UNIVERSIDADE FEDERAL DE MINAS GERAIS
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
Departamento de Botânica
Programa de Pós-Graduação em Biologia Vegetal

VINÍCIUS COELHO KUSTER

MORFOFISIOLOGIA E COMPORTAMENTO FENOLÓGICO DE PLANTAS EM DOIS NÍVEIS ALTITUDINAIS NA SAVANA NEOTROPICAL

Tese apresentada ao Programa de Pós-Graduação em Biologia Vegetal do Departamento de Botânica do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, como requisito parcial à obtenção do título de Doutor em Biologia Vegetal.

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Área de Concentração: Morfologia, Sistemática e Diversidade Vegetal

Orientador: Prof. Dr. Fernando Henrique Aguiar Vale
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2014

UFMG

Programa de Pós-Graduação em Biologia Vegetal
Universidade Federal de Minas Gerais
ICB - Departamento de Botânica

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Dedico esse trabalho ao meu orientador, Fernando Henrique Aguiar Vale. O seu entusiasmo e simplicidade cativam e moldam os seus orientados.

AGRADECIMENTOS

A Universidade Federal de Minas Gerais (UFMG), através do Departamento de Botânica, pela oportunidade.

A CAPES pela bolsa Reuni, sem a qual não teria conseguido concluir essa etapa. A oportunidade de obter experiência didática a partir de aulas ministradas para os cursos de graduação da UFMG me fez mais confiante como profissional.

Aos professores da UFMG que me auxiliaram no aperfeiçoamento e na resolução de questionamentos obtidos ao longo da minha vida acadêmica. Em especial ao meu orientador, Fernando Henrique Aguiar Vale, por não ser apenas um mero leitor de frases. A amizade e companheirismo do “Fernandinho” foram fundamentais para continuação e encerramento do meu doutorado, além de me fazer enxergar uma vida social em paralelo ao trabalho.

A professora Rosy Mary dos Santos Isaias pelo apoio, indicações de trabalho e amizade. Apesar de não ser seu orientado sempre me senti com liberdade para discussões, parcerias e desabafos. Considero-a uma profissional modelo. Ao professor João Renato Stehmann pela identificação das plantas utilizadas como modelo na minha tese. Aos professores José Pires de Lemos-Filho e Geraldo Wilson Fernandes pelo empréstimo dos equipamentos de fisiologia vegetal e pela autorização e apoio no trabalho em uma das áreas estudadas, respectivamente.

A minha família pelo apoio, amor, confiança e múltiplas ajudas. O apoio se estabelece baseado no conhecimento e apesar de não entenderem exatamente o que faço minha família sempre acreditou e confiou em mim. Assim agradeço minha mãe Rodivalda Coelho Kuster, meu pai Arlindo Kuster e meu irmão Maicon Coelho Kuster por TUDO.

Aos meus amigos do laboratório pelos momentos divertidos durante os vários cafés, em especial a Ariane Castro por me apresentar Belo Horizonte, a dança, auxiliar ativamente na tese e por ser uma grande amiga. A Lucimara Amélia de Souza por ser uma excelente companheira, me ajudando nos momentos mais complexos do meu doutorado. Ao André Portugal, Deborah Aragão, Marcele Laux, Narah Vitarelli e Vinícius Morais pela amizade e carinho. Ao Renê Carneiro por auxiliar nas traduções e intervenções no texto. A Silvana Aparecida de Castro e Bárbara Vieira pela contribuição fundamental na fisiologia vegetal.

A Cristiane Trindade Teixeira por simplesmente morar comigo. Em casa eu tive o melhor exemplo de simplicidade, humildade, caráter, amizade e dedicação. Cristiane, Lila e eu formamos uma linda família. Sentirei saudades.

Aos meus amigos extra institucionais Derick Henrique, Eliton Botelho, Júlia Chálabi, Maycon Martins e Rodrigo Macedo por tornarem meus anos em Belo Horizonte os melhores da minha vida.

Aos meus irmãos de orientação Edvânia Freires, Luciana Seabra, Marcos Hanashiro, Maria Tereza Campos, Mateus Marcato por compartilhar e auxiliar nos momentos bons e difíceis dessa trajetória. Em especial ao Mateus Marcato que me ajudou ativamente na parte laboratorial.

Aos meus alunos que participaram da minha formação como professor acadêmico, as pessoas que me acompanharam no campo e as que direta ou indiretamente, com o trabalho ou simples amizade, auxiliaram nesse trabalho.

Muito obrigado a todos, sozinho nada teria sido concretizado.

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Photosynthetic and anatomical responses of three plant species in two altitudinal levels of the Neotropical savannah

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Physiological responses of Neotropical savannah plants during dry and rainy seasons

RESUMO

Savanas Neotropicais são ecossistemas sazonais e ocorrentes nas Américas, sendo denominada no Brasil de Cerrado. Apresenta fitofisionomias diferenciadas pelo porte dos indivíduos, com destaque para os Campos Rupestres por ocorrerem em áreas de elevada altitude (> 900 metros). Para esse estudo utilizou-se duas áreas modelos: um campo sujo presente no topo do campo rupestre (CSRF), a 1.400 metros de altitude, e outro campo sujo típico (TCS) na base do campo rupestre, a 700 metros de altitude. Essas áreas foram selecionadas para avaliar as respostas das plantas perante as condições abióticas, relacionadas a três abordagens: altitude, fenologia foliar e sazonalidade. Dados de fenologia, fisiologia e anatomia vegetal foram obtidos de *Byrsonima verbascifolia*, *Roupala montana* e *Solanum lycocarpum*. Esses táxons foram escolhidos baseados na ocorrência nas áreas modelo, número amostral satisfatório e por se enquadrarem em dois grupos fenológicos foliares: sempreverde (*R. montana* e *S. lycocarpum*) e brevidecídua (*B. verbascifolia*). Os dados abióticos encontrados para o campo sujo em campo rupestre evidenciam habitat menos estressante do que era esperado, com plasticidade morfofisiológica foliar em respostas as diferenças abióticas, como umidade, temperatura e vento. Em relação a fotossíntese, foi encontrada pequena diferença fisiológica entre as áreas, que pode ser reflexo da alta alteração anatômica. As espécies de mesma fenologia foliar não apresentaram o mesmo comportamento fisiológico nas estações seca e chuvosa, sendo contrária a hipótese inicial.

Palavras-chave: Cerrado, fenologia foliar e sazonalidade.

ABSTRACT

Neotropical savannas are seasonal ecosystems that occur in the Americas and are denominated Cerrado in Brazil. It presents phytophysiognomies distinguished by the size of the individuals, especially the Rupestrian fields that occur in areas of high altitude (> 900 meters). For this study, two areas were chosen as models: the campo sujo at 1.400 meters of altitude on the top of the Rupestrian field (CSRF), and the typical and continuous campo sujo (TCS) at the base of the Rupestrian field, at 700 meters of altitude. These areas were selected to evaluate the plant responses related to three parameters: altitude, leaf phenology and seasonality. The phenology, plant anatomy and physiology data were obtained using *Byrsonima verbascifolia, Roupala montana* and *Solanum lycocarpum*. These *taxa* were selected based on the occurrence in the model areas, the satisfactory sample size and to fit into two leaf phenology: evergreen (*R. montana* and *S. lycocarpum*) and brevideciduous (*B. verbascifolia*). The abiotic data found for the dry *campo sujo* on Rupestrian fields show a less stressful habitat than what was expected, with leaf morphophysiological plasticity in response to abiotic factors, such as humidity, temperature and wind. A few physiological differences were found between areas, which may reflect the high anatomical modifications. The species of the same leaf phenology did not present the same physiological behavior in dry and wet seasons, being contrary to the initial hypothesis.

Keywords: Cerrado, leaf phenology and seasonality.

Introdução geral

Savanas são ecossistemas compostos por estrato herbáceo, contínuo ou compartilhado com estratos arbustivos e arbóreos, que variam na intensidade de cobertura (Furley & Newey, 1983; Solbrig, 1996). O clima apresenta variações sazonais de temperatura e pluviosidade, com estações chuvosas e secas bem definidas (Solbrig, 1996). As savanas tropicais encontram-se nos continentes Americano, Africano, Asiático e a Oceania, enquanto as savanas neotropicais estão presentes nas Américas (Solbrig, 1996), sendo denominada no Brasil de Cerrado.

O Cerrado compreende uma faixa contínua em Goiás, Bahia, Minas Gerais e Mato Grosso e algumas áreas disjuntas em outros Estados (Eiten, 1972). Ocupa 23% do território brasileiro (Oliveira-Filho & Ratter, 1995; Ratter *et al.*, 1997), sendo o segundo maior bioma do país, superado apenas pela Floresta Amazônica (Ribeiro & Walter, 1998). É ocupado por um complexo vegetacional que inclui diferentes fisionomias, determinadas pela ação do fogo, pela distribuição dos tipos de solo (Coutinho, 1982), disponibilidade de água e nutrientes, geomorfologia, topografia, latitudes e impacto de atividades antrópicas (Ribeiro & Walter, 1998).

Coutinho (1978) sugere a presença de cinco fitofisionomias para o Cerrado, denominadas de campo limpo, campo sujo, campo cerrado, cerrado *stricto sensu* e cerradão. Dentre essas fitofisionomias, ocorrem dois extremos: o cerradão, no qual predominam espécies arbóreo-arbustivas, e campo limpo, onde há predomínio do componente herbáceo-subarbustivo. As demais fisionomias encontradas: campo sujo, campo cerrado e cerrado *sensu stricto* podem ser considerados ecótonos entre o cerradão e o campo limpo.

O Cerrado apresenta sazonalidade na temperatura e na precipitação ao longo do ano, com dois períodos climáticos bem definidos: um quente e úmido de outubro a março (estação chuvosa) e outro frio e seco de abril a setembro (estação seca) (Klink & Machado, 2005). Normalmente ocorre sobre latossolos e neossolos quartzarênicos profundos, bem drenados, distróficos, ácidos e álicos, raramente sobre solos mesotróficos (Haridasan, 1992). O latossolo geralmente é profundo e abastecido de água nas camadas mais profundas, o que limita a deficiência hídrica apenas aos dois primeiros metros de profundidade (Rizzini, 1997). A sazonalidade hídrica e características peculiares do solo resultam numa vegetação típica e com caracteres adaptativos próprios.

Dentre as estratégias adaptativas observadas no Cerrado, destacam-se a existência de estruturas que conservam água, como alguns xilopódios, e um sistema radicular bem desenvolvido, que pode alcançar as camadas profundas do solo onde há maior disponibilidade hídrica (Jackson *et al*., 1999; Oliveira *et al.,* 2005). O ciclo de vida e/ou perda temporal da parte aérea de algumas espécies de Cerrado, tais como hemicriptófitas, terófitas e geófitas, são mecanismos que possibilitam sua sobrevivência durante o período de estiagem (Mantovani & Martins, 1988; Batalha *et al.,* 1997). Além disso, os padrões fenológicos e síndromes de dispersão normalmente encontram-se ajustados à estacionalidade climática. As espécies sempreverdes mantêm folhas na copa o ano todo (Oliveira, 1998, Franco e*t al*. 2005), enquanto que espécies brevidecíduas e decíduas perdem completamente a folhagem durante o período seco (Morais *et al*. 1995, Franco *et al*. 2005). A floração e frutificação das espécies do estrato herbáceo ocorrem normalmente no final da estação chuvosa e as do estrato arbustivo-arbóreo no início da mesma (Batalha *et al.*, 1997; Batalha & Mantovani, 2000).

Do ponto de vista ecofisiológico, as respostas de muitas plantas também refletem a sazonalidade do Cerrado. Perez & Moraes (1991) relatam diminuição acentuada dos valores de potencial hídrico e condutância estomática durante a época seca. Na estação chuvosa, com a reposição de água no solo, esses valores aumentaram. Esses resultados foram corroborados por Naves-Barbiero *et al.* (2000) que também encontraram grande controle estomático na estação seca, uma vez que as espécies estudadas não foram capazes de extrair água suficiente do solo.

Dentre as fitofisionomias existentes no Cerrado, Menezes & Giulietti (1986) incluíram ainda os campos rupestres, caracterizados pela sua ocorrência, normalmente, em altitudes superiores a 900 m, predomínio de espécies herbáceo-arbustivas e presença eventual de arvoretas pouco desenvolvidas. Geralmente ocorrem sobre grandes extensões de afloramentos rochosos, com solos pouco profundos, litólicos, de relevo bastante íngreme e montanhoso, com maciços rochosos quartzíticos ou calcáreos, que podem assumir formas de grandes blocos de rochas e escarpas acentuadas (Romero, 2002).

