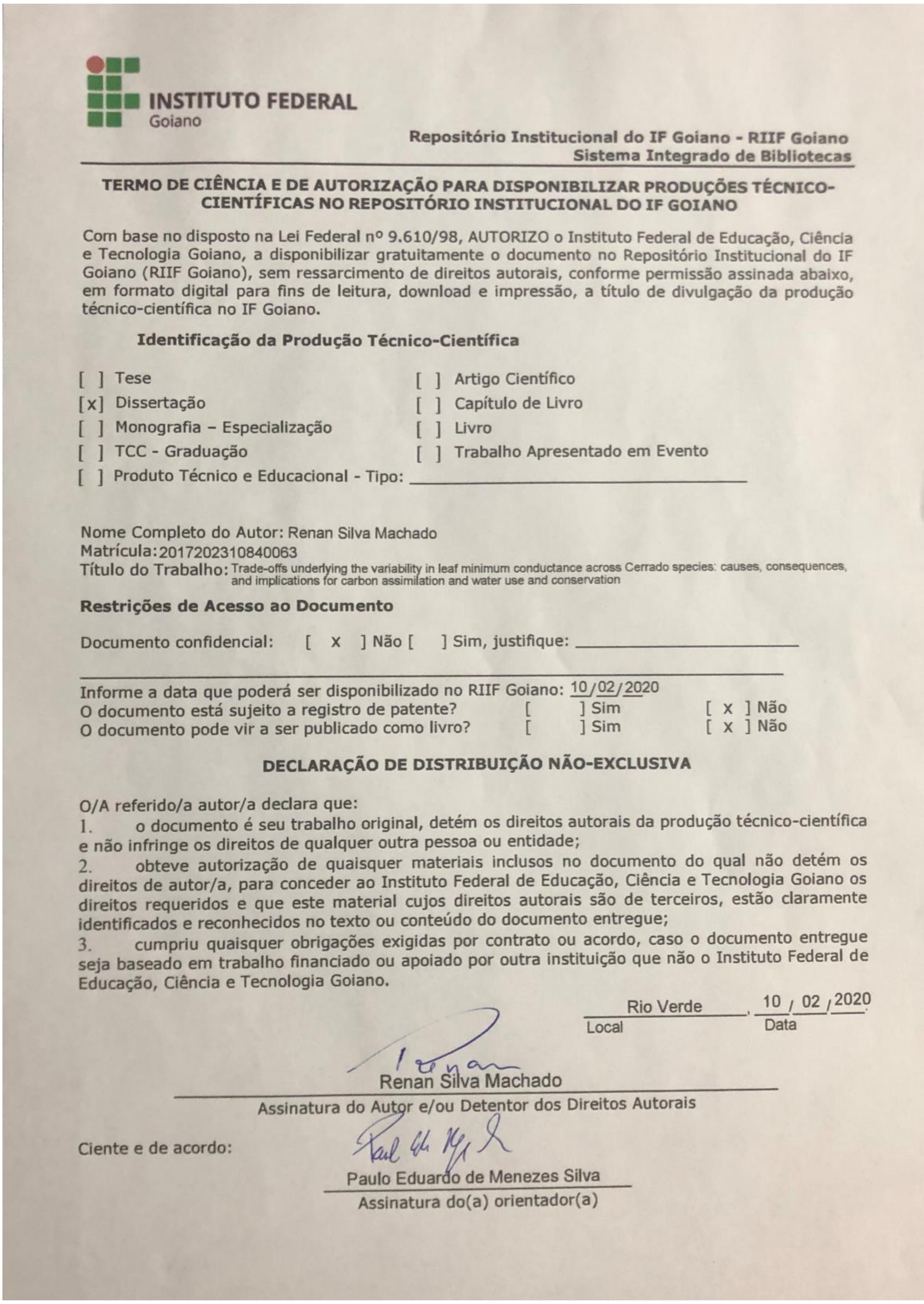

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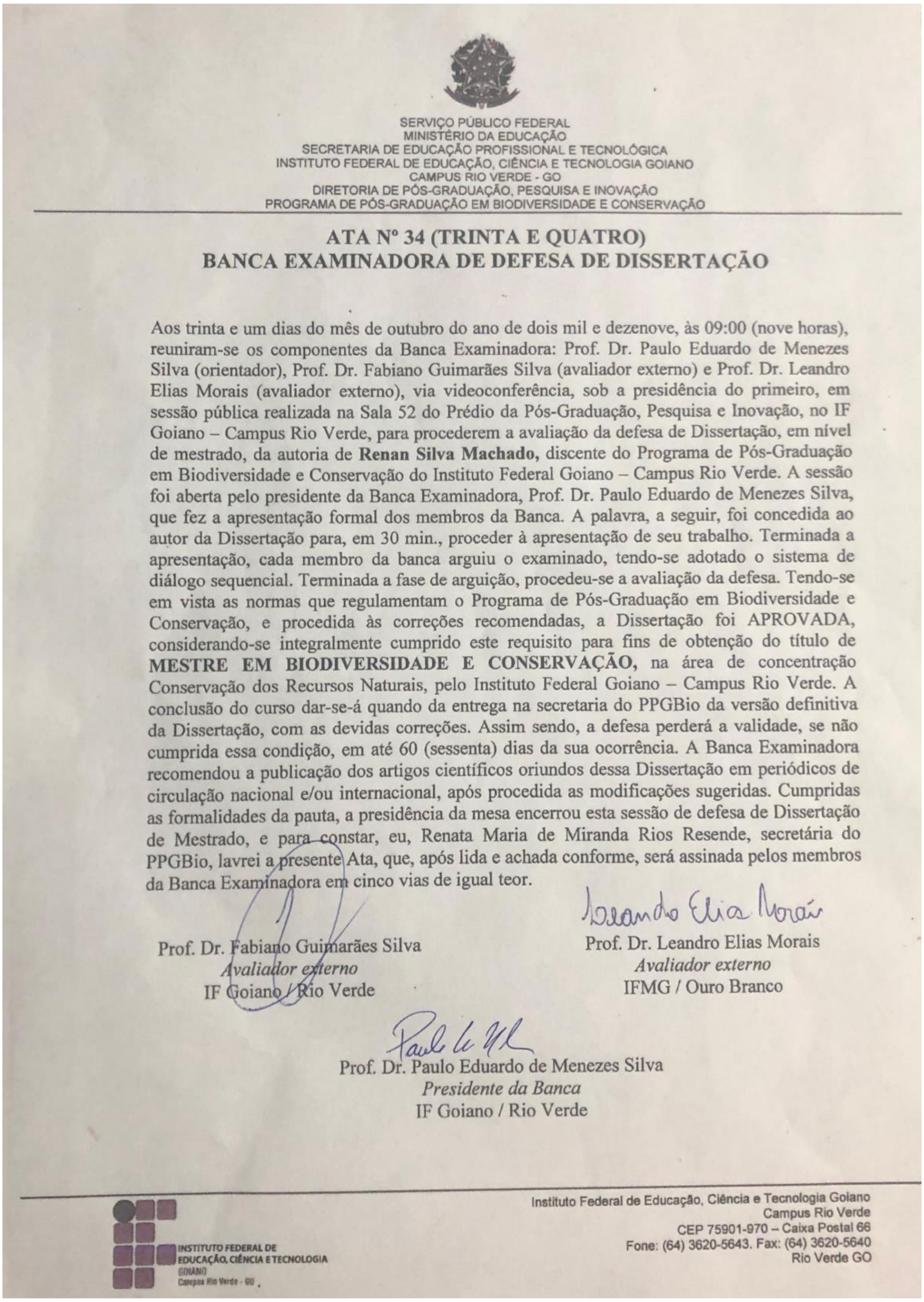

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ATA Nº 34 (TRINTA E QUATRO)
BANCA EXAMINADORA DE DEFESA DE DISSERTAÇÃO

Aos trinta e um dias do mês de outubro do ano de dois mil e dezenove, às 09:00 (nove horas), reuniram-se os componentes da Banca Examinadora: Prof. Dr. Paulo Eduardo de Menezes Silva (orientador), Prof. Dr. Fabiano Guimarães Silva (avaliador externo) e Prof. Dr. Leandro Elias Morais (avaliador externo), via videoconferência, sob a presidência do primeiro, em sessão pública realizada na Sala 52 do Prédio da Pós-Graduação, Pesquisa e Inovação, no IF Goiano – Campus Rio Verde, para procederem a avaliação da defesa de Dissertação, em nível de mestrado, da autoria de **Renan Silva Machado,** discente do Programa de Pós-Graduação em Biodiversidade e Conservação do Instituto Federal Goiano – Campus Rio Verde. A sessão foi aberta pelo presidente da Banca Examinadora, Prof. Dr. Paulo Eduardo de Menezes Silva, que fez a apresentação formal dos membros da Banca. A palavra, a seguir, foi concedida ao autor da Dissertação para, em 30 min., proceder à apresentação de seu trabalho. Terminada a apresentação, cada membro da banca arguiu o examinado, tendo-se adotado o sistema de diálogo sequencial. Terminada a fase de arguição, procedeu-se a avaliação da defesa. Tendo-se em vista as normas que regulamentam o Programa de Pós-Graduação em Biodiversidade e Conservação, e procedida às correções recomendadas, a Dissertação foi APROVADA, considerando-se integralmente cumprido este requisito para fins de obtenção do título de **MESTRE EM BIODIVERSIDADE E CONSERVAÇÃO,** na área de concentração Conservação dos Recursos Naturais, pelo Instituto Federal Goiano – Campus Rio Verde. A conclusão do curso dar-se-á quando da entrega na secretaria do PPGBio da versão definitiva da Dissertação, com as devidas correções. Assim sendo, a defesa perderá a validade, se não cumprida essa condição, em até 60 (sessenta) dias da sua ocorrência. A Banca Examinadora recomendou a publicação dos artigos científicos oriundos dessa Dissertação em periódicos de circulação nacional e/ou internacional, após procedida as modificações sugeridas. Cumpridas as formalidades da pauta, a presidência da mesa encerrou esta sessão de defesa de Dissertação de Mestrado, e para constar, eu, Renata Maria de Miranda Rios Resende, secretária do PPGBio, lavrei a presente Ata, que, após lida e achada conforme, será assinada pelos membros da Banca Examinadora em cinco vias de igual teor.