Messias *et al.* (1997) destacaram dois tipos vegetacionais para o campo rupestre: os campos quartzíticos e os campos ferruginosos. O termo campo quartzítico é designado para o tipo de vegetação associada a afloramentos quartzíticos, muito característico na Cadeia do Espinhaço (Viana & Lombardi, 2007), enquanto o campo ferruginoso encontra-se sobre a canga, com presença de vegetação herbácea ou arbustiva que se desenvolve num ambiente com alta incidência solar, pouca matéria orgânica disponível e solo com altas concentrações de metais pesados (Messias *et al.*, 1997). Essas fitofisionomias podem apresentar cinco tipos básicos de formações vegetacionais: capões de mata, que acompanham os cursos d’água; capões de mata das encostas secas; campos graminosos secos; graminosos úmidos e afloramentos rochosos quartzíticos (Peron, 1989).

O complexo mosaico de vegetação que caracteriza a região de campo rupestre é resultado de variações na topologia e declividade, além da natureza do substrato e do microclima do local. Flutuações diárias extremas da temperatura e umidade; o efeito de ventos fortes e frequentes e altos níveis de insolação são características típicas do campo rupestre (Giulietti *et al.*, 2002). Durante a estação seca, muitas regiões sofrem o efeito de queimadas, iniciadas naturalmente ou por ação antrópica. O estresse hídrico, predominante nessa estação, muitas vezes é amenizado pela captação de umidade da neblina (Giulietti *et al.*, 2002).

A Cadeia do Espinhaço, que se estende de Minas Gerais a Bahia, é composta por um conjunto de serras e chapadas pertencentes aos biomas Caatinga, Mata Atlântica e Cerrado, com destaque para os dois últimos biomas por serem considerados *hotspots* mundiais (Mittermeier *et al.*, 1999). Os campos rupestres apresentam ampla distribuição nas porções altas da Cadeia do Espinhaço, ocorrendo elevado endemismo e presença de grande número de espécies ameaçadas de extinção (Mendonça & Lins, 2000). Inserida na porção sul da Cadeia do Espinhaço, a Serra do Cipó destaca-se por apresentar um dos mais ricos complexos rupestres quartzíticos do Brasil, sendo uma região de transição (ecótono) entre os biomas Cerrado a oeste e Mata Atlântica a leste (Schaefer *et al.*, 2009).

A base geológica da Serra do Cipó é constituída predominantemente por uma matriz de quartzito onde se desenvolvem quase que exclusivamente espécies herbáceas. Em contrapartida, escrubes e capões de matas são observados inseridos apenas sobre cambissolo húmico, cambissolo hálico e latossolo vermelho-amarelo, originados da intemperização das rochas metabásicas ou filito. Esses solos são mais férteis e com maior capacidade de retenção de água, em comparação ao quartzítico (Schaefer *et al.*, 2009). A área sobre latossolo, de porte predominantemente herbáceo, foi denominado por Giulietti *et al.* (1983) como campo Cerrado, sendo subsequentemente enquadrada na fitofisionomia campo sujo por Vitta (1995). Nessa porção de campo sujo inserida no campo rupestre quartzítico, Vitta (1995) relatou a ocorrência de espécies exclusivas, em comparação com áreas de campo rupestre próximas, mas que são frequentes em outras fitofisionomias do Cerrado. Esse enclave encontra-se a 1.400 metros de altitude, enquanto que o Campo sujo típico (*sensu* Coutinho, 1978) mais próximo está a 700 metros de altitude. Está diferença altitudinal leva a uma variação nos fatores abióticos, que afetam as respostas morfofisiológicas das plantas.

Oliveira-Filho & Fontes (2000) sugeriram que variáveis geoclimáticas, particularmente sazonalidade pluvial e temperatura, além do tipo de solo, clima e altitude, têm tido ao longo do tempo influência na evolução e especiação de táxons no sudeste do Brasil, o que pode estar ocorrendo com as espécies nos enclaves ou ilhas insulares (Conceição *et al.*, 2007) em meio ao campo rupestre. Além disso, a possibilidade de isolamento dessas áreas pode inviabilizar a troca gênica e consequentemente influenciar em inúmeros processos biológicos.

Face a diversidade de estruturas e processos presentes no Cerrado, esta tese foi diagramada para responder a influência nas plantas de três fatores: Altitude, fenologia e sazonalidade. Utilizaram-se duas áreas de campo sujo e três espécies modelos. Uma área presente no topo do campo rupestre e outra na base do campo rupestre (Figura 1A-C). Dados de fenologia, fisiologia e anatomia vegetal foram utilizados procurando entender as respostas das plantas aos temas supracitados. Para tal, as espécies *Byrsonima verbascifolia* (L.) Rich. (Malpighiaceae), *Roupala montana* Aubl. (Proteaceae) e *Solanum lycocarpum* A. St.-Hil. (Solanaceae) foram utilizadas nesse estudo (Figura 2A-C).

Figura 1. Ambientes estudados. A- Imagem das áreas de estudo, com campo sujo em rupestre (seta amarela) e campo sujo típico (setas vermelhas). B- Visão geral do campo sujo em campo rupestre; C- Visão geral do campo sujo típico.

Figura 2. Espécies estudadas. A- *Byrsonima verbascifolia*; B- *Roupala montana*; C- *Solanum lycocarpum*.

Os temas trabalhados foram inclusos em três capítulos, todos em formato de artigo científico, assim distribuídos:

1. Photosynthetic and anatomical responses of three plant species in two altitudinal levels of the Neotropical savannah;

2. Morphophysiological responses of plants to distinct altitudes in the Neotropical savannah: Water parameters;

3. Physiological responses of Neotropical savannah plants during dry and rainy seasons.

A ocorrência de campo sujo em dois níveis altitudinais distintos nos leva a acreditar que as espécies modelos apresentarão alterações morfofisiológicas às condições abióticas das duas áreas, com destaque para o campo sujo em campo rupestre, pelo posicionado atípico dessa fitofisionomia. Além disso, será avaliada as respostas fisiológicas das espécies modelos em relação as estações seca e chuvosa do Cerrado, no qual esperamos que tenham relação com os padrões de fenologia foliar.

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Capítulo I

Photosynthetic and anatomical responses of three plant species in two altitudinal levels of the Neotropical savannah

Abstract

The phytophysignomies from the Neotropical Savannah may be differentiated by the size of the individuals, as plants may be subjeted to direct light irradiance or different levels of shading. The focus of this study are the functional traits of leaves from plants growing in phytophysiognomies at different altitudes. We evaluated the leaf anatomy, the quantum yield of photosystem II, and the photosynthetic pigments in plants occurring in two *Campo Sujo* areas separated by 700 meters of altitude during the rainy season. The physiological traits of the leaves did not vary much between the two areas, but the same was not true for anatomical aspects, as the thickness of the leaves was conspicuously different.

Keywords: *Byrsonima verbascifolia, Campo sujo, Roupala montana,* rupestrian field and *Solanum lycocarpum.*

Introduction

Plant development is influenced both by biotic factors, such as competition and parasitism, and by abiotic ones, such as the availability of nutrients in the soil and light irradiance (Larcher 2000). Light radiation is essential for the biosynthesis of carbohydrates and the consequent self-sufficiency of the plants, and also influences the processes of germination and the tropism (Barber & Anderson 1992). Nevertheless, depending on the quality and quantity of light radiation, it may become photodestructive (Barber & Anderson 1992). Plant plasticity at the levels of morphology, physiology and biochemistry allow the plants to to adapt to the great variety of light gradients found in nature (Bradshaw 1965; Oguchi et al. 2005).

The ability to alter the metabolism of the leaf, i.e., the rate of carbon assimilation, the light compensation point, and the photosynthetic capacity, can be noticed in plant species able to grow under a wide range of light irradiance (Ronquim et al 2003), as can be found in the Neotropical savannah. In this biome, plants are also subjected to variations in water availability, normally with a dry season from April to September and a rainy season from October to March (Klink & Machado 2005). Associated with the levels of light radiation, temperature, CO_2 concentration, the content of nitrogen in the leaves, and the moisture in the soil may affect the photosynthetic activity of plants (Marenco & Lopes 2005). High photosynthetic rates can determine faster growth (Devall 1992), common in pioneer plants (Greaver & Herbert 2004), while lower photosynthetic rates are observed usually in the dry seasons (Greaver & Herbert 2004), as a consequence of less water availability (Franco 2008), common in Neotropical savannah.

In the Neotropical savannah (*Cerrado sensu lato*), at *Campo limpo* (grasslands) and at the Shrub savannah , plant species receive direct light radiation, and are rarely shaded (Coutinho 1978; Ronquim et al. 2003). Menezes and Giulietti (1986) also included the Rupestrian fields into the phytophysiognomies of the Neotropical savannah, which are restricted to areas above 900 meters. In the Rupestrian fields, the main type of soil is the Quartzarenic neosoil, but patches of red-yellowish latosol (oxisol) are also reported to occur at such sites (Schaefer et al. 2009). The vegetation at these particular patches is very similar to the phytophysiognomies described by Coutinho (1978), and both the *Cerrado sensu stricto* (Moura et al. 2010) and the *Campo sujo* (Vitta 1995) may occur. The base of mountains are warmer and more humid than the tops, and the air temperature decreases about 0.6 °C per 100 meters up on the tropical mountains (Sarmiento 1986). The distinct environmental characteristics between the base and the top of the Rupestrian fields may modulate different responses in the leaf functional traits. This assumption has been tested by Slayter & Morrow (1977) along an altitudinal gradient in Australia, where changes in the photosynthetic apparatus of *Eucalyptus pauciflora* were registered.

Taking for granted that differences in altitudinal levels should influence the photosynthetically active photon flux density, and consequently the photosynthetic and anatomical leaf features, three plant species, *Byrsonima verbascifolia, Roupala montana* e *Solanum lycocarpum,* co-occurring in disjunct areas of *Campo sujo* at two different altitudinal levels were studied.

Material and methods

Sampling and photosynthetic analyses were performed in two areas on sunny: typical *campo sujo* (TCS) at the altitude of 700 meters (19°22’01’’S and 43°37’10’’W), and a *campo sujo* in rupestrian field (CSRF) at the altitude of 1400 meters (20º01’90”S and 43°34’105”W). Cloudless days during the rainy season, in February 2012, when the three plant species were producing new leaves. Vouchers of individuals of *Byrsonima verbascifolia* (L.) Rich. (Malpighiaceae), *Roupala montana* Aubl. (Proteaceae) and *Solanum lycocarpum* A. St.-Hil. (Solanaceae) from the two areas were deposited at the herbarium of the Universidade Federal de Minas Gerais (BHCB) under the registration numbers of 161583 to 161587. *R. montana* did not flowered at the CSRF during the period of the study, and, thus was not deposited in the herbarium.

Anatomical analyses were performed in leaves of the third node from the shoot apex in five individuals per environment. Samples were collected, fixed in F.A.A (formalin, acetic acid, 50% ethanol, 1:1:18 v/v/v), stored in 70% ethanol (Johansen 1940) and processed for light microscopy. Fragments from the intercostal region from the middle of the leaf lamina were embbeded in 2-hydroxyethyl methacrylate Resin Leica® or Paraplast® (Kraus & Arduin 1997), and sectioned in rotary microtome (5 and 10 μm, respectively). Resin embbeded material was stained with 0.05% Toluidine blue - pH 4.7 (O'Brien et al., 1964), and Paraplast® embbeded material was stained with 1% safranin and astra blue (Kraus & Arduin 1997). Slides were mounted with Entellan® (Kraus & Arduin 1997), and photographed in a light micrcoscope (Primo Star Zeiss® coupled with Canon camera model A650). Images were used for histometric analyses. The thickness of the abaxial and adaxial surfaces of the epidermis, and of the chlorophyllous parenchyma was mensured using the software Image Pro-Plus, version 4.1 for Windows® (Media Cybernetics).

Photosynthetically active photon flux density (μmol $m^{-2}s^{-1}$) was registered using a Datta Logger (LI-COR model LI-1400), which was programmed to register at every 5 seconds, and to calculate the mean values at every hour between 8:00am and 7:00pm.

Photosynthesis quantum yield and pigment dosage were evaluated in leaves of the third node from the shoot apex, in 4 individuals of each species at the two different areas. The effective quantum yield (ΔF/Fm') was evaluated between 8:00am and 4:00pm, and the potential quantum yield (Fv/Fm) from 5:00am to 4:00pm by measuring modulated fluorescence using a MINI-PAM (Walz).