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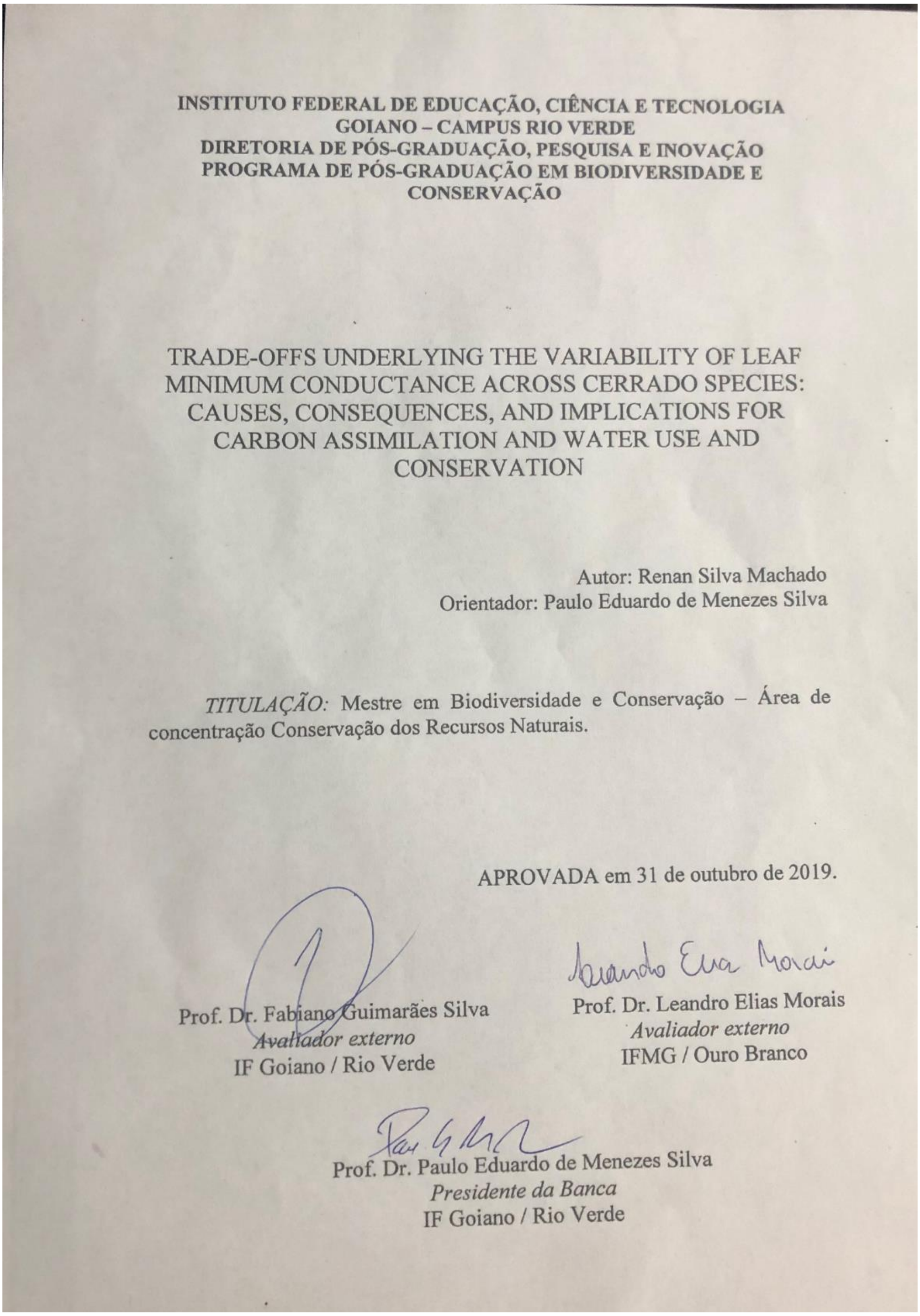

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TITULAÇÃO: Mestre em Biodiversidade e Conservação – Área de concentração Conservação dos Recursos Naturais.

APROVADA em 31 de outubro de 2019.

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DEDICO ESTE TRABALHO:

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MUITO OBRIGADO!

BIOGRAFIA

Renan Silva Machado, natural de Brasília – DF, filho de Eurípedes Flávio Machado e Ironi Silva Machado. Sua formação profissional iniciou em 2000, no curso de Licenciatura Plena em Ciências Biológicas pela Universidade Estadual de Goiás - UEG. Pós-graduado em Tratamento e Disposição Final em Resíduos Sólidos e Líquidos pela Universidade Federal de Goiás – UFG, Pós-graduado em Perícia, Auditoria e Gestão Ambiental pelo Instituto de Pós-Graduação de Goiás – IPOGO, Pós-graduado em Planejamento Urbano e Ambiental pela Universidade de Rio Verde – UniRV. Em 2017, iniciou a pós-graduação *Stricto sensu* - Mestrado em Biodiversidade e Conservação – IF Goiano – Campus Rio Verde, com prazo para concluir em outubro de 2019.

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CHAPTER I

Trade-offs underlying the variability in leaf minimum conductance across Cerrado species: causes, consequences, and implications for carbon assimilation and water use and conservation

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RESUMO

Mesmo após o completo fechamento estomático as plantas continuam a perder água de suas folhas para a atmosfera. Essa transpiração residual, também conhecida como condutância mínima (g_{min}), possui grandes impactos sobre as estratégias de conservação e uso de água entre as espécies. No entanto, apesar da importância de g_{min} para o balanço hídrico geral de uma planta, especialmente em condições de seca, pouco se sabe sobre como diferenças em caracteres funcionais foliares estão relacionados com a grande variabilidade desse parâmetro entre espécies e grupos funcionais. O principal objetivo do presente estudo foi promover uma profunda caracterização da variação de g_{min} entre espécies e grupos funcionais do Cerrado brasileiro, bem como elucidar os caracteres morfo-anatômicos foliares envolvidos com a variabilidade desse parâmetro entre espécies de um dos mais importantes biomas brasileiros. Apesar de ambas as faces do limbo foliar terem explicado de forma significativa a variação total de g_{min} entre espécies, a contribuição relativa deles variou substancialmente, sendo que a transpiração peristomatal representou aproximadamente 67% da transpiração residual total. A alta variabilidade nas taxas de g_{min} também estiveram diretamente associadas com os grupos funcionais das espécies estudadas, sugerindo a existência de um comprometimento entre a assimilação de carbono e perdas de água através de g_{min}. Esse comprometimento, por sua vez, esteve ligado à alocação diferencial de estômatos na epiderme. Para maximizar a difusão de CO_2, espécies de crescimento rápido (decíduas) investiram em estômato menores e mais numerosos, o que resultou em uma maior capacidade de difusão de CO_2. No entanto, apesar dessa estratégia ter maximizado a fixação de carbono, ela também resultou em maior perda de água por transpiração residual. Por outro lado, a estratégia de crescimento mais conservativa das espécies sempre-verdes envolveu a construção de folhas com estômatos maiores e menos numerosos, estratégia que resultou em menor fixação de carbono, mas que preveniu a perda excessiva de água por transpiração residual. A variabilidade de g_{min} também teve impacto direto sobre a taxa de sobrevivência das espécies de diferentes grupos funcionais em um cenário de déficit hídrico severo, sendo que plantas sempre-verdes sobreviveram 28 dias a mais do que plantas decíduas nessas condições simuladas. No entanto, apesar do grande impacto das variações morfo-anatômicas dos estômatos sobre a variação geral de g_{min} entre espécies, contribuições significativas das propriedades estruturais da cutícula não foram encontradas.

ABSTRACT

Even after complete stomatal closure, plants keep losing water from their leaves to the atmosphere. This minimum transpiration rate, also known as minimum conductance (g_{min}), represents an inevitable water leak, which has a great impact on the strategies of water use and conservation across species. However, despite the importance of g_{min} to overall water balance, especially under drought conditions, few is known about how differences in leaf functional properties are related to the high variability of this trait across species of the same site, and also across biomes. The main objective of the present study was to make a deep characterization of the g_{min} variability among species from Brazilian Cerrado, and to better elucidate which are the main morphoanatomical characteristics that may drive the presumably high variability of water leaks among species and functional groups from this important Brazilian domain. Although both leaf sides had significantly explained the high g_{min} variability across species, their relative contribution differed substantially, with peristomatal transpiration representing 67% of the total minimum transpiration. The high variability in g_{min} rates was tightly associated with the growth strategies of the studied species, suggesting the existence of a trade-off between carbon assimilation and water loss thought minimum conductance. This trade-off, by its turn, seemed to be linked to the differential stomata allocation in the epidermis. In order to allow high CO_2 diffusion, fast-growing species (deciduous) invested in small and numerous stomata, which resulted in higher CO_2 diffusion capacity. However, although this strategy maximizes the CO_2 assimilation rate, it also led to higher water loss thought stomata leaks. On the other hand, the more conservative strategy of evergreen species involved the construction of leaves with fewer and larger stomata, a strategy that led to a lower CO_2 assimilation capacity (A), but also prevented excessive water leaks (g_{min}) thought the leaves. The variability in g_{min} rates between functional groups had a substantial impact on the time to hydraulic failure (mortality) under our modeled severe drought conditions, with evergreen species surviving 28 days longer than the deciduous species. However, besides the high impact of stomata morphoanatomy to the overall g_{min} variability across species and functional groups, significative contributions of cuticle properties were not found.

INTRODUCTION

From all the resources needed to sustain plant growth and development, water is probably the most abundant and, at the same time, the most limiting. In fact, the water availability is the main factor determining the performance, distribution, and survival of plant species worldwide (Choat *et al.*, 2012b; Anderegg *et al.*, 2015; Trueba *et al.*, 2017). Under conditions of water restriction, plants typically reduce the aperture of their stomata (reduction in stomata conductance – g_s), a strategy that significantly reduces water loss through transpiration, but at the same time limit the CO_2 diffusion for photosynthesis (Galmés *et al.*, 2013; Xiong *et al.*, 2016). Thus, plants are constantly exposed to the paradox of maximizing the CO_2 diffusion while minimizing the water lost through transpiration (Mcadam & Brodribb, 2012). However, even after complete stomatal closure, plants keep losing water to the atmosphere. This minimum transpiration rate, also known as minimum conductance (g_{min}), represents an inevitable water leak, which has a great impact on the strategies of water use and conservation across species (Kerstiens, 1996a; Martin-StPaul *et al.*, 2017; Duursma *et al.*, 2019). In fact, recent studies have suggested g_{min} as a key trait determining the survival under drought conditions, in such way that species with lower g_{min} tend to sustain a better water status, postponing hydraulic failure and drought-induced mortality, when compared to plants with higher g_{min} rates (Gleason *et al.*, 2014; Cochard, 2019; Duursma *et al.*, 2019). However, despite the impact of g_{min} on the strategies of water use and conservation, few is known about how differences in leaf functional properties are related to the high variability of this trait across species of the same site, and also across biomes.

Several pieces of evidence suggest that, morphoanatomically, the water loss from minimum transpiration arises from leaks at two main sites: cuticle and stomata (Kerstiens, 1996b,a; Richardson *et al.*, 2007; Bueno *et al.*, 2019). Plant cuticle act like a shield, which covers the outer cell walls of leaf epidermis, preventing the uncontrolled water loss thought the atmosphere, the attach of microorganisms, and the damages induced by UV light (Kerstiens, 1996a; Schuster *et al.*, 2017). Chemically, the cuticle can be described as a polymer membrane composed of a cutin matrix and cuticular waxes (Schuster *et al.*, 2017). The water loss thought the cuticle only (when leaks from stomata are disconsider) is termed cuticular transpiration ($g_{cuticle}$) and involves the dissolution of water molecules inside the leaves into the medium of the cuticle, diffusion through the solid matrix and, finally, the desorption from the cuticular waxes at the outer portion of this membrane

(Richardson *et al.*, 2007; Duursma *et al.*, 2019). Given the diversity of ecological functions assumed by the cuticle, it is expected that differences in the relative investment in this structure, as a consequence of contrasting life-history strategies, would largely influence the variability in g_{min} rates across species, a hypothesis not always experimentally supported (Kerstiens, 1996a; Richardson *et al.*, 2007).

The contribution of stomatal leakiness to the g_{min} rates is far less studied than those from the cuticle. In fact, although it was already shown that in hypostomatous leaves g_{min} rates tend to be much higher than g_{cuticle}, few studies have focused on the factors that drive such differences in water resistance between the adaxial (solid cuticle) and abaxial side (stomatous surface) of such leaves (Duursma *et al.*, 2019). Some evidence suggests that this lower water resistance in the stomatous leaf surface arises from incomplete stomatal closure, which leads to a higher stomatal residual transpiration (g_{stomata}) (Schuster *et al.*, 2017). Thus, if stomata have such an impact on determining g_{min} rates, it could be expected that morphoanatomical variations in its size (*SS*) and density (*SD*) would largely affect this trait. In fact, strong positive correlations between *SD* and g_{min} were already reported for 10 Sorghum varieties, a result that support the "leaky stomata" hypothesis (Šantrůček *et al.*, 2004). Besides that, as changes in *SS* and *SD* are major determinants of the maximum leaf diffusive conductance to CO_2 and water vapor (g_{wmax} and g_{cmax}, respectively), we also could expect that contrasting strategies of carbon assimilation and water use should be reflected on g_{min} rates. Although never experimentally tested, this observation suggests the existence of a trade-off between carbon assimilation and minimum conductance.

As both cuticle and stomata are extremely responsive to variations in environmental conditions, especially water availability, species with inhabiting regions with periodic drought episodes should present high variability in g_{min} rates, which would reflect their contrasting strategies of water use and conservation. This might be the case of the plants which inhabit the Cerrado, the second-largest Brazilian biome and one of the world's largest biodiversity hotspots (Strassburg *et al.*, 2017). The climate of Cerrado is characterized by a long period of water restriction, approximately five months, that possibly acted as an important selective pressure that shaped several plant lineages that successfully invaded savannah environments, which contrast significantly on their morphophysiological traits (e.g. relative investment on wood, bark, and leaves) (Pellegrini *et al.*, 2017; Rossatto & Franco, 2017). One of the most remarkable differences in the life-history characteristics across Cerrado plant species can be found in their leaf habit. Most of the plants of this domain are drought deciduous, and thus shed their leaves during periods of water shortage (Franco *et al.*, 2005). As differences in leaf habit usually reflect contrasting strategies of carbon assimilation and water use among species from the same site (Poorter *et al.*, 2009; De La Riva *et al.*, 2016; John *et al.*, 2017), we can expect that differences in deciduousness in the Cerrado vegetation possibly involve a differential investment in cuticle and stomata, with a direct impact on g_{min} rates across species. However, to the best of our knowledge, there have been no studies to data that already explored the variability in g_{min} across Cerrado plant species, and neither how this variation can be explained by morphoanatomical differences on cuticle and stomata of species with contrasting life-history strategies (e.g. deciduous and evergreen).

The main objective of the present study was to make a deep characterization of the g_{min} variability among Cerrado species, and to better elucidate which are the main morphoanatomical characteristics that drive this presumably high variability. To achieve this objective, 31 native species, widely distributed in the Cerrado, were analyzed to respond to the following questions: *i*) How variations in the relative investment in cuticle and stomata affect the g_{min} rates across species? As the cuticle represents the main barrier, and stomata the main leaks to water loss, we expect that a combination of a thinner leaf cuticle in association with lower *SS* and higher *SD* will be associated with higher g_{min} rates. ii) What are the benefits and costs associated with high g_{min} rates? Considering that both *A* and g_{min} rates are affected by stomata dimensions and frequency, we believe that the variability in g_{min} rates arises from an inevitable trade-off between carbon assimilation and water loss. In this regard, in order to achieve higher *A* rates, fast-growing species will cover their leaf epidermis with a high number of small stomata, which ultimately will lead to high g_{min} rates, due to stomata leakiness. iii) If g_{min} is related to carbon economics and water use and conservation, will this trait be reflected in different life-history strategies? We expect that, as a result of their fast-growing strategy, deciduous species will have a set of morphoanatomical characteristics, including cuticle and stomata, which will enable higher carbon assimilation but, at the same time, will lead to higher water loss when compared to evergreen species.

MATERIAL AND METHODS

Study site and species selection

To understand the ecological consequences of the variability in g_{min} rates across Cerrado plants, 31 representative species from this biome were sampled. All samplings and analyses were performed on a natural population of a Cerrado fragment belonging to the ecological reserve of the University of Rio Verde, Rio Verde, Goiás (GO), Brazil (17°47'09.2" S 50°57'50.63" W). The mean annual precipitation in the reserve is 1700 mm, with the dry season extending from May to September, and the mean annual temperature is 23 ºC. The species selected were the most abundant in the region, and five adult plants of each species were analyzed. The selected species cover a great diversity of families (Table 1) and encompass substantial structural and functional variability, which ensured a broad representation of the Cerrado tree flora. All the morphological and physiological analyses were conducted during the rainy season, between the months of February and April, using the youngest, fully expanded leaves, which correspond to the third or fourth leaf pair from the apex of lateral branches.

Table 1. List of the studied species and their leaf habit.

Species name	Family	Leaf_habit
Acosmium subelegans	Fabaceae	Deciduous
Acrocomia aculeata	Arecaceae	Evergreen
Anacardium humile	Anacardiaceae	Evergreen
Arrabidaea brachypoda	Bignoniaceae	Deciduous
Bacopa monnierioides	Plantaginaceae	Deciduous
Bauhinia curvula	Fabaceae	Deciduous
Bowdichia virgilioides	Fabaceae	Deciduous
Brosimum gaudichaudii	Moraceae	Deciduous
Butia capitata	Arecaceae	Evergreen
Byrsonima coccolobifolia	Malpighiaceae	Deciduous
Byrsonima sericea	Malpighiaceae	Evergreen
Campomanesia pubescens	Myrtaceae	Deciduous
Casearia decandra	Flacourtiaceae	Deciduous
Cecropia pachystachya	Urticaceae	Evergreen
Cochlospermum regium	Cochlospermaceae	Deciduous
Cupania vernalis	Sapindaceae	Deciduous
Curatella americana	Dilleniaceae	Deciduous
Diospyros hispida	Ebenaceae	Deciduous
Dipteryx alata	Fabaceae	Evergreen
Genipa americana	Rubiaceae	Deciduous
Guatteria sellowiana	Annonaceae	Evergreen
Handroanthus ochraceus	Bignoniaceae	Deciduous
Himatanthus obovatus	Apocynaceae	Deciduous
Hymenaea courbaril	Fabaceae	Deciduous
Machaerium villosum	Bignoniaceae	Evergreen
Micropholis venulosa	Sapotaceae	Evergreen
Qualea grandiflora	Vochysiaceae	Deciduous
Roupala montana	Proteaceae	Evergreen
Solanum lycocarpum	Solanaceae	Deciduous
Syagrus oleracea	Arecaceae	Evergreen
Terminalia argentea	Combretaceae	Deciduous

MORPHOANATOMY

Leaf mass area

The leaf mass area (LMA) was calculated as leaf area⁄dry mass. We took care that the leaves sampled for the measurements of SLA were representative, healthy, fully expanded leaves that did not show clear signs of mechanical or pathogen damage (Markesteijn *et al.*, 2011; Menezes-Silva *et al.*, 2017).

Stomatal characterization

The epidermal impression technique was used to determine the stomatal morphoanatomical properties (Franks *et al.*, 2009; Martins *et al.*, 2014; Menezes-Silva *et al.*, 2015). For this analysis, a small amount of instant glue was placed on a histological slide, and the vegetable material was pressed against the slide for a few minutes. Then, the slides were observed at 20x magnification with the aid of a light microscope (model AX70TRF, Olympus Optical, Tokyo, Japan) equipped with the U-Photo system.

For analysis of the slide, 30 fields of 0.171 mm^2 were chosen randomly to determine the stomatal density (*SD*), guard cell length (L), pore area (p) and guard cell width (*W*) using the ImageJ software (Franks *et al.*, 2009; Galmés *et al.*, 2013) on both the abaxial and adaxial sides of leaves that appeared after the initiation of the treatments. The stomatal size (*SS*) was determined using the L and W measurements. The theoretical maximum stomatal conductance (g_{wmax}) was calculated based on these data as proposed by Franks et al. (2009):

$$g_{\mathrm{wmax}} = \frac{\mathrm{DE}\, d_{w\,a}}{\frac{v(l+\pi)}{2\sqrt{\frac{a}{\pi}}}}$$

where d_{w} represents the diffusion of water vapor into the air, a is the maximum pore area of a fully open stomata, v is the molar volume of air, and l represents the pore depth of a fully opened stomata. For normalization of the values, the constants d_{w} and v represent the values at 25°C (24.9×10^{-6} m^2 s^{-1} and 24.4×10^{-3} m^3 mol^{-1}, respectively). The value of a was calculated as $\pi\ (\rho/2)^2$, where ρ represents the length of the stomatal pore, which is approximated $L/2$ according to Franks and Farquhar (2009). The value of l for a fully opened stomata was calculated as $L/4$ (Doheny-Adams *et al.*, 2012; Martins *et al.*, 2014; Menezes-Silva *et al.*, 2015). The average fraction of the leaf epidermis that is allocated to stomata (f_{gc}) was determined by the average size of the guard cell pair and average stomatal density (de Boer *et al.*, 2016).

Cuticle thickness

Leaf cuticle thickness was determined by hand sectioning fresh leaves. One section of the middle lamina was stained with Sudan III solution. After the staining, the segments were observed and photographed with the above-mentioned microscope (Bacelar *et al.*, 2004; Gotsch *et al.*, 2010). The thickness of the adaxial ($\mathrm{CT}_{\mathrm{adaxial}}$) and abaxial ($\mathrm{CT}_{\mathrm{abaxial}}$) cuticle were made at multiple points along the leaf cross-section and reported as averages per leaf. The total cuticle thickness (CT_{total}) was expressed as the some of the mean values of $CT_{adaxial}$ and $CT_{abaxial}$.

WATER LEAKS

Minimum transpiration rates

Leaf minimum water conductance (g_{min}) was determined gravimetrically from the consecutive weight loss of desiccating leaves following the proposed by Bueno et al., 2019. High-melting-point (68 °C) paraffin wax was used to seal the wounds of cut petioles of water-saturated leaves and leaflets. The transpiration rate (J) was calculated from the change in fresh weight (ΔFW) with time (t) divided by the total projected leaf area (sum of the adaxial and abaxial projected areas).