The Fv/Fm was analyzed in leaves previously adapted to the dark during 30 minutes; *Fm* is the maximum fluorescence emmited after a saturating pulse of light, and $Fv=F_m-F_0$ (F_0 is the minimal fluorescence of the leaf adapted to the dark). The ΔF/Fm' was obtained by measuring leaves instantly exposed to direct light radiation and calculated as (Fm'-F)/Fm', with *F* being the fluorescence at the stationary state and *Fm'* as the maximum fluorescence after a saturating pulse of light (Genty et al., 1989).

The photosynthetic pigments were quantified using leaf discs of 1cm² immersed in 80% acetone for 48 h and centrifuged at 1000 rpm for 5 minutes using a centrifuge (Fanen model 206BL). The supernatant was analyzed in a spectrophotometer (Thermo Spectronic Mod. Genesys 10UV) at the wavelengths of 470, 646 e 663nm, and the quantification of chlorophyll *a, b,* total chlorophylls, carotenoids and the ratio between them followed the equations proposed by Lichtenthaler & Wellburn (1983).

The significance of the quantitative data was evaluated using the software JMP 5.0 (SAS Institute). The t-test or Dunnett's test were employed after the tests of normality (Shapiro-Wilk´s test) and heterocedasticity (Levene test). Both tests had 5% of significance. The principal component analysis (PCA) was performed with the software STATISTICA 7, and was based on the matrix of correlation between all variables. Data were logarithmic transformed to attend to parametric analyses (Zar 1996).

Results

The photosynthetically active photon flux density (μmol $m^{-2}s^{-1}$) was similar from the beginning through the middle of the day, but differed between the areas from 4:00 pm to 7:00pm (Fig. 1).

Comparative Anatomical Leaf Traits

The epidermis of *S. lycocarpum* and *R. montana* is uniseriate with thick cuticle (Fig. 2A-F), and that of *B. verbascifolia* is two-layered (Figure 2A-B). The cuticle is thick in the first two species, and thin in *S. lycocarpum* (Fig. 2E-F). The leaves are hypostomatic with dorsiventral mesophyll in *B. verbascifolia* (Fig. 2A-B) and *R. montana* (Fig. 2C-D), and amphistomatic with isobilateral mesophyll in *S. lycocarpum* (Fig. 2E-F). Secondary metabolites accumulated in the epidermis of *B. verbascifolia* (Fig. 2A-B) and throughout the mesophyll of *R. montana*, including in the sclereids (Fig. 2C-D). Druses were observed in *B. verbascifolia* (Fig. 2A) and *S. lycocarpum* (Fig. 2E).

The anatomical differences were not so conspicuous on the populations between the areas (Fig. 2A-F), but the thickness of the epidermis and of the chlorophyllous parenchyma were quantitatively distinct (Fig. 3). The adaxial epidermis of the three species was thicker at the CSRF. *B. verbascifolia* and *S. lycocarpum* had thicker adaxial palisade parenchyma at the CSRF, and for *B. verbascifolia* the spongy parenchyma was also thicker (Fig. 3). The leaves of *B. verbascifolia* and *R. montana* were thicker (> 200 μm), while the leaves of *S. lycocarpum* were slender (< 200 μm) (Fig. 3).

Comparative Physiological Leaf Traits

The Fv/Fm did not vary between the species and generally presented higher mean values at the TCS. *R. montana* differed the most from the other species, but only two points were significantly different (Fig. 4A-C). *B. verbascifolia* had Fv/Fm higher than 0.80 at down, in both areas. No differences were observed between the areas along the day, but the values decreased the most at the CSRF, especially around 10:00 am (Fig. 4A). *R. montana* had Fv/Fm higher than 0.80 at dawn and remained stable during the day at the TCS, while the individuals from the CSRF had Fv/Fm under 0.80 at dawn and decreased considerably around 10:00 am (Fig. 4B). *S. lycocarpum* from both areas had Fv/Fm higher than 0.80 at dawn with little variations during the day. The values for this species did not decrease (~ 0.78) as occurred for the other two species at the CSRF, even during the period of highest light exposure (Fig. 4C).

The ΔF/Fm’ had little fluctuation along the day for the three species in both areas (Fig. 4D-F), with. *R. montana* presenting higher ΔF/Fm’ during the afternoon at the TCS (Fig. 4E), and the biggest drop in the values of ΔF/Fm’ along the day.

The contents of chlorophyll *a*, *b*, carotenoids, total chlorophylls, chlorophyll *a/b* ratio, and the total chlorophyll/carotenoids ratio were similar between the areas for two species (Table 1). *S. lycocarpum* had the higher content of chlorophyll *b*, and the lower chlorophyll *a/b* ratio at the TCS (Table 1).

Comparative Anatomical vs. Physiologial traits

The first two axes of the PCA explained 52.33% of the total variation, and separated the three species. *B. verbascifolia* and *R. montana* were also separated by the areas of occurrence (Fig. 5). The histometric data, with the exception of the adaxial epidermis, correlated negatively to the axis 1 (Table 2). The ΔF/Fm’ and Fv/Fm and the chlorophylls *a* and *b* correlated positively to the axis 1 of the PCA (Table 2), while the carotenoids had an inverse correlation (Table 2). The anatomical features approximated the most *S. lycocarpum* and *B. verbascifolia*, and the physiological features approximated the most *B. verbascifolia* and *R. montana* (Fig. 5).

Discussion

Anatomical traits

The values for the thickness of the leaves, of the adaxial epidermis, and of the chlorophyllous parenchyma were predominantly higher for the three species at the CSRF. Despite the similarity of the PPFD between the areas, the higher light radiation, especially after 4:00pm, and the higher frequency of fogs and clouds at the CSRF (Körner 2007) may have influenced such responses during the formation of the leaves. Sims & Pearcy (1992) affirmed that the thickness of the leaves is both determined by the light irradiance during leaf development and by little changes after the process of maturation, which could be observed in *B. verbascifolia* and *S. lycocarpum.*

The high thickeness of the leaves of *B. verbascifolia* and *S. lycocarpum* at the CSRF is due to the thicker adaxial epidermis and to the more elongated palisade parenchyma. Such type of cell growth homogenizes light distribution within the leaf and enhances the efficiency of the use of light (Vogelmann & Martin 1993). The thickness of the epidermal cells is influenced by light intensity (Coupe et al. 2006) and also by humidity (Serna & Fenoli 2000). This feature is particularly evidenced at the plants at the CSRF, and may act as a filter, concentrate and/or direct light towards the chlorophyllous parenchyma, as proposed by Dickson (2000).

Besides thicker adaxial epidermis and palisade parenchyma, *B. verbascifolia* also presented thicker spongy parenchyma at the CSRF, which is usually associated with enhanced stomatal conductance and photosynthetic rates (Flexas et al. 2008; Niinemets et al. 2009), due to the higher CO_2 flux and lower resistance of the mesophyll. The conductance of the mesophyll is measured by the diffusion of carbon dioxide from the subestomatic spaces until the chloroplasts for carboxylation (Flexas et al. 2012). Such conductance is finite and variable, and play fundamental role on the productivity of plants (Niinemets et al. 2009).

Physiological traits

The physiological functional traits of the leaves of the three species did not present great differences between the areas. However, *R. montana* presented lower quantum yield of the PSII and higher photoinhibition at the CSRF, and *S. lycocarpum* presented higher chlorophyll *b* content at the TCS. The main physiological feature of *R. montana* was evidenced by Lüttge et al. (1998) and Lemos-Filho (2000) with plants from the Neotropical savannah, including the rupestrian fields. As a novelty, *B. verbascifolia* and *S. lycocarpum* had a recovery at 5:00am, which did not occur to *R. montana*. This species had higher values of Fv/Fm at the TCS probably as a response to the shading of the population, while the continuous photoinhibition at the CRSF could be explained by the continuous and direct light radiation. As the most prominent effect, the photoinhibition of *R. montana* may have determined the absence of flower production during the period of study.

The higher production of chlorophyll *b* and lower chlorophyll *a/b* ratio at the TCS does which correlates the highest production of chlorophyll *b* occurs to shading, as an adaptive mechanism of light absorption at a different wavelength from that of chlorophyll *a* (Lüttge, 1997). On the other hand, higher levels of chlorophyll *b* alters the chlorophyll *a/b* ratio and is typicalof plants grown under lower light intensities

(Boardman 1977). These patterns of responses demonstrate that the production of photosynthetic pigments in *S. lycocarpum* may be susceptible to differences on the time of exposure to PPFD, with the production of chlorophyll *b* enhanced in plants grown under lower rates of PPFD by the end of the daylight.

Anatomical vs. Physiologial traits

Despite the modulation of the anatomical parameters, *B. verbascifolia* and *S. lycocarpum* did not present differences on the PSII quantum yield and consequently on the photosynthesis rate.

The abiotic factors of the studied areas lead to modifications on the leaf functional traits, especially for *B. verbascifolia* and *R. montana,* as evidenced by the tests of means and PCA, which separated them by the area of occurrence. The magnitude and type of response varied between species solely on the anatomical basis in *B. verbascifolia,* but *R. montana* and *S. lycocarpum* presented anatomical and physiological variations. *R. montana* presented higher physiological plasticity when compared to *S. lycocarpum*, but lower anatomical responses.

Conclusion

The little anatomical variations in *R. montana* should determine the difference on the quantum yield, which is an expected response. The thicker adaxial epidermis at the CSRF was a common response for the 3 species. The palisade parenchyma was thicker at the CSRF in *B. verbascifolia* and *S. lycocarpum,* while the spongy parenchyma was thicker only in *B. verbascifolia*. Thus, it has been observed that the higher the quantitative structural modifications the lower were physiological alterations.

These results also show clearly that the three species had different types and magnitude of responses.

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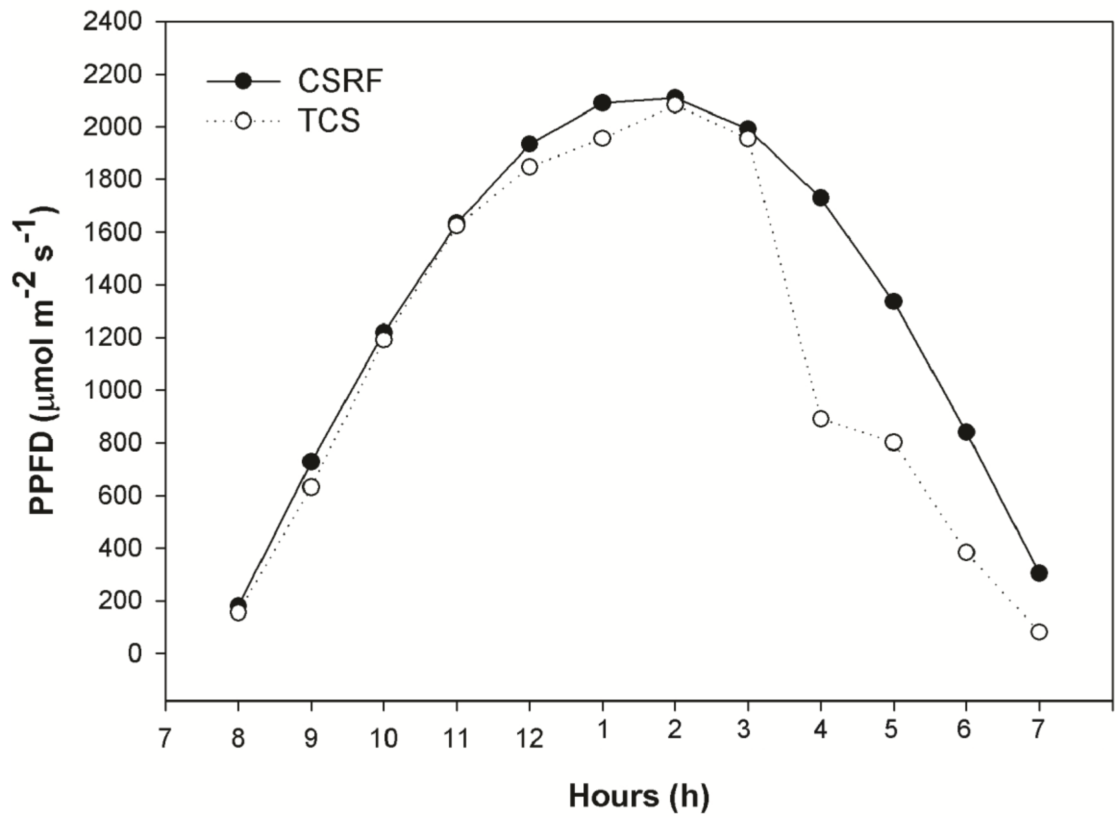

Figure 1. Photosynthetically active photon flux density (PPFD) at the *Campo Sujo* in Rupestrian Field (CSRF) and at the Typical *Campo Sujo* (TCS).

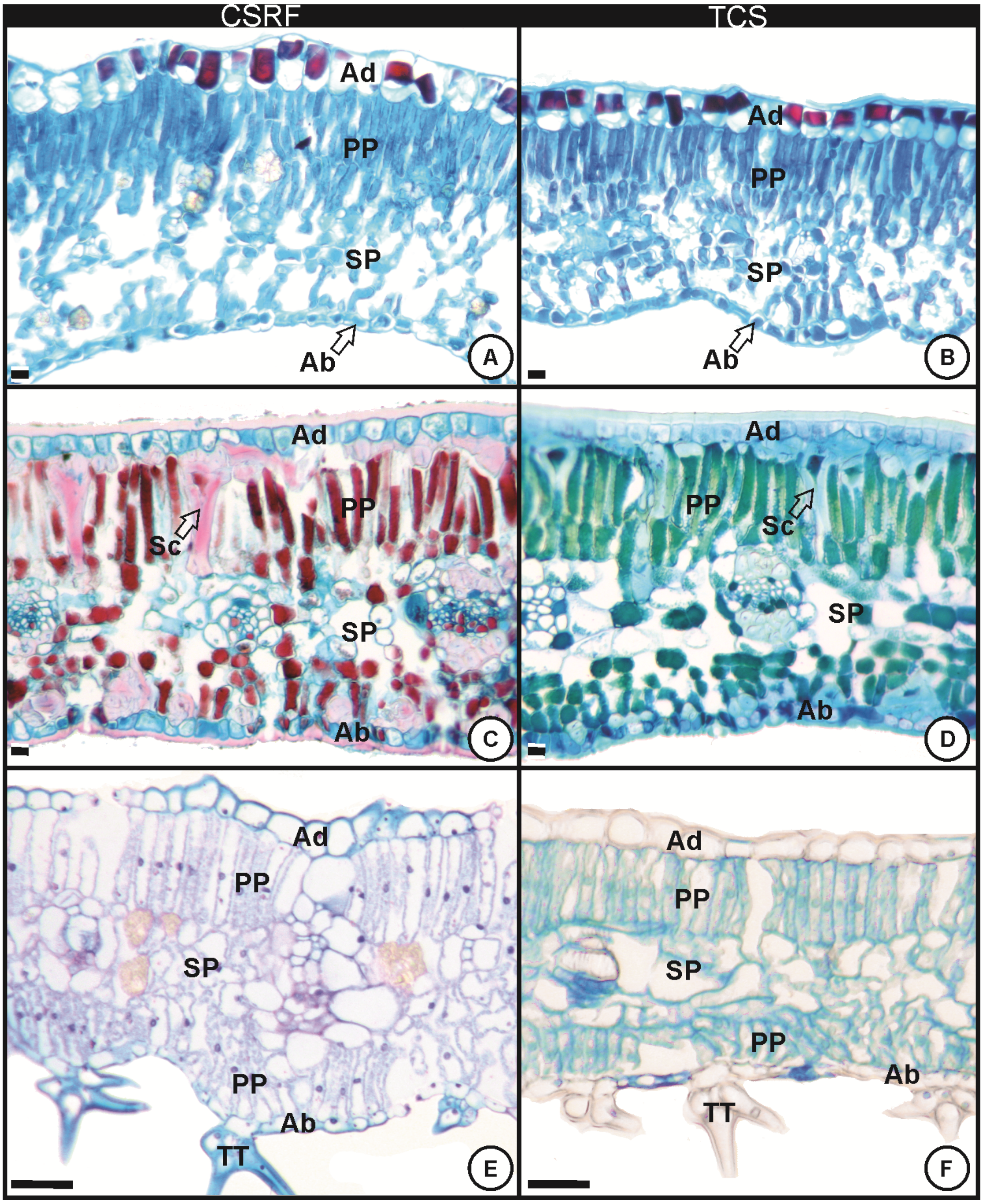

Figure 2. Transverse sections of the intercostal region of the leaves of *Byrsonima verbascifolia* (A-B), *Roupala montana* (C-D) and *Solanum lycocarpum* (E-F) from two altitudinal levels of the Neotropical savannah. Note: CSRF- *Campo Sujo* in Rupestrian Field (1.400m); TCS- Typical *Campo Sujo* (700m); Ad- Adaxial epidermis; PP- Palisade parenchyma; SP- Spongy parenchyma; Sc- Sclereid; TT- Tector trichome, Ab- Abaxial epidermis. Bar=50 μm.

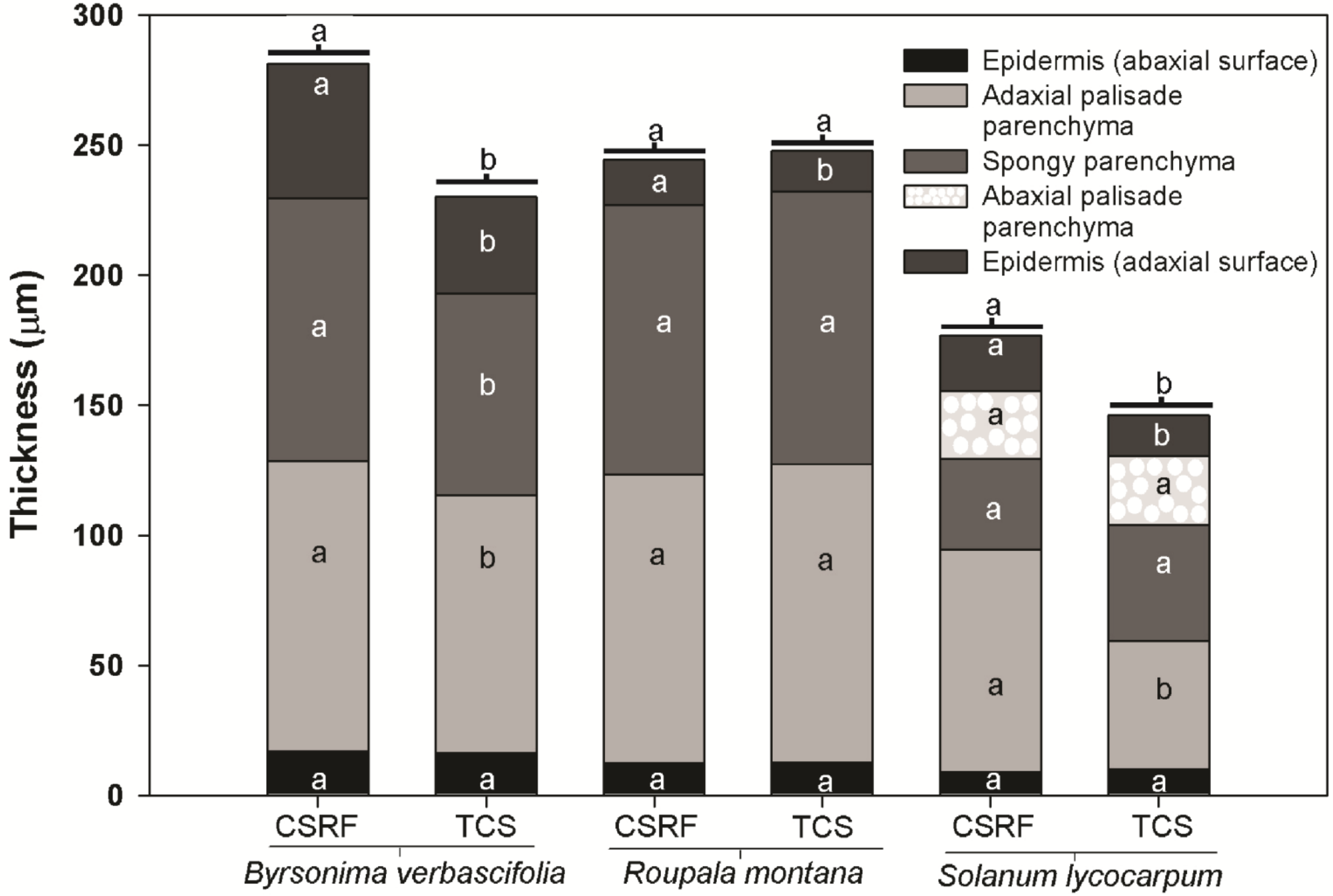

Figure 3. Thickness of the leaf tissues of *Byrsonima verbascifolia*, *Roupala montana* and *Solanum lycocarpum* at two altitudinal levels in the Neotropical savannah. Note: CSRF- *Campo Sujo* in Rupestrian Fields (1.400m); TCS- Typical *Campo Sujo* (700m). *Means followed by the same letters do differ statistically.

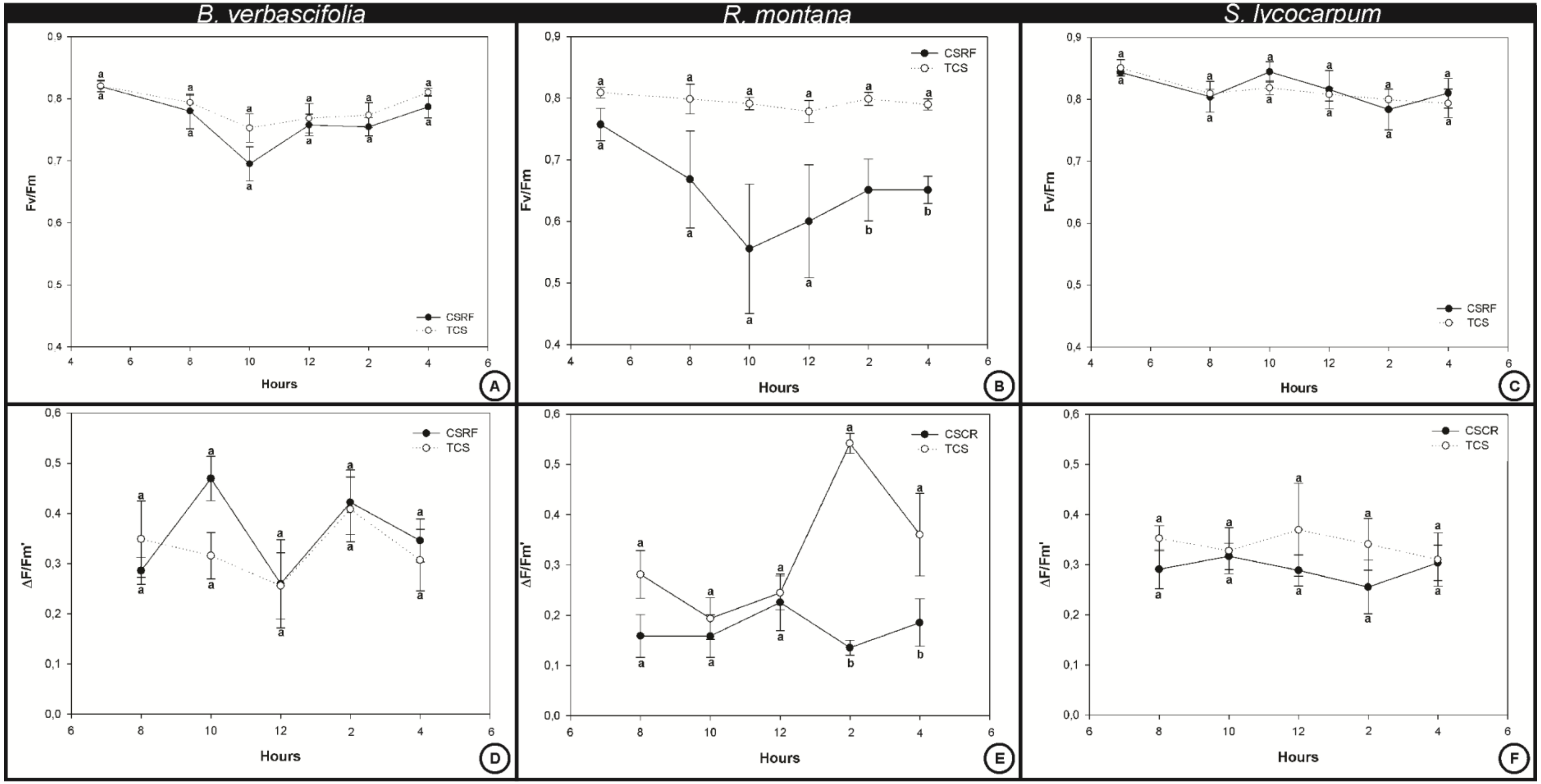

Figure 4. Mean effective quantum yield (A-C) and potential quantum yield (D-F) ± EP of *Byrsonima verbascifolia* (A, D), *Roupala montana* (B, E) and *Solanum lycocarpum* (C, F) at two altitudinal levels from the Neotropical savannah. Note: CSRF- *Campo Sujo* in Rupestrian Field (1.400m); TCS- Typical *Campo Sujo* (700m). *Means followed by the same letters do differ statistically.