In order to disentangle the relative contribution of each side of the leaves to the overall g_{min} variation, a subset of species was selected to run a second experiment. In this experiment, leaves were allowed to dehydrate exactly as described before and, after g_{min} was reached, multiple layers of petroleum jelly were applied to the abaxial surface, and water loss was measured again until a new stabilization point was reached (Brodribb *et al.*, 2014). As all the selected species were hypostomatous, the sealing of the abaxial (stomatous) surface allowed to achieve the minimum transpiration through the cuticle only (cuticular transpiration - $g_{cuticle}$). Finally, multiple coats of petroleum jelly were added to the adaxial surface, and the water leaks through the jelly were measured (Brodribb *et al.*, 2014). The minimum transpiration of the abaxial leaf side (peristomatal transpiration – $g_{stomata}$) was obtained as follows:

$$g_{\mathbf{stomata}} = g_{\mathbf{min}} - g_{\mathbf{cuticle}}$$

GAS EXCHANGE

The net carbon assimilation rate *(A)*, stomatal conductance (gs) and the transpiration rate (E) were determined in an open system under saturated light conditions (1500 µmol photons m^{-2} s^{-1}), temperature of 25 °C and a CO_2 partial pressure of 40 Pa using an infrared gas analyzer (LI-6800, LI-COR Inc., Nebraska, USA) equipped with a blue/red light source (Menezes-Silva *et al.*, 2017; Peguero-Pina *et al.*, 2017). Gas exchange measurements were conducted between 08:00 and 11:00 am in sun-exposed, fully expanded leaves (four leaves per species).

STATISTICAL ANALYSIS AND MODELING APPROACH

Before analysis, species traits were $\log_{10}$-transformed if necessary, to improve homoscedasticity and normality. Differences in morphoanatomical and physiological traits between life-history groups (deciduous and evergreen species) were assessed by independent *t*-tests on the mean species values. Pearson's linear correlation analyses were used to investigate the relationship between the water leaks through minimum transpiration with the traits that reflect aspects of carbon assimilation and water use among the species. All the analyses were performed in R v.3.5.1 (R. Development Core Team, 2018).

In order to predict the impact of the g_{min} variability on the survival of plants under a scenario of water deprivation, we used the *SurEau* model, developed by Dr. Hervé Cochard, which simulates water transport in the soil-plant-atmosphere continuum, and includes a detailed representation of capacitance in stems and leaf tissues. In this model, tree mortality is assumed to be triggered by hydraulic failure, and a plant is considered dead when its loss 99% of hydraulic conductivity (Martin-StPaul *et al.*, 2017).

RESULTS

Variability in g_{min} rates across species and its association with cuticle and stomata morphoanatomical properties

Cerrado species differed markedly on their g_{min} rates (~6 fold) (Table 3). However, although this high variability was associated with leaks arising from both adaxial and abaxial leaf sides, their relative contribution differed substantially. In fact, we found that g_{stomata} contributed 67% of the total g_{min}, whereas g_{cuticle} contributed with only 33% (Fig. 1). This higher leakiness from the abaxial leaf side was directly related to the stomatal morphoanatomical properties. In general, we found a negative correlation between stomatal density (*SD*) and size (*SS*) (Table 4), and these constrain of space allocation to epidermis affected directly the overall variability in g_{min} rates (Table 4, Figs. 1 and 3). Both g_{min} and g_{stomata} were positively correlated with *SD*, and negatively correlated with *SS* (Fig. 3). In addition, significative positive correlations were also found between g_{min} and %fgc (Fig. 3). On the other hand, variations in g_{min} were not associated with any of the measured cuticle traits, except for the unexpected positive correlation found between g_{min} and $\mathrm{CT}_{\mathrm{adaxial}}$ (Table 4, Fig. 4). Although cuticle traits were poorly related to the g_{min} variability across species, variations in this membrane were tightly associated with increases in LMA (Table 4).

The variability of water leakiness and morphoanatomical traits of cuticle and stomata were also reflected in contrasting life-history groups (Table 4). In general, the much higher g_{min} rates of deciduous species were associated with their higher *SD* and %fgc and lower *SS*, when compared to evergreen species (Table 3, Fig. 3). However, despite the high variability in stomata traits, significative differences between life-history groups were not found in any of the cuticle properties (Tables 3, Figure 4).

Growth strategies and its implications for carbon assimilation and water loss among species

The Cerrado species differed substantially on their carbon assimilation capacity (*A*) (Table 3), and this difference across species was mainly associated with diffusive aspects, as denoted by the high positive correlation found between *A* with g_{s} (Fig. 2). In addition to the physiological regulation of the stomatal movements, represented by g_{s}, the CO_2 diffusion capacity, and thus *A* rates, among species were also affected by stomatal morphological aspects (Tables 3 and 4, Fig 2). In fact, *A* rates was positively correlated with *SD*, %fgc, and g_{wmax}, and negatively correlated with *SS* (Table 4). The differences in *A* rates were directly related to the growth strategy of the studied species (Table 3, Fig. 2). Deciduous species showed a fast-growing strategy, which involved physiological and morphoanatomical aspects that allowed for high CO_2 diffusion (higher gs, SD, %fgc and gwmax, and lower SS), resulting in higher *A* rates, but also increased water loss (*E*) (Tables 3 and 4, Figs. 2 and 3). Evergreen species, on the other hand, was characterized by a more conservative growth strategy, represented by lower *A* and *E* rates, due to lower g_{s}, *SD*, %fgc and g_{wmax} and higher *SS* (Tables 3 and 4, Figs. 2 and 3).

The fast-growing strategy of deciduous species, although allowed for higher carbon assimilation (*A*), also resulted in higher water leaks (g_{min}), when compared to the more conservative strategy of evergreen species (Table 3, Fig. 5). The high positive correlation found between *A* and g_{s} with g_{min} (Table 3, Fig. 5) represents the base of the trade-off between carbon assimilation and water leaks, which is linked by stomata morphological properties, as denoted by the high positive correlations between SD, %fgc and gwmax with both *A* and g_{min} (Table 3, Fig. 5).

Table 2. Diffusive and morphoanatomical traits for 31 species from Brazilian Cerrado. Mean ± (n = 4) of light-saturated CO2 assimilation rate (*A*), stomatal conductance (g_s), transpiration (*E*), minimum transpiration (g_{min}), leaf mass area (LMA), stomata density (*SD*), stomata size (*SS*), fraction of epidermis allocated to stomata (%fgc), maximum stomatal conductance (g_{wmax}), adaxial cuticle thickness ($CT_{adaxial}$), abaxial cuticle thickness ($CT_{abaxial}$) and total cuticle thickness (CT_{total}).