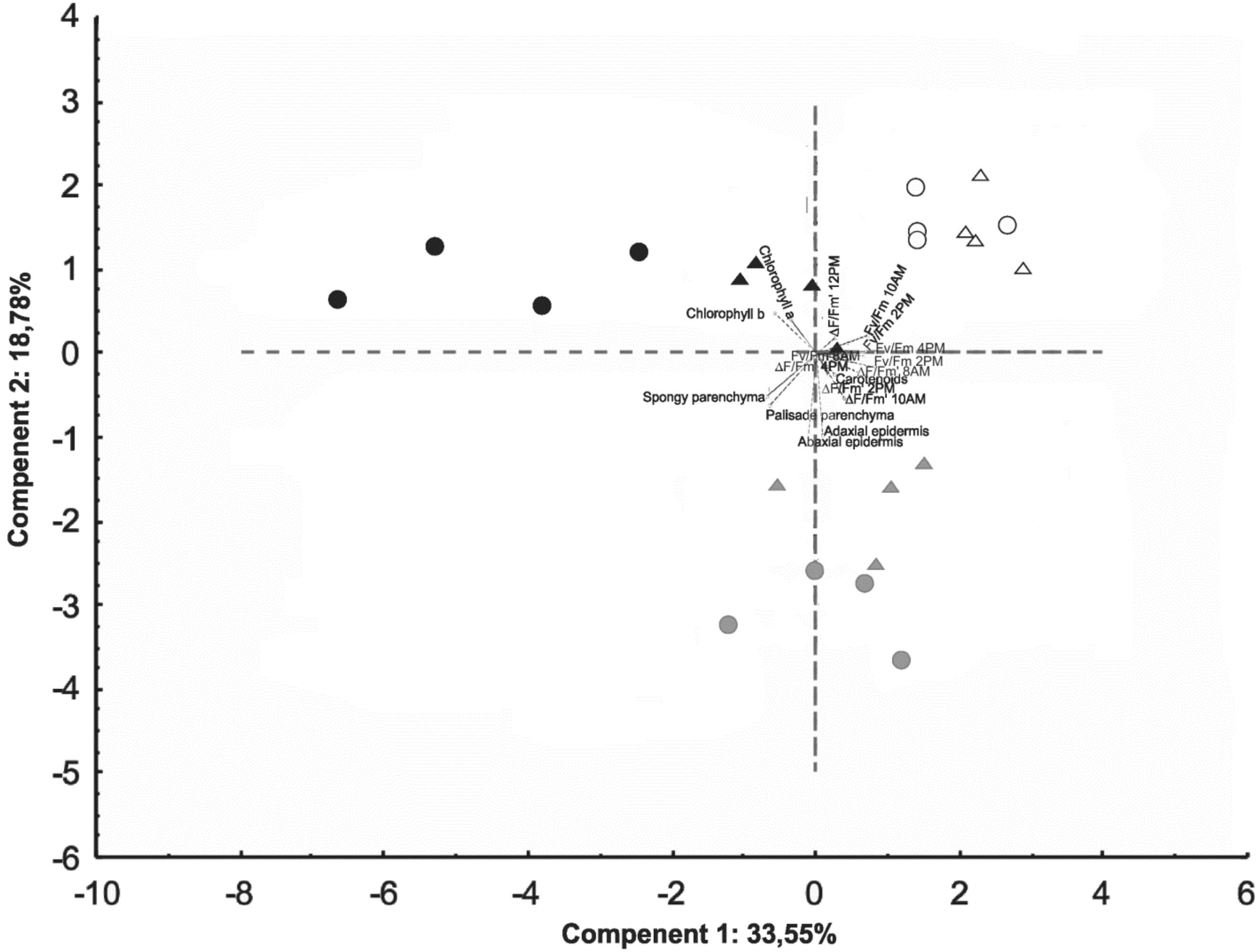

Figure 5. Scores of the two first axes of the principal component analysis (PCA) obtained from mofphophysiological traits of the leaves of *Byrsonima verbascifolia* (gray symbols), *Roupala montana* (white symbols) and *Solanum lycocarpum* (black symbols) occurring at the *Campo Sujo* in Rupestrian field (circles) and Typical *Campo sujo* (triangles) from the Neotropical savannah.

Table 1. Photosynthetic pigments of *Byrsonima verbascifolia*, *Roupala montana* and *Solanum lycocarpum* at two altitudinal levels from the Neotropical savannah. Note: CSRF- *Campo Sujo* in Rupestrian field (1.400m); TCS- Typical *Campo Sujo* (700m); Chl. – Chlorophyll; Carot.- Carotenoids. * Means followed by the same letters do differ statistically.

Species	**Environments / Pigments (mg/cm^2)**											
	CSRF	**TCS**	**CSRF**	**TCS**	**CSRF**	**TCS**	**CSRF**	**TCS**	**CSRF**	**TCS**	**CSRF**	**TCS**
	Chl. *a*		Chl. *B*		Carot.		Total Chl.		Chl. *a*/ Chl. *b*		Total Chl. / Carot.	
Byrsonima verbascifolia	7,62 ± 0,64 **a**	7,71 ± 0,45 **a**	4,26 ± 0,37 **a**	4,44 ± 0,37 **a**	507,47 ± 8,64 **a**	490,55 ± 24,51 **a**	519,36 ± 9,51 **a**	502.72 ± 24,65 **a**	1,79 ± 0,02 **a**	1,75 ± 0,11 **a**	1,02 ± 0,002 **a**	1,02 ± 0,002 **a**
Roupala montana	9,02 ± 0,59 **a**	10,02 ± 0,84 **a**	6,70 ± 0,64 **a**	6,86 ± 0,68 **a**	456,99 ± 23,40 **a**	427,76 ± 41,12 **a**	472,71 ±24,05 **a**	444,65 ± 42,25 **a**	1,36 ± 0,10 **a**	1,47 ± 0,10 **a**	1,03 ± 0,002 **a**	1,03 ± 0,002 **a**
Solanum lycocarpum	8,39 ± 0,45 **a**	10,56 ± 0,91 **a**	5,21 ± 0,29 **a**	7,76 ± 0,39 **b**	483,32 ± 42,68 **a**	538,38 ± 50,36 **a**	496,93 ± 43,39 **a**	556,71 ± 51,65 **a**	1,61 ± 0,03 **a**	1,35 ± 0,053 **b**	1,28 ± 0,001 **a**	1,03 ± 0 **a**

Table 2. Principal components of the populations of *Byrsonima verbascifolia*, *Roupala montana* and *Solanum lycocarpum* obtained from the matrix of correlation of the leaf parameters at two altitudinal levels from the Neotropical savannah. Note: ΔF/Fm' - Effective quantum yield; Fv/Fm- Quantum yield potential.

Parameters	**Component 1**	**Component 2**
Adaxial epidermis	0,083526	0,958906
Abaxial epidermis	-0,117052	-0,939359
Palisade parenchyma	-0,674899	-0,610113
Spongy parenchyma	-0,715183	-0,495500
ΔF/Fm' 8am	0,593534	-0,216969
ΔF/Fm' 10am	0,400354	-0,521728
ΔF/Fm' 12pm	0,269244	0,193492
ΔF/Fm' 2pm	0,255202	-0,242230
ΔF/Fm' 4pm	0,461126	-0,074731
Fv/Fm 5am	0,875290	0,009889
Fv/Fm 8am	0,678034	-0,020638
Fv/Fm 10am	0,743841	0,220670
Fv/Fm 12pm	0,846375	0,018792
Fv/Fm 2pm	0,702867	0,032086
Fv/Fm 4pm	0,817398	-0,154068
Chlorophyll a	-0,371564	0,407908
Chlorophyll b	-0,604903	0,484129
Carotenoids	0,162562	-0,252675
Variation explained (%)	**33,55%**	**18,78%**

Capítulo II

Morphophysiological responses of plants to distinct altitudes in the Neotropical savannah: Water parameters

Abstract

Rupestrian fields are located above 900 meters of altitude and have many endemic and endangered plant species. Also, they may present patches of *campo sujo* (shrub savannah), which is a phytophysiognomy commonly found in Neotropical savannah at lower altitudes. The altitudinal gradient observed on the distribution of *campo sujo* areas allowed the studied of the influence of abiotic factors on the morphophysiology of plant species. Tree models species were selected and used leaves during the rainy season of 2012. The abiotic conditions of the *campo sujo* in higher altitudes are less stressfull than that in the lower altitudes. In higher altitude the anatomical data varied little, while the physiological data presented ample variation. The hypothesis that the *campo sujo* in higher altitudes would be more limiting was not corroborated. A high morphophysiological changes between the areas, but with different strategies among species models, and consequently the plants of these environments did not show the same a pattern of responses.

Keywords: *Byrsonima verbacifolia*, *Campo sujo*, *Roupala montana*, rupestrian fields, *Solanum lycocarpum.*

Introduction

Brazilian Neotropical savannah is known as *Cerrado* and include the rupestrian fields according to Menezes and Giulietti (1986), which strictly occur above 900 meters of altitude, with variable topology and steepness (Giulietti et al., 2002). Litholic neosoils are predominant at these sites (Schaefer et al., 2009), which also present extreme humidity and temperature variations, besides high irradiation levels and strong and frequent winds (Giulietti et al., 2002). Rupestrian fields present many endemic and endangered plant species (Mendonça and Lins, 2000).

Two different annual seasons occur in the Neotropical savannah, a dry season normally from April to September, and a rainy season normally from October to March (Klink and Machado, 2005). The success of a given plant species in such a seasonal environment is strictly related to the capacity of maintaining adequate water balance during dry periods, which sustains the balance of carbon intake (Franco 2002). Perez and Moraes (1991) had also evidenced that water potential and stomatal conductance highly decreased during dry season, but were restored to normal levels after water was available in the soil.

The physiological performance of the plants in the Rupestrian fields are related both to plant morphology and anatomy, which directly modulate growth rates and plant survival (Chazdon and Kaufmann, 1993; Grassi and Bagnaresi, 2001; Hanba et al., 2002). For such survivorship, the control of transpiration flux accordingly to the number and degree of ostiole aperture are crucial (Hetherington and Woodward, 2003). This control seems to occur on the leaves of *Miconia albicans*, where the stomatal density changed among populations from distinct phytophysiognomies of the Cerrado during dry and rainy season. The production of trichomes, and the thickness of epidermis, cuticle and chlorophyllous parenchyma were also affected under these conditions (Bedetti et al., 2011).

The disjunct pattern of distribution of soil types constitutes another probable factor influencing the establishment of plants in different areas of the Rupestian fields. Litholic humic neosoils are frequently found, but red-yellowish latosol (oxisol) are also reported to occur (Schaefer et al., 2009). The red-yellowish latosol occurs on the patches of short woodlands, in areas of *Cerrado sensu stricto* (Moura et al., 2010) and *Campo sujo* (Vitta, 1995) with similar floristic structure. *Campo sujo* is characterized by the predominance of herbs and sparse short-sized trees (Coutinho, 1978), and was reported to occur in high lands within Rupestrian fields (up to 1.400 m), but also in lower altitudes (700 m) (Coutinho, 1978). The premise that the type of the soil together with the other abiotic factor will influence on the floristic structure of a given area is herein tested based on the comparison of the morphophysiology of leaves. The morphology and physiology of *Byrsonima verbascifolia*, *Roupala montana* and *Solanum lycocarpum* are analyzed on two areas with distinct altitudes, on the perspective of checking the alterations affected by water parameters of each environment.