	A	g_s	*E*	g_{min}	LMA	*SD*	*SS*	fgc	g_{wmax}	$CT_{adaxial}$	$CT_{abaxial}$	CT_{total}
Species	μmol CO_2 m^{-2} s^{-1}	mmol H_2O m^{-2} s^{-1}	mmol H_2O m^{-2} s^{-1}	mmol H_2O m^{-2} s^{-1}	g cm^{-2}	stomata mm^2	$μm^2$	%	mol H_2O m^{-2} s^{-1}	μm	μm	μm
Micropholis venulosa	13.9 (1.07)	360.1 (0.01)	6.10 (0.17)	2.22 (1.07)	69.5 (0.82)	346.6 (25.1)	158.1 (7.97)	8.61 (0.43)	0.73 (0.08)	1.88 (0.04)	1.30 (0.09)	3.18 (0.11)
Acosmium subelegans	18.7 (0.25)	580.5 (0.02)	9.33 (0.18)	10.29 (1.23)	120.2 (19.5)	1056.1 (90.2)	118.0 (4.89)	19.5 (0.81)	1.97 (0.31)	7.57 (1.04)	2.33 (0.14)	9.90 (0.94)
Acrocomia aculeata	12.9 (0.82)	200.5 (0.02)	3.74 (0.42)	2.53 (0.62)	94.4 (5.57)	231.5 (30.5)	297.7 (14.8)	10.8 (0.54)	1.04 (0.19)	3.35 (0.19)	2.26 (0.05)	5.61 (0.22)
Anacardium humile	11.4 (0.99)	140.8 (0.02)	2.91 (0.34)	2.35 (0.78)	99.3 (14.1)	777.2 (60.1)	93.9 (2.45)	11.4 (0.30)	0.48 (0.05)	4.31 (0.15)	2.26 (0.18)	6.58 (0.33)
Arrabidaea brachypoda	20.8 (0.81)	490.4 (0.15)	5.18 (1.01)	5.06 (0.45)	101.1(9.51)	524.8 (43.2)	198.9 (6.01)	16.3 (0.49)	1.8 (0.25)	4.45 (0.27)	2.26 (0.19)	6.71 (0.42)
Bacopa monnierioides	14.2 (0.14)	371.7 (0.03)	6.15 (0.40)	3.94 (0.58)	90.8 (3.89)	1562.0 (85.4)	67.2 (4.83)	16.5 (1.19)	0.43 (0.06)	1.63 (0.04)	0.88 (0.04)	2.51 (0.02)
Bauhinia curvula	21.8 (0.76)	530.6 (0.07)	8.43 (0.90)	5.41 (0.29)	77.2 (3.64)	1708.3 (18.4)	46.3 (2.37)	12.4 (0.64)	1.14 (0.17)	3.58 (0.11)	1.25 (0.10)	4.83 (0.16)
Bowdichia virgilioides	22.2 (1.19)	590.8 (0.25)	6.30 (1.66)	6.69 (0.62)	132.5 (10.9)	358.9 (41.5)	129.1 (9.14)	7.27 (0.51)	1.01 (0.12)	4.59 (0.22)	1.96 (0.25)	6.55 (0.11)
Brosimum gaudichaudii	25.3 (0.89)	460.2 (0.04)	7.31 (0.55)	9.45 (2.11)	169.0 (12.5)	1156.2 (54.6)	106.1 (11.5)	19.2 (2.10)	2.25 (0.21)	6.36 (0.49)	2.98 (0.19)	9.34 (0.37)
Butia capitata	19.5 (0.44)	470.7 (0.01)	6.91 (0.08)	2.72 (0.56)	197.8 (6.99)	366.8 (31.2)	248.2 (19.1)	14.2 (1.09)	2.11 (0.22)	4.89 (0.20)	3.55 (0.15)	8.45 (0.26)
Byrsonima coccolobifolia	23.9 (0.75)	530.1 (0.03)	7.19 (0.72)	7.96 (1.38)	188.1 (6.30)	811.6 (70.4)	198.7 (9.07)	25.3 (1.16)	2.99 (0.29)	12.67 (0.17)	2.2 (0.21)	14.8 (0.26)
Byrsonima sericea	13.9 (0.28)	220.9 (0.01)	4.09 (0.23)	3.97 (0.19)	98.1 (2.34)	130.0 (12.2)	329.1 (2.39)	6.72 (0.05)	0.7 (0.02)	3.94 (0.14)	2.02 (0.08)	5.96 (0.21)
Campomanesia pubescens	15.6 (0.95)	300.2 (0.01)	4.80 (0.26)	4.48 (0.49)	108.6 (3.86)	402.9 (35.4)	149.0 (7.41)	9.42 (0.47)	0.99 (0.07)	4.05 (0.11)	2.59 (0.14)	6.64 (0.24)
Casearia decandra	10.9 (0.93)	370.8 (0.05)	6.12 (0.62)	4.51 (0.34)	105.6 (11.1)	747.5 (58.1)	90.6 (6.77)	10.6 (0.79)	0.37 (0.03)	3.52 (0.18)	2.32 (0.13)	5.84 (0.31)
Cecropia pachystachya	14.9 (0.88)	250.6 (0.03)	4.35 (0.42)	4.59 (0.38)	101.1 (3.76)	538.4 (37.8)	136.5 (4.13)	11.5 (0.35)	0.47 (0.04)	4.02 (0.15)	1.45 (0.04)	5.48 (0.18)
Cochlospermum regium	17.7 (0.45)	500.7 (0.00)	8.73 (0.20)	4.12 (0.35)	68.4 (3.56)	255.1 (19.5)	156.8 (8.96)	6.28 (0.36)	0.7 (0.11)	4.55 (0.27)	2.73 (0.28)	7.29 (0.29)
Cupania vernalis	18.7 (0.66)	340 5(0.03)	6.19 (0.49)	4.90 (0.11)	82.3 (1.23)	796.1 (12.5)	124.6 (5.27)	15.5 (0.66)	1.63 (0.25)	2.95 (0.07)	1.78 (0.11)	4.74 (0.17)
Curatella americana	16.7 (0.63)	40.32 (0.04)	6.30 (0.50)	7.02 (1.19)	107.3 (12.9)	734.5 (60.1)	118.0 (4.20)	13.6 (0.48)	2.07 (0.25)	2.58 (0.19)	1.24 (0.18)	3.82 (0.27)
Diospyros hispida	14.5 (0.34)	450.4 (0.00)	7.14 (0.06)	7.24 (1.22)	122.7 (10.9)	711.5 (18.5)	245.1 (9.55)	27.3 (1.07)	3.51 (0.07)	6.27 (0.71)	3.04 (0.26)	9.32 (0.95)
Dipteryx alata	13.0 (0.26)	301.8 (0.01)	5.23 (0.23)	3.51 (0.91)	55.2 (1.93)	332.4 (40.2)	127.4 (7.62)	6.65 (0.40)	0.56 (0.02)	1.42 (0.01)	0.86 (0.04)	2.28 (0.05)
Genipa americana	11.5 (0.77)	201.6 (0.01)	3.93 (0.21)	2.97 (0.35)	102.4 (7.97)	528.3 (21.0)	190.5 (6.98)	15.8 (0.58)	1.63 (0.02)	3.17 (0.28)	1.43 (0.15)	4.61 (0.40)
Guatteria sellowiana	12.0 (0.97)	200.5 (0.05)	3.59 (0.76)	2.35 (0.54)	90.36(5.77)	404.2 (48.2)	190.1 (7.16)	12.0 (0.45)	0.7 (0.10)	2.66 (0.04)	1.38 (0.11)	4.04 (0.15)
Handroanthus ochraceus	20.9 (0.49)	530.8 (0.04)	7.77 (0.61)	6.15 (1.40)	144.9 (7.44)	1325.0 (74.1)	89.8 (2.07)	18.6 (0.43)	1.51 (0.12)	2.62 (0.10)	0.74 (0.02)	3.37 (0.10)
Himatanthus obovatus	15.4 (0.22)	350.4 (0.02)	5.79 (0.25)	4.92 (0.47)	137.3 (6.97)	524.6 (46.1)	118.9 (2.42)	9.79 (0.20)	0.68 (0.02)	7.20 (0.25)	4.91 (0.22)	12.1 (0.43)
Hymenaea courbaril	17.0 (1.22)	201.5 (0.04)	4.21 (0.57)	6.06 (1.15)	110.5 (5.72)	359.3 (28.1)	235.6 (14.5)	13.2 (0.82)	2.84 (0.16)	4.20 (0.17)	2.30 (0.19)	6.51 (0.25)
Machaerium villosum	17.1 (0.30)	380.5 (0.02)	6.51 (0.22)	3.34 (0.11)	113.1 (13.0)	462.2 (21.6)	101.4 (6.11)	7.36 (0.44)	0.61 (0.08)	2.77 (0.12)	1.70 (0.07)	4.47 (0.10)
Qualea grandiflora	13.2 (0.18)	260.8 (0.02)	4.69 (0.24)	5.43 (0.84)	74.1 (6.09)	371.7 (47.9)	146.3 (8.21)	8.54 (0.48)	0.9 (0.17)	3.01 (0.08)	1.37 (0.03)	4.38 (0.07)
Roupala montana	12.0 (1.07)	220.7 (0.02)	3.97 (0.40)	3.54 (0.30)	119.5 (5.39)	320.1 (15.4)	515.6 (22.2)	25.9 (1.12)	3.01 (0.22)	5.91 (0.25)	1.93 (0.12)	7.85 (0.35)
Solanum lycocarpum	14.7 (0.20)	570.4 (0.01)	8.61 (0.21)	11.06 (2.89)	87.9 (9.41)	760.6 (11.8)	110.0 (6.19)	13.1 (0.74)	1.72 (0.16)	2.90 (0.16)	1.62 (0.14)	4.52 (0.30)
Syagrus oleracea	10.4 (0.42)	160.5 (0.01)	3.00 (0.11)	1.75 (0.21)	117.1 (5.81)	257.1 (19.8)	181.7 (10.1)	7.33 (0.41)	0.7 (0.06)	4.02 (0.24)	1.86 (0.12)	5.88 (0.35)
Terminalia argentea	19.0 (1.21)	460.4 (0.06)	7.48 (0.62)	5.08 (0.25)	96.3 (4.86)	875.7 (48.3)	173.7 (13.4)	23.8 (1.85)	1.99 (0.36)	4.19 (0.06)	2.63 (0.12)	6.83 (0.13)

Table 3. Variation in diffusive and morphoanatomical traits among species and functional groups (deciduous and evergreen species) of plants from Brazilian Cerrado.

	Differences among species				Deciduous	Evergreen	*t*-test	
Traits	Min	Max	Mean ± SE	Ratio	Mean ± SE	Mean ± SE	*t*	*P*
A	10.1	24.7	16.3 (1.50)	2.45	17.7 ± 0.87	13.8 ± 0.83	2.87	**
g_{s}	165.1	585.6	366.9 (31.2)	3.55	419.5 ± 23.8	271.2 ± 30.4	3.77	***
E	2.91	9.32	5.81 (4.59)	3.20	6.46 ± 0.33	4.62 ± 0.44	3.30	**
g_{min}	1.75	11.06	4.93 (4.01)	6.32	6.09 ± 0.46	2.99 ± 0.26	4.66	***
LMA	55.2	197.8	108.7 (15.7)	3.58	110.7 ± 6.66	105.1 ± 10.9	0.46	ns
SD	130.1	1708.3	614.5 (51.4)	13.13	773.9 ± 91.6	324.5 ± 36.3	3.52	**
SS	46.3	515.6	171.5 (18.9)	11.14	136.5 ± 11.2	234.9 ± 36.6	-3.2	**
%fgc	6.28	23.9	12.8 (11.8)	3.81	14.5 ± 1.20	9.75 ± 0.84	2.72	**
g_{wmax}	0.37	3.54	1.34 (0.10)	9.57	1.57 ± 0.19	0.94 ± 0.14	2.23	*
$\mathrm{CT}_{\mathrm{adaxial}}$	1.42	12.6	4.24 (0.35)	8.87	4.62 ± 0.55	3.56 ± 0.39	1.30	ns
$\mathrm{CT}_{\mathrm{abaxial}}$	0.74	4.91	2.03 (0.19)	6.64	2.12 ± 0.20	1.87 ± 0.21	0.78	ns
$\mathrm{CT}_{\mathrm{total}}$	2.28	14.8	6.28 (0.52)	6.49	6.75 ± 0.69	5.44 ± 0.56	1.28	ns

The table shows overall minimum (Min), maximum (Max) and mean traits values (± SE) among species and functional groups; and the ratio of the maximum: minimum value (Ratio). For differences between functional groups, mean values of the traits are given with the *t*-test statistics for differences between deciduous and evergreen species; ^{ns}, nonsignificant, ***, $P < 0.001$, **, $P < 0.01$, *, $P < 0.05$. Trait abbreviation as in table 2.

Table 4. Pearson correlation between diffusive and morphoanatomical traits among 31 species from Brazilian Cerrado

	A	g_{s}	E	g_{min}	LMA	SD	SS	g_{wmax}	fgc	$CT_{adaxial}$	$CT_{abaxial}$
g_{s}	0.75***										
E	0.64***	0.93***									
g_{min}	0.61***	0.73***	0.67***								
LMA	0.39*	0.23ns	0.14^{ns}	0.24^{ns}							
SD	0.51**	0.74**	0.73***	0.60***	0.18^{ns}						
SS	-0.37*	-0.55**	-0.60***	-0.39*	0.14^{ns}	-0.74***					
g_{wmax}	0.49**	0.40*	0.30^{ns}	0.48**	0.44*	0.23^{ns}	0.25^{ns}				
%fgc	0.40*	0.54**	0.46**	0.48**	0.43*	0.69***	-0.06^{ns}	0.67***			
$CT_{adaxial}$	0.37*	0.19^{ns}	0.11^{ns}	0.38*	0.68***	0.03^{ns}	0.28^{ns}	0.49**	0.35^{ns}		
$CT_{abaxial}$	0.15^{ns}	0.06^{ns}	0.02^{ns}	0.10^{ns}	0.46**	-0.23^{ns}	0.36*	0.30^{ns}	0.07^{ns}	0.75***	
CT_{total}	0.33^{ns}	0.17^{ns}	0.10^{ns}	0.31^{ns}	0.66***	0.04^{ns}	0.32^{ns}	0.46**	0.29^{ns}	0.98***	0.86***

The table shows the correlation coefficients and their significance; ^{ns}, nonsignificant, ***, $P < 0.001$, **, $P < 0.01$, *, $P < 0.05$. Trait abbreviation as in table 2.

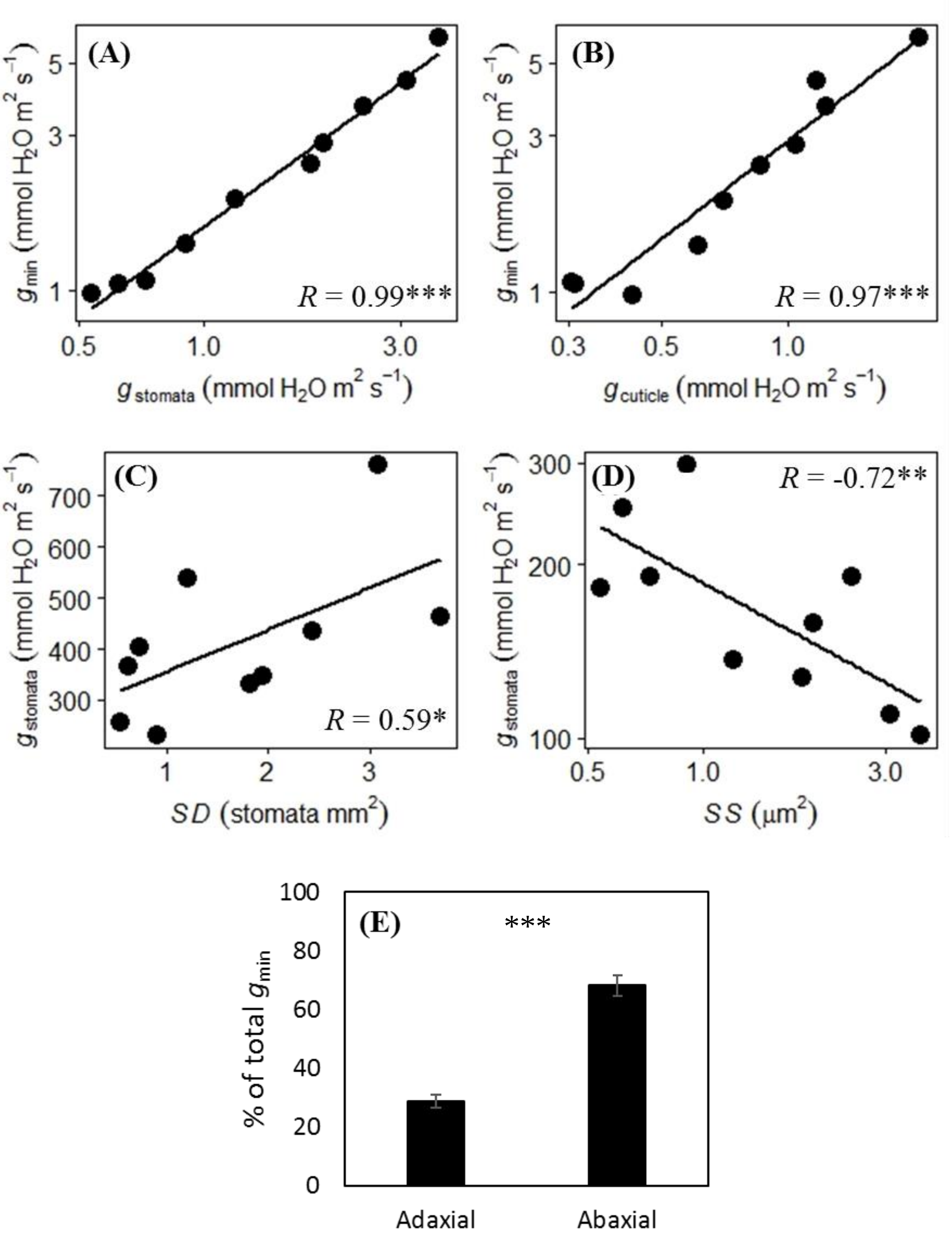

Figure 1. Relationships between (A) leaf minimum conductance (g_{min}) and the peristomatal conductance ($g_{stomata}$), (B) g_{min} and cuticle conductance ($g_{cuticle}$), (C) $g_{cuticle}$ and stomatal density (*SD*), (D) $g_{stomata}$ and stomata size (*SS*), and (E) the relative contribution of the conductances of the adaxial and abaxial leaf sides to the overall g_{min} variation across Cerrado species. Significance of Pearson correlation coefficients: ***, $P < 0.001$, **, $P < 0.01$, *, $P < 0.05$. In E, asterisks denote statistical difference at $P < 0.001$ as denoted by *t*-test.

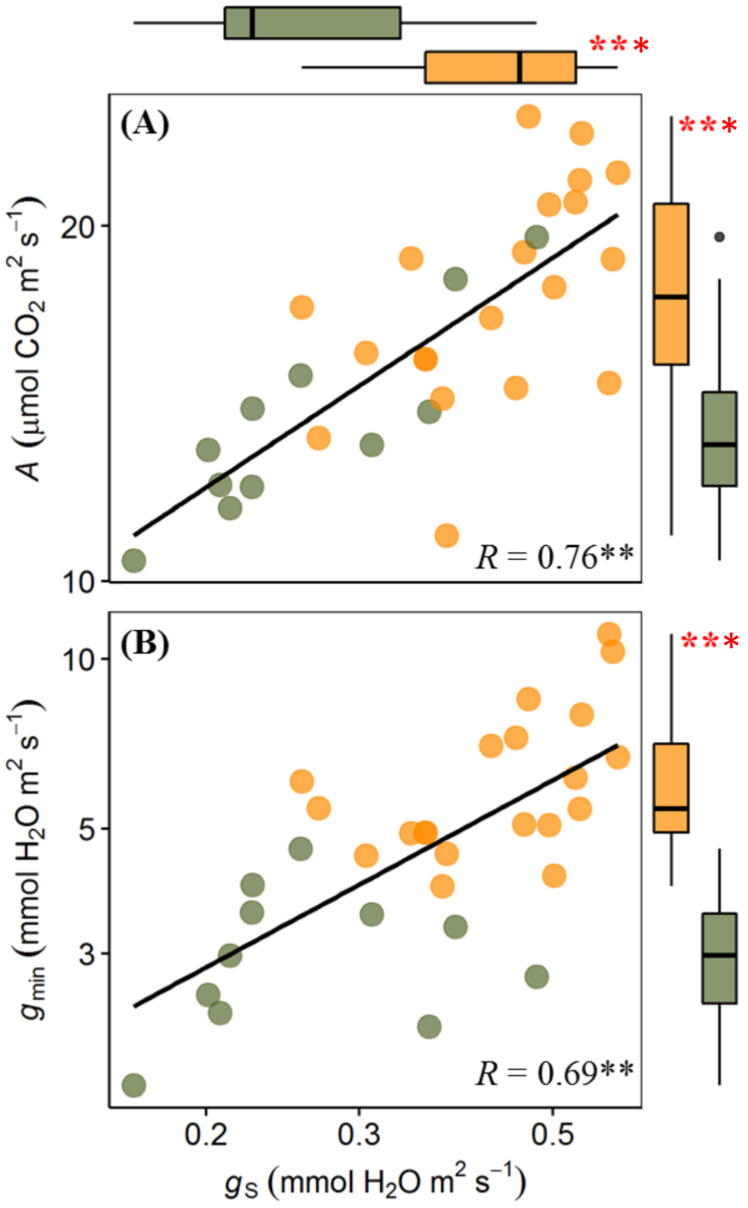

Figure 2. Relationships between (A) the light-saturated CO_2 assimilation rate (*A*) and the (B) leaf minimum conductance (g_{min}) with stomatal conductance (g_s) for 31 species from the Brazilian Cerrado. Significance of Pearson correlation coefficients: ^{ns}, nonsignificant, ***, $P < 0.001$, **, $P < 0.01$, *, $P < 0.05$. The graph also shows the differences in diffusive and stomatal morphoanatomical traits between functional groups, as denoted by *t*-test. Significance of t-test *P* values: ***, $P < 0.001$. Green and orange circles and bars, evergreen and deciduous species, respectively

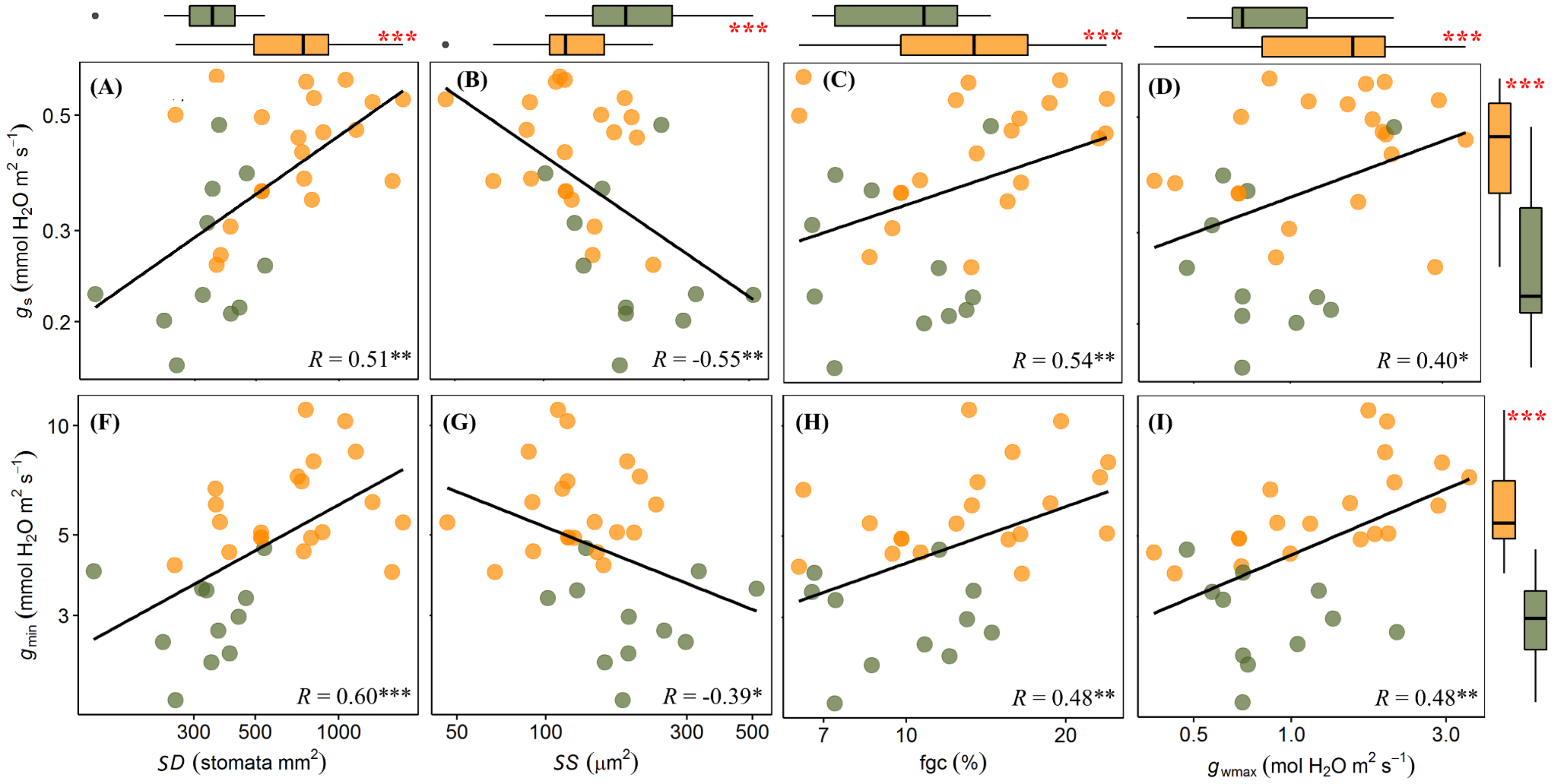

Figure 2. Relationships between stomatal conductance and leaf minimum conductance with stomata morphoanatomical traits for 31 species from the Brazilian Cerrado. Significance of Pearson correlation coefficients: ^{ns}, nonsignificant, ***, $P < 0.001$, **, $P < 0.01$, *, $P < 0.05$. The graph also shows the differences in diffusive and stomatal morphoanatomical traits between functional groups, as denoted by *t*-test. Significance of t-test P values: ***, $P < 0.001$. Green and orange circles and bars, evergreen and deciduous species, respectively. Trait abbreviation as in table 2.