Material and methods

The study was conducted at Serra do Cipó, within the mountain range of Cadeia do Espinhaço, Minas Gerais state, Brazil. The two collection areas are located 100Km northeast from Belo Horizonte, and characterized as typical *Campo sujo* (TCS) *sensu* Coutinho (1978) at the altitude of 700 meters (19°22’01’’S and 43°37’10’’W), and a *Campo sujo* in Rupestrian field (CSRF) at the altitude of 1400 meters (20º01’90”S and 43°34’105”W).

Fertile branches of *Byrsonima verbascifolia* (L.) Rich. (Malpighiaceae), *Roupala montana* Aubl. (Proteaceae) and *Solanum lycocarpum* A. St.-Hil. (Solanaceae) were collected at both study areas and deposited in the Hebarium of Universidade Federal de

Minas Gerais (BHCB) under the registration numbers: 161583 and 161587. Samples of *Roupala montana* at CSRF were not deposited as the accompanied individuals did not produce flowers during the period of analyses.

Environmental, physiological and anatomical parameters were assessed during sunny days on the rainy season (February 2012), since most of the sampled individuals produced new leaves strictly during this period. Temperature (°C), and air humidity (%) were measured using a Datta Logger LI-COR model LI-1400, which recorded the values every 5 seconds (means *per* hour) from 8:00 am to 4:00 pm. The measurements were done during the same days of the ecophysiological assessements.

The soil was sampled at the CSRF and TCS from the 0 a 100 mm profile, at five sites with no vegetation covering. The samples were homogeneized in plastic bags and sent to the laboratório de solos of the Universidade Federal de Viçosa. The results were compared to Alvarez *et al.* (1999) for cultivated plants.

The area of 5 leaves from the third nodes of 6 individuals of each species from both sites was measured using the software Image Pro-Plus, version 4.1 for Windows® (Media Cybernetics).

For anatomical analyses, samples (n = 5) were fixed in F.A.A (formalin, glacial acetic acid, ethanol 50%, 1:1:18 v/v), stored in 70% ethanol (Johansen, 1940) and processed for light microscopy. Permanent slides with transverse sections of 2 leaves of the third nodes from 5 individuals of each species occurring on both sites were mounted in Entellan®. Fragments of epidermis were detached using 10% sodium hypoclorite, and stained with 1% safranin (Kraus and Arduin, 1997). Images were captured in a photomicroscope Zeiss® Primo Star coupled with a camera Canon model A650.

Leaf fragments of the midrib portions were embedded either in 2-hydroxyethyl methacrylate Resin Leica® or Paraplast® (Kraus and Arduin, 1997), and sectioned in a rotatory microtome. Resin embedded sections (5 µm) were stained with 0,05% toluidine blue, pH 4,7 (O'Brien et al., 1964). Paraplast® embedded sections (10 µm) were stained with 0,5% safranin and Astra blue, 2:8 (Kraus and Arduin, 1997). Photomicrographs of the transverse sections were used for histometry.

Data on stomatal density (stomata/mm²), stomatal index (Cutter, 1978), area of the midrib tissues (occupied by vessel elements, sieve elements and fibers), were obtained with the software Image Pro-Plus®. Stomatal quantification was based on the abaxial surface of the leaf epidermis of *B. verbascifolia* and *R. montana*, and on the adaxial surface of the leaf epidermis of *S. lycocarpum,* as the abaxial leaf surface of this species is deeply hairy.

Measurements of water potential (Ψ) and stomatal conductance (Gs) were taken on the field from leaves of the third node in four individuals of each species. Water potential was evaluated using leaves collected before *predawn*, at *midday* and also by the end of the daylight (between 4:00–5:00pm) using a PMS pressure chamber, model 1000. The diameter of the petioles of *B. verbascifolia* did not fit the equipment and could not be evaluated. Stomatal conductance was measured using a porometer LI-COR model LI-1600, between 8:00 am and 4:00 pm.

Data were analyzed with JMP 5.0 (SAS Institute) after being tested for normality (Shapiro-Wilk's test) and homogeneity (Levene's test). T test was applied to normal data and Dunnett's test was used for non-normal data at 5% of significance. Principal components analysis (PCA) used a matrix of correlation between all variables, using the software STATISTICA 7. Data were logarithmic transformed to attend to parametric analyses (Zar, 1996).

Results

Environmental parameters

The CSRF presented lower temperatures and higher relative humidity during the day, with the exception of the humidity value taken at 8:00 am, which was 3% lower than that at the TCS (Fig. 1A-B). The difference in temperature was higher at 8:00 am (12º C), and maintained a difference of 9º to 10º C along the day (Fig. 1A). The humidity varied the most at 9:00 am, but presented more proximate values around 4:00 pm (Fig. 1B). The soil of the CSCR was classified as red latosoil, and that of the TCS was classified as quartzarenic neosol. The nutrient content of the soils of both sites were similar, based on the classification of Alvarez et al. (1999) (Table 1). The levels of potassium andd zinc were the most distinct (Table 1).

Morphoanatomical traits

The leaf area of *R. montana* was significantly smaller on the CSRF. The other two species presented variation on leaf size between the sites (Fig. 2A), with *B. verbascifolia* presenting a wide range of variation (108 cm^2 to 771 cm^2).

Stomata position on leaf surface, types of trichomes, and sinuosity of the anticlinal cell walls of the ordinary epidermal cells were similar among the plants and sites (Fig. 3A-L). *B. verbascifolia* (Fig. 3A-D) and *R. montana* (Fig. 3E-H) were hypostomatic, with paracytic stomata and straight to sinuous anticlinal cell walls on both leaf surfaces (Fig. 3A-H). The non-glandular trichomes of *B. verbascifolia* were long, branched, and occur on the whole leaf surface (Fig. 3A-D). *R. montana* presented calcium oxalate crystals in each ordinary epidermal cell on both leaf surfaces at the TCS, which was rarely observed on the plants of the CSRF (Fig. 3E-H). *S. lycocarpum* was amphistomatic with mainly anisocytic and eventually paracytic stomata (Fig. 3I-L). The anticlinal cell walls of the ordinary epidermal cells on the adaxial surface were straight to slightly sinuous, but conspicuously sinuous on the abaxial surface (Fig. 3I-L). The non-glandular trichomes were stellate and concentrated on the abaxial leaf surface (Fig. 3I-L). Stomatal density was lower for *R. montana* and higher for *S. lycocarpum* from the CSRF, but not significantly different for *B. verbascifolia* between the sites (Fig. 2B). Stomatal index did not vary significantly between the sites for *R. montana*, but it was higher for *B. verbascifolia* and *S. lycocarpum* from the CSRF (Fig. 2C).

The vascular tissues at the midrib were structurally similar in the species from both sites (Fig. 4A-F), except for the presence of phloem-associated fibers in *S. lycocarpum* from the TCS (Fig. 4F). *B. verbascifolia* (Fig. 4A-B) and *R. montana* (Fig. 4C-D) presented ramified vascular system with collateral bundles, which were concentrically arranged in *B. verbascifolia* (Fig. 4A-B). The midrib of *B. verbascifolia* presented parenchymatic pith with included amphixylic bundles (Fig. 4A-B).

All parameters evaluated for the vascular system of *B. verbascifolia* from both sites were significantly different, with the lowest values on plants from the CSRF. *R. montana* and *S. lycocarpum* presented an inverted tendency, with the highest values on the plants from the CSRF (Fig. 5A).

Physiological analyses

Individuals from the CSRF generally presented lower diary variation on the stomatal conductance, and lower absolute values, with the exception of *B. verbascifolia* from the TCS, which presented the lowest values at 4:00 pm (Fig. 5B-D). *R. montana* and *S. lycocarpum* presented the higher difference on the stomatal conductance between the sites at the beginning of the morning, but similar values by the end of daylight (Fig. 5C-D).

Water potential was significantly different for *R. montana* and *S. lycocarpum* from both sites, as plants from the CSRF presented lower values at *predawn* and higher ones from 4.00 pm to 5.00 pm. Both species presented similar values at *midday* (Fig. 5E-F). The individuals of *R. montana* from the CSRF started to recover their water potential at *midday*, while the ones from the TCS still presented no recovering sings (Fig. 5E). *S. lycocarpum* from the TCS presented the most negative values at *midday*, but the individuals from both sites presented recovering between 4.00-5.00 pm (Fig. 5F). *R. montana* presented the lowest water hydric potential when compared to *S. lycocarpum*, reaching values near -2,8 MPa at *midday*, while *S. lycocarpum* from the TCS reached -1,4 MPa (Fig. 5E-F).

Multivariate analysis

The two first axes of the PCA explained 64% of the total variation, which separated all four studied groups (Fig. 6). The populations of *S. lycocarpum* was the closest the others. Histometric and leaf area parameters were similiar and positively correlated to the two first axis, with the exception of the stomatal density and the area of fibers (Table 2). As far as the physiological data are concerned, only the Ψ (*predawn*), the Gs at 8.00 am, and the Gs at 2.00 pm were negatively correlated with the axis 1 (Table 2).

Discussion

Environmental parameters

The abiotic conditions of the CSRF are less stressfull than that of the TCR, but the individuals of the three species had the greatest physiological variation at the CSRF. Both areas had structurally distinct soils, but with similar nutrients composition. The anatomical data varied little, while the physiological data presented ample variation on the plants of the CSRF.

The low temperatures at the altitute of 1.400 meters was similar to those of other environments at high altitudes (Körner, 2007), nevertheless the lower relative humidity at such site was unexpected. The high variation on the climatic conditions at *Serra do Cipó* depends not only on the differences in altitudes but on the position of the mountains (França and Ribeiro 2008), as should be noted at the CSRF over the TCR In fact, the inexistence of global patterns on the humidity and radiation parameters for high altitude environments (Körner 2007), and could have been influenced by the differences in water availability, and the constant clouds concentrated near the mountais on the CSRF.

Structural adaptive features

Morphoanatomical aspects are diretly related to the environmental conditions to which the plant organs are subjected to during their development (Dickison, 2000), while physiological traits tend to vary due to minor variations observed during the days and along the year (Larcher, 2000). The expansion of leaf lamina is regulated by sunlight (Muroya et al. 1997, Niinemets et al. 1998, Rôças et al. 2001) which was observed only for *R. montana,* whose leaf area was higher for the individuals of the TCS than for those of the CSRF. Peculiarly, the higher leaf area was determined by the end of daylight due to the shading of lower areas by the mountains of *Serra do Cipó* nearby the typical *campo sujo* areas.

The data on number and index of stomata and ordinary epidermal cells indicated uniform response of *B. verbascifolia* (abaxial surface) and *S. lycocarpum* (adaxial surface) on both sites, even with the highest values at the TCS. Epidermal cells are known to vary in response to as humidity (Serna and Fenoli, 2000), light intensity (Lake et al., 2001; Coupe et al., 2006) and temperature (Teskey et al., 1987), among other factors. So, differences on water availability between the areas are believed to have affected the turgor pressure of plant cells, that may grow larger at the CSRF than at the TCS, as reported by Pessoa and Calbo (2004).

The three model plant species presented leaf strategies commonly related to mesic or xeric environments. Wide and long vessel elements (Bresta et al., 2011) that occupy minimal area (Larcher, 2000) constitute an efficient type of xylem, which has been evolutionarily selected in mesophytes. On the other hand, a type of xylem which best resists to cavitation, with narrow and short vessel elements occurs in plants from xeric environments (Bresta et al., 2011), that occupy large tissue area (Larcher, 2000). The highest area occupied by vessel elements observed on the individuals of *B. verbascifolia* from the CST follows the pattern described for plants from mesic environments. This group of plants usually present higher leaf area and stomatal conductance (Farquhar and Sharkey, 1982), which together with leaf vascularization, controls water flow inside leaf tissues (Franco, 2008). Nevertheless, *R. montana* and *S. lycocarpum* produced less xylem on similar environmental conditions, following the pattern for xeric environments, which indicates that unidentified factors may influence this response.

The absence of phloem fibers in the third node leaves of *S. lycocarpum* from the CSRF indicates that there might be a temporal difference on the ontogenesis of such tissues on the studied populations. Phloem fibers differentiated earlier on the individuals of *S. lycocarpum* from the TCS, which favors leaf rigidity to avoid withering. This differentiation should be attributed to different abiotic stresses, such as mineral deficiency, drought, UV-B radiation, and low temperatures, which are believed to cause changes in the lignin contents of several plants (Cabane et al. 2012), and may have influenced the fibers *S. lycocarpum.*

Physiological behaviors

Despite the similiarity in epidermal data, the stomata seemed to close at critical moments, as on the TCS by the end of daylight. This morphological strategy possibly occurred due to the lower humidity on the TCS when compared to the CSRF, and is independent of water availability, as proposed by Meinzer et al. (1999) and, Mattos et al. (2002). The lower absolute values of stomatal conductance of the individuals of the CSRF, even under higher humidity, was probably due to the windy conditions at the rupestrian fields (Giulietti et al., 2002), which reduce the limitroph air layer on the leaves, thus affecting stomatal conductance.