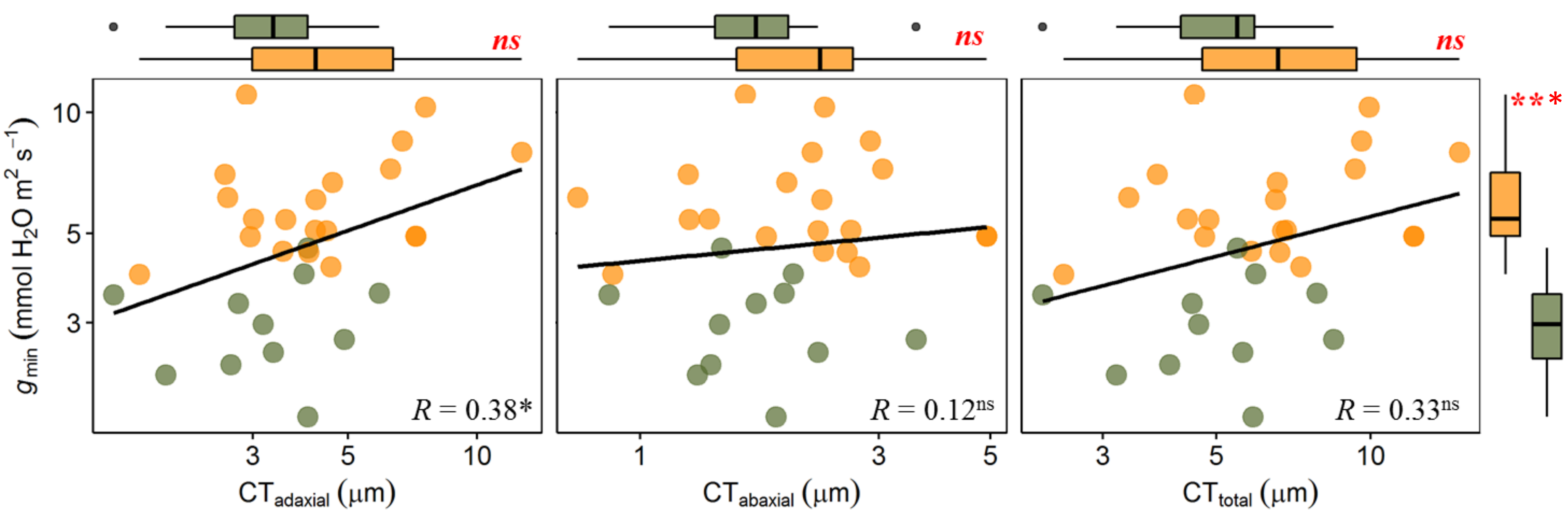

Figure 4. Relationships between the leaf minimum conductance (g_{min}) and (A) the thickness of the cuticle from the adaxial ($CT_{adaxial}$), (B) abaxial ($CT_{abaxial}$), and (C) both leaf sides (CT_{total}) for 32 species from the Brazilian Cerrado. Significance of Pearson correlation coefficients: ^{ns}, nonsignificant, *, $P < 0.05$. The graph also shows the differences in diffusive and stomatal morphoanatomical traits between functional groups, as denoted by *t*-test. Significance of t-test P values: ***, $P < 0.001$. Green and orange circles and bars, evergreen and deciduous species, respectively.

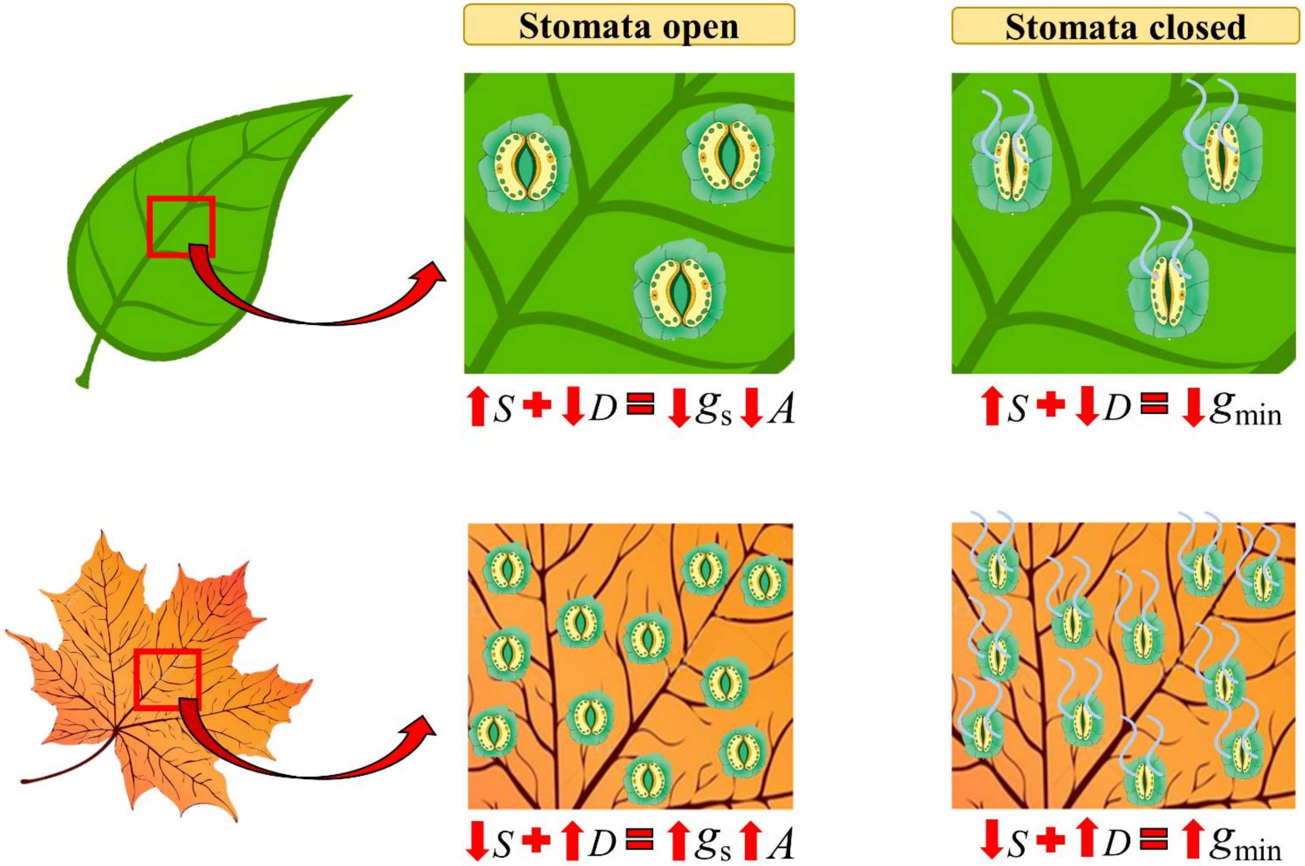

Figure 5. Conceptual model showing the trade-offs underlying the variability in leaf minimum conductance across species from the Brazilian Cerrado. (A) Evergreen species tend to present a more conservative growth strategy, which involves the construction of leaves with fewer and larger stomata (lower SS and higher SD, respectively), resulting in a lower CO2 diffusion (g_s). Although this strategy leads to a lower CO_2 assimilation capacity (*A*), it also prevents the excessive water leaks (g_{min}). (B) On the other hand, the fast-growing strategy of deciduous species involves the investment in numerous small stomata (higher *SD* and lower *SS*), which result in higher CO_2 diffusion capacity. However, although this strategy maximizes the CO_2 assimilation rate, it also leads to higher water loss thought stomata leaks.

Water leaks and tree mortality

Although the water loss thought g_{min} represented only 0,01% of the total water loss by transpiration, our modeling approach demonstrated that these small leaks can have a great impact on tree survival under drought stress. The fast-growing strategy of deciduous species, which was associated with higher g_{min} rates (Tables 2,3 and 4, Figs. 2, 3 and 5), resulted in a mean time to hydraulic failure of 49 days (Fig. 6), whereas the more conservative strategy of evergreen species resulted in a substantially longer survival time under the modeled scenario of severe drought stress (21 days) (Fig. 6).

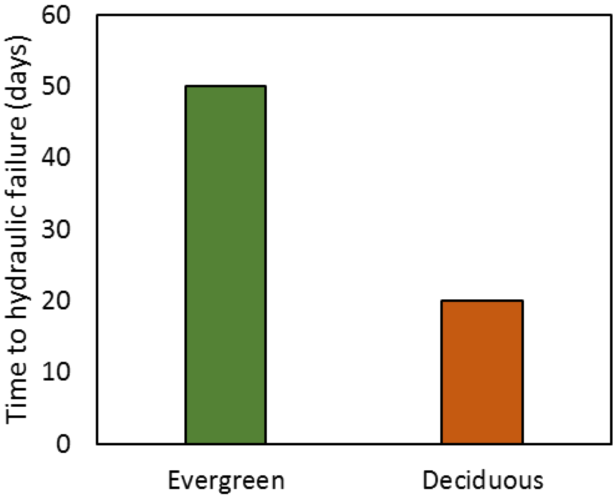

Figure 6. Modeled drought-induced mortality between functional groups of plant species from the Brazilian Cerrado. Tree mortality was considered to be triggered by hydraulic failure (reduction in 99% of hydraulic conductivity of the main stem).

DISCUSSION

Even after complete stomatal closure, plants keep losing water from their leaves to the atmosphere (Duursma *et al.*, 2019). However, although these water leaks have important implications for plant performance and survival, especially under drought conditions (Blackman *et al.*, 2016; Bueno *et al.*, 2019), few is known about the causes and consequences underlying the variability in g_{min} across species. In this study, by analyzing 31 native species from the Brazilian savannah (Cerrado), we demonstrate for the first time that the variability in g_{min} rates across Cerrado species arises as a consequence of their contrasting growth strategies. We also show that these water leaks, although representing a small fraction of water loss through transpiration, can have a huge impact on the strategies of water use and conservation between life-history groups.

Where does the water leaks come from?

Our results demonstrate that, although both g_{cuticle} and g_{stomata} had significantly explained the high g_{min} variability across the studied species, their relative contribution differed substantially. In fact, we found that the abaxial side of the leaf contributed 67% of the total minimum transpiration (Fig. 1). This result complements the yet scarce, but growing body of evidence which suggests that the overall minimum transpiration arises from heterogeneous leaks around the leaves (Šantrůček *et al.*, 2004; Brodribb *et al.*, 2014). In addition, as all the studied species are hypostomatous, we also expected that this higher permeance of the abaxial side of the leaves would be strongly related to morphoanatomical characteristics of the stomata, especially their density and size. More specifically, considering that stomata can be particularly leaky (Kerstiens, 1996a; Šantrůček *et al.,* 2004), even when they were supposed to be closed (e.g. drought conditions), the increase in stomata density should result in increased g_{min} rates. These hypotheses seem to be confirmed since the species with the highest g_{min} and g_{stomata} rates were those which invested in smaller and more numerous stomata (Table 4, Fig. 3). The strong negative correlation found between *SS* and *SD* (Table 4), by its turn, reflects the already well-documented constraints of epidermis space allocation (Franks *et al.,* 2009; Franks & Beerling, 2009a; de Boer *et al.*, 2016).

In addition to the leaks that arise from the stomata pore, the higher permeance of the abaxial leaf surface can also be related to the lower resistance to water flux from the guard cells (Schuster *et al.*, 2017). In fact, it was already shown that the cuticle layer that covers the guard cells lack important chemical components, which may increase their permeance (Duursma *et al.,* 2019). Accordantly, a detailed study on *H. helix* demonstrated that a significative proportion of the water flux from the abaxial surface of the leaves occurs through the non-porous surface (Osborn & Taylor, 1990), another indicator of the higher permeance of guard cells. If guard cells present higher water permeance, it could be expected that increases in the allocation of epidermis to stomata should result in higher g_{min} rates. Our results seem to confirm this hypothesis, since we found significative positive correlations between fgc with g_{min} (Table 4, Fig. 3). Thus, considering that the abaxial leaf side had a significative higher contribution to the overall minimum transpiration, and assuming that the leaks on this surface were associated with incomplete stomatal closure and/or lower resistance to water flux from the guard cells, the high g_{min} variability found among Cerrado species (~6 fold) was probably a reflex of their contrasting allocation of epidermis to stomata, as a result of variation in *SD* and *SS* (Table

3 and 4, Fig 3). When taken together, these results reinforce the "leaky stomata" hypothesis (Kerstiens, 1996a; Šantrůček *et al.*, 2004; Duursma *et al.*, 2019), and suggest that the ecological strategies that result in a differential distribution of stomata in the epidermis can have a significative impact on the overall g_{min} rates across species.

Given the high contribution of g_{cuticle} to the overall variation in g_{min} among species, we expected that increases in the relative investment in cuticle would be associated with reductions in g_{min} rates. However, contrary to our hypothesis, although we have found substantial differences in cuticle thickness in both adaxial and abaxial sides among species (Tables 2 and 3), the expected negative correlation between cuticle thickness and g_{min} was not observed (Table 4, Fig. 4). Contradictorily, a small, but significative, positive correlation between $\text{CT}_{\text{adaxial}}$ and g_{min} was found (Table 4, Fig. 4). If the cuticle represents the main barrier against water loss (Schuster *et al.*, 2017; Bueno *et al.*, 2019), how could the increase in its thickness be not associated with reductions in minimal transpiration? The most likely answer to this question resides in the fact that the main diffusion barrier of the cuticle consists of a thin layer of wax (the limit skin), deposed at or near the outer surface of this membrane (Schreiber, 2001). This helps to explain why variations in g_{min} are better explained by differences in cuticle composition rather than its thickness (Bueno *et al.*, 2019). Considering that increases in cuticle thickness were not associated with reduction in g_{min} rates, what could explain the substantial differences in the relative investment of this structure among Cerrado species? The high positive correlations found between cuticle thickness with LMA (Table 4) is a good indication of other possible ecological functions that may be fulfilled by the cuticle. As LMA is a trait that reflects the trade-off between carbon gain and longevity (Poorter *et al.*, 2009; John *et al.*, 2017), the differences in cuticle thickness among species may reflect their contrasting carbon investment in leaf construction and protection against biotic and abiotic stressors.

Trade-off underlying the g_{min} variability among Cerrado species

We hypothesized that contrasting ecological strategies, which result in a differential stomata distribution across the leaf epidermis, would also reflect the g_{min} variability between life-history groups. This hypothesis also seems to be confirmed since the large variation in g_{min} rates between deciduous and evergreen species (~3 fold) was tightly related to the substantial differences found in their stomata size and distribution (Tables 3 and 4, Fig. 3). In addition, the differences in stomata properties among life-story groups also reflected their contrasting growth strategies, as denoted by the marked differences in their A rates (Table 3, Fig. 2). Thus, as stomata morphology had a direct impact on both CO_2 diffusion and water leaks (represented by g_s and g_{min}, respectively), we hypothesized the existence of a trade-off between carbon assimilation and water loss though minimum transpiration. The positive correlations found between A and g_{min} with g_s and g_{wmax} (Table 4) seems to confirm this hypothesis and suggest that variations in g_{min} between functional groups arise as a consequence of their contrasting growth strategies.

In order to maximize carbon acquisition through their short growing season, deciduous species typically tend to maximize photosynthesis during the wet period, at the expense of higher water loss through transpiration; whereas evergreen species tend to show a more conservative strategy of carbon assimilation and water use (Sack *et al.*, 2003; Franco *et al.*, 2005; Markesteijn *et al.*, 2011). The photosynthetic potential of a given species, in its turn, is mainly determined by diffusive and biochemical components (Galmés *et al.*, 2013). Since all the measurements were conducted during the raining season, and that biochemical limitations are usually triggered by extreme drought conditions (Flexas *et al.*, 2009; Tosens *et al.*, 2016), the high positive correlations found between A and g_s (Fig. 2) suggest that the main limitation to photosynthesis on the studied species involved diffusive aspects. Although g_s rates can be affected within a few minutes, through dynamic alterations in stomatal pore aperture (Drake *et al.*, 2013; Vanlerberghe *et al.*, 2016), the physiological limits of CO_2 diffusion (g_{wmax}) are set morphoanatomically, though alterations in *SS* and *SD* (Franks & Beerling, 2009b; Haworth *et al.*, 2018; Bertolino *et al.*, 2019). The high positive correlations found between g_s with g_{wmax} (Table 4, Fig 3) reinforce the role of morphoanatomical adjustments in determining the rates of CO_2 diffusion and suggest that differences in photosynthetic potential among contrasting life-history strategies were mainly determined by different combinations of *SS* and *SD* (Tables 3 and 4, Figs. 2, 3 and 5). In fact, the higher A rates of deciduous species, when compared to evergreen ones, were tightly associated with the investment in smaller and more numerous stomata, which possibly resulted in increased CO_2 diffusion capacity, as evidenced by their higher values of g_s and g_{wmax} (Tables 3 and 4, Figs 2, 3 and 5). However, although this strategy allows deciduous species to achieve higher growth potential during the rainy season (Franco *et al.*, 2005), under drought conditions, the higher allocation of epidermis to stomata will inevitably result in higher g_{min} rates, as denoted by the positive correlations found between g_{min} and fgc (Table 4, Fig 3). On the other hand, the more conservative growth strategy of evergreen species, although involve lower A rates due to lower g_s and g_{wmax}, as a result of the investment in higher *SS* and lower *SD*, has as one of the main benefits the reduction in water loss under drought conditions (Tables 3 and 4, Figs 2, 3 and 5).

Consequences of the g_{min} variability across species: implications for strategies of water use and conservation

Our results demonstrate that the contrasting growth strategies between deciduous and evergreen species had a direct impact on their g_{min} rates. However, considering that the water loss from minimum transpiration found in the present study represented just a small fraction, less than 1%, of the total water loss through transpiration, what could be the real impact of those small leaks to the overall water balance of the studied species? The ecologic implications of the variability in g_{min} rates are fully appreciated under drought, a condition periodic experience by Cerrado species. As the soil dries, the tension in the xylem vessels tends to increase considerably, which may reduce the stability of the water column and, ultimately, lead to cavitation (breakage of the water column) (Schuldt *et al.*, 2016; Hochberg *et al.*, 2019). As a consequence, cavitation can lead to extensive hydraulic failure (HF), reducing a plant's ability to replenish the water lost through transpiration and resulting in extreme desiccation and death (Choat *et al.*, 2012a; Mitchell *et al.*, 2013; Hartmann *et al.*, 2018). In this way, the ability to avoid excessive water loss significantly reduce the drop in the water potential, and thus postpone the time to hydraulic failure (Mcculloh *et al.*, 2014; Anderegg *et al.*, 2019). Our modeling approach clearly demonstrates that, although small, the water leaks from minimum transpiration can have a great impact on the THF between deciduous and evergreen species. The fast-growing strategy of deciduous species, which involved higher carbon assimilation and thus higher water leaks (Fig. 5), resulted in a THF approximately 43% lower than the more conservative strategy of evergreen species (Figs. 5 and 6). Thus, is evident that the benefits of growing fast come with the cost of being more prone to hydraulic dysfunction.

Although our simulations suggest that the higher g_{min} rates of deciduous species would make them more vulnerable to drought, other important physiological aspects must be considered when interpreting these results. We do not know, for example, if deciduous species from the Brazilian Cerrado present the hydraulic segmentation strategy, in which organs that represent a lower carbon investment (e.g. leaves and small roots) are more vulnerable to cavitation than more basal organs (e.g. branches and trunks). Several studies already showed that the cavitation of leaves can act as a hydraulic valve, protecting the spread of embolism to the trunks (Charrier *et al.*, 2016; Wolfe *et al.*, 2016; Zhang *et al.*, 2016). From this perspective, is possible that deciduous species shed their leaves as a strategy to avoid excessive water loss due to their higher g_{min} rates. Unfortunately, due to the lack of information regarding the vulnerability to drought of Cerrado species, these hypotheses cannot be confirmed. Thus, in order to better understand the impact of climate change on Cerrado vegetation, is of paramount importance the increase of studies that focus on the water relations of the flora of this domain.

CONCLUSIONS

The results obtained in the present study provide a new perspective of the main drivers of g_{min} variability across species with contrasting growth strategies. We demonstrated that, in Cerrado plant species, water loss thought stomata leakiness is as important as cuticle permeability in determining the overall g_{min} rates. In addition, our results also provide strong evidence that the g_{min} variability across species and functional groups arise as a result of a trade-off between carbon assimilation and water loss, and that this variability may have a huge impact on the time to hydraulic failure, and thus in the survival time under drought conditions. Overall, the present study significantly increases the knowledge regarding the water leaks after stomatal closure of the plants that compose one of the largest and most diverse Brazilian biomes.

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