The stomatal conductance of the three model plant species herein studied presented divergent patterns, with slight variations at the beginning and at the end of the daylight. At the tropical savannah, for instance, lower values of leaf area and xylem area are believed to buffer fluctuations on daily stomatal conductance and water potential (Bucci, 2001). Such results are most probably due to different values of water potential during the same period under particular environmental conditions. The elevated values of water potential at the *predawn* on the TCS should be related to higher rates of stomatal conductance. On the other hand, the recovering of water potential at the end of the daylight on the individuals from the CSRF should be explained by the higher wateravailability at this site.

Integrative analysis

The modulation of morphophysiological traits by abiotic factors was demonstrated to occur on the individuals of *R. montana* e *S. lycocarpum*, which could be distinguished on the PCA analyses due to their site of occurrence. Nevertheless, the populations of *S. lycocarpum* seemed to be less affected by the abiotic parameters of the different sites when compared to *R. montana*. Current results on anatomical similarities but with histometric differences are similar to those of Bedetti et al. (2011) for populations of *Miconia albicans* occurring at *Campo sujo*, *Cerrado sensu stricto* and *Cerradão*, which could also be distinguished only by histometric traits during rainy season.

The hypothesis that the CSRF would be more limiting was not corroborated. A high morphophysiological changes between CSRF and TCS, but with different strategies among species models, and consequently the plants of these environments did not show the same a pattern of responses.

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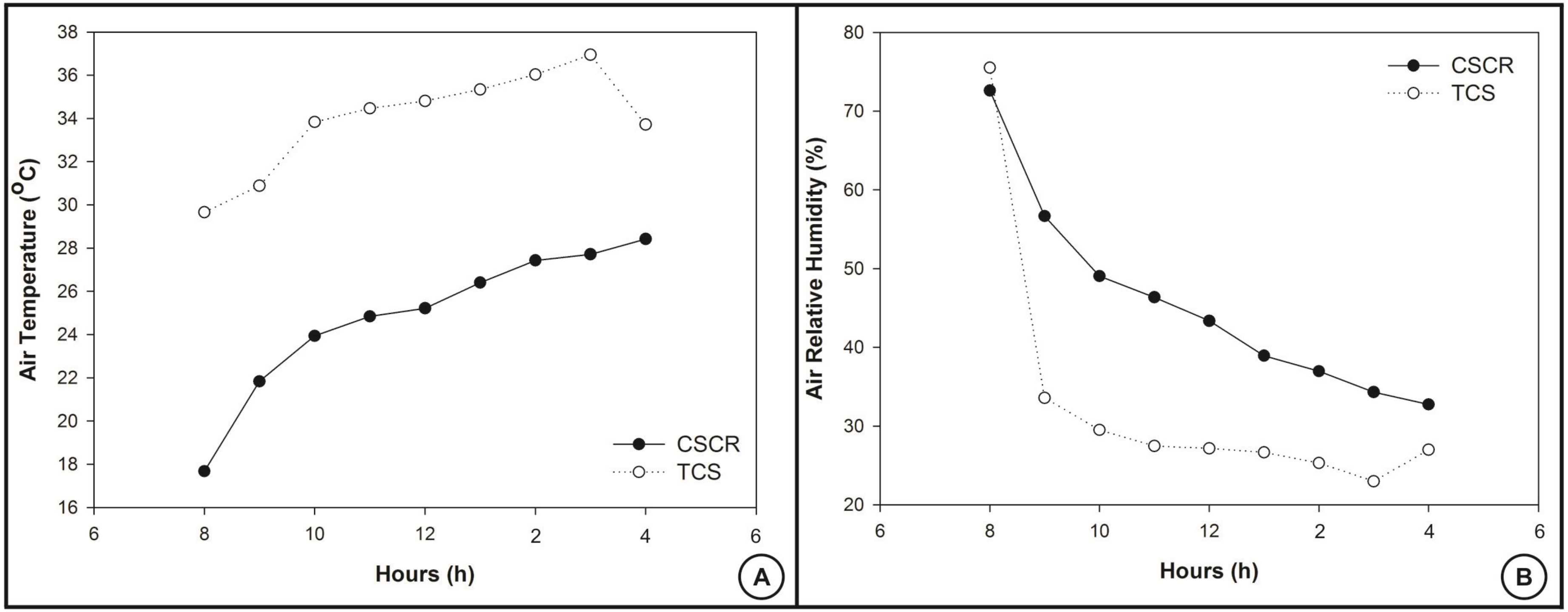

Figure 1. Abiotic parameters at two altitudinal levels from the Neotropical savannah. A- Air temperature; B- Air humidity; CSRF - *Campo Sujo* in Rupestrian field; TCS – Typical *Campo sujo*.

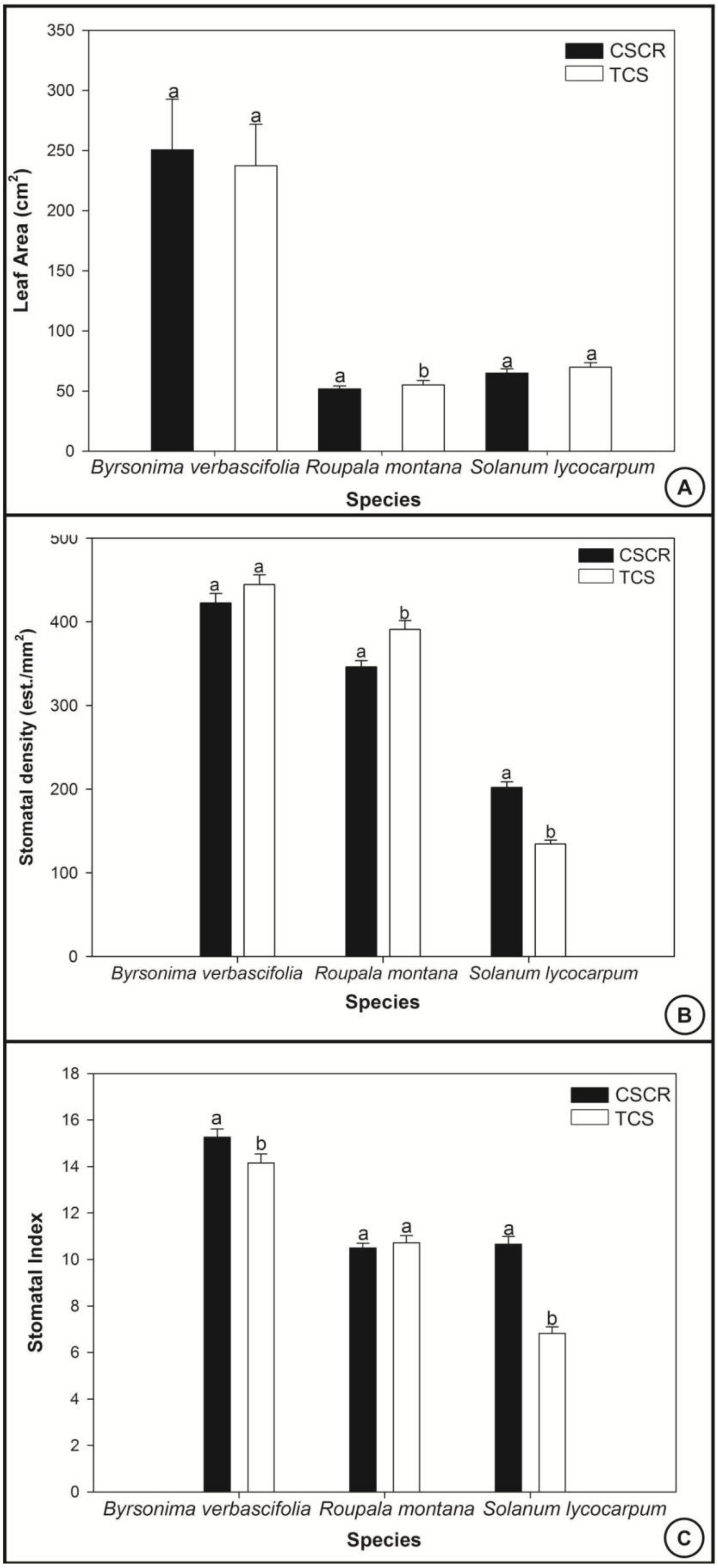

Figure 2. Morphoanatomical variables of three plant species at two altitudinal levels from the Neotropical savanna. Note: A – Leaf area ± SE; B- Stomatal density ± SE; C- Stomatal index ± SE; CSRF - *Campo Sujo* in Rupestrian field; TCS – Typical *Campo sujo*. * Means followed by equal letters do not differ statistically.

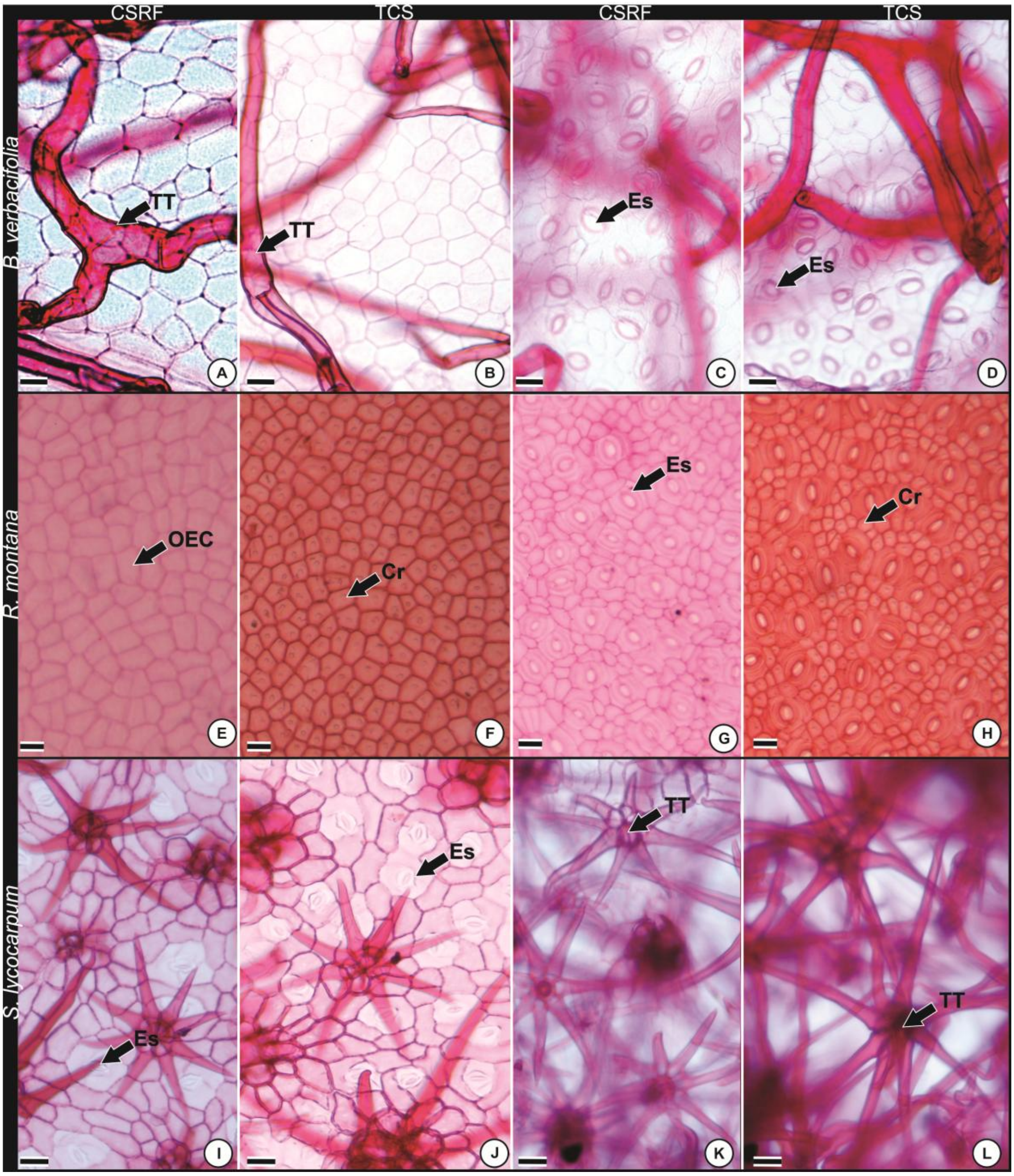

Figure 3. Frontal view of the epidermis from the adaxial (A-B/E-F/I-J) and abaxial leaf surfaces (C-D/G-H/K-L) of three species at two altitudinal levels from the Neotropical savannah. A-D- *B. verbascifolia*; E-H- *R. montana*; I-L- *S. lycocarpu*. Note: CSRF - *Campo Sujo* in Rupestrian field; TCS – Typical *Campo sujo*; TT- Tector trichome; St- Stomata; OEC- Ordinary epidermal cell; Cr- Crystal. Bars=50 µm.

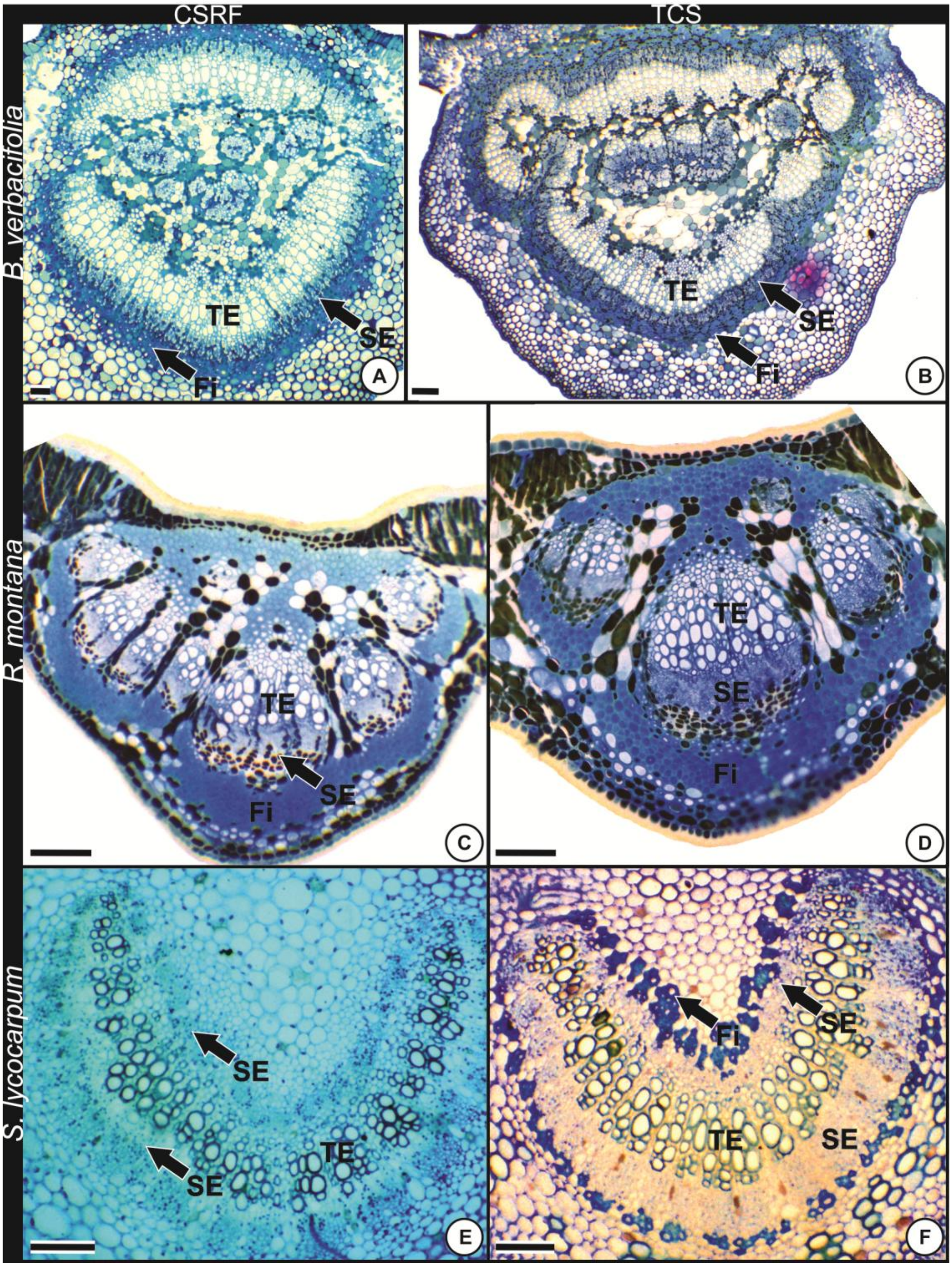

Figure 4. Cross sections of the midrib of *B. verbascifolia* (A-B), *R. montana* (C-D) and *S. lycocarpum* (E-F) at two altitudinal levels from the Neotropical savannah. Note: CSRF - *Campo Sujo* in Rupestrian field; TCS – Typical *Campo sujo*; SE- Sieve elements; TE- Tracheary elements; Fi- Fibers. Bars=100 µm.

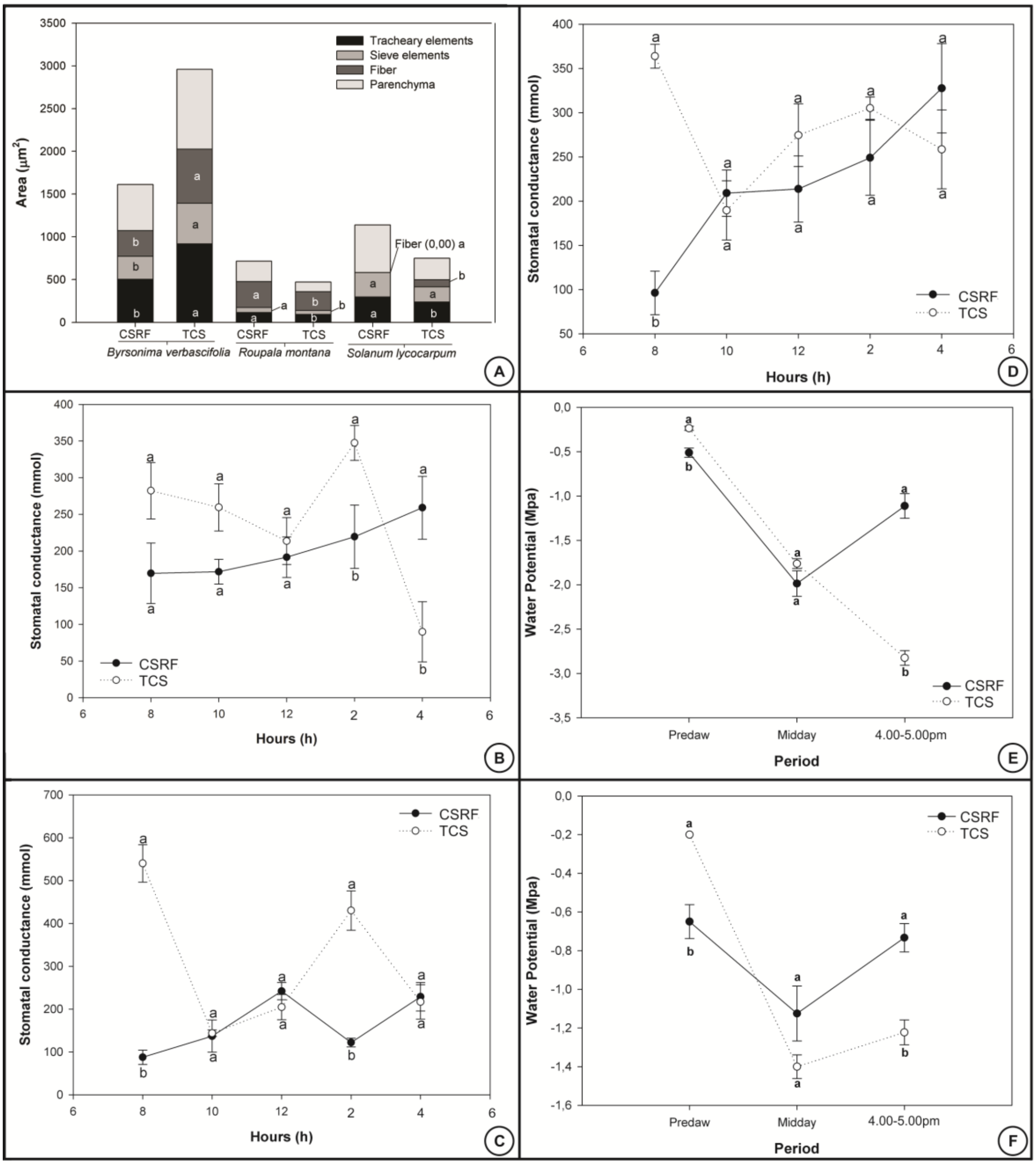

Figure 5. Mean area of the midrib tissues (A), stomatal conductance ± SE (B-D) and hydric potential ± SE (E-F) of three species at two altitudinal levels from the Neotropical savannah. B- *B. verbascifolia*; C/E- *R. montana*; D/F- *S. lycocarpum*. Note: CSRF - *Campo Sujo* in Rupestrian field; TCS – Typical *Campo sujo*. * Means followed by equal letters do not differ statistically.

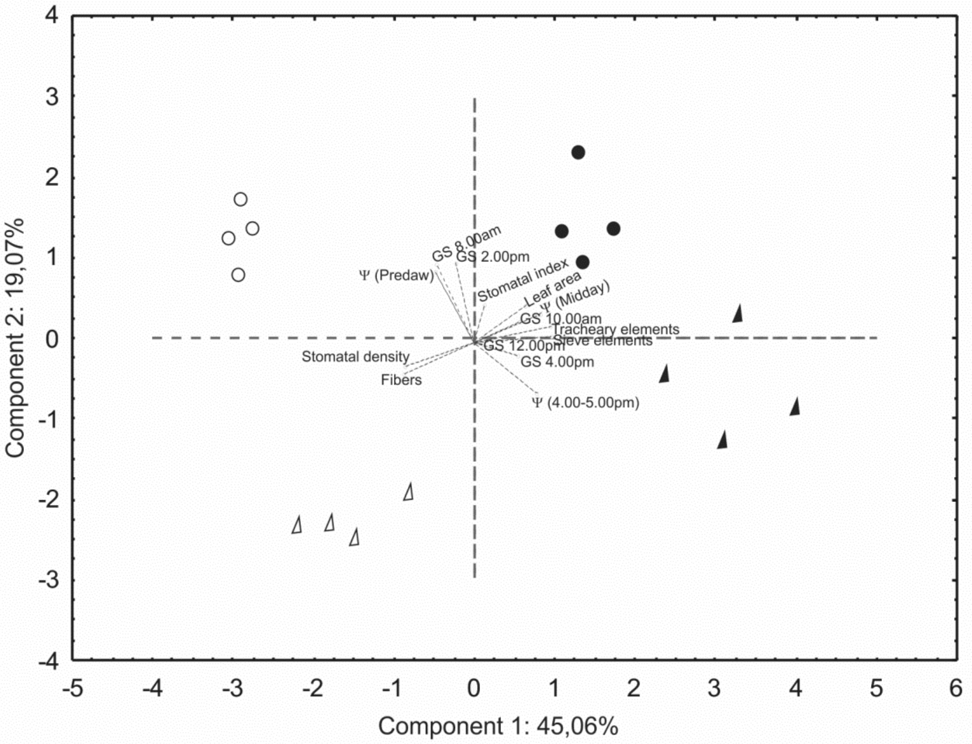

Figure 6. Relation of scores from the two first axes of the principal component analysis (PCA) obtained from morphophysiological leaf traits of *R. montana* (white symbols) and *S. lycocarpum* (black symbols) occurring at *Campo Sujo* in Rupestrian field (Circles) and Typical *Campo sujo* (Triangles).

Table 1. Availability of chemicals of the soil at two altitudinal levels from the Neotropical savanna, according to Alvarez et al. (1999). Note: CSRF - *Campo Sujo* in Rupestrian field; TCS – Typical *Campo sujo*.

Parameters	**CSCR**	**Alvarez et al. (1999)**	**CST**	**Alvarez et al. (1999)**
pH	5,08	High acidity (4,5-5,0)	4,34	Very high acidity (<4,5)
P	0,8	Very low (≤12,5)	1,9	Very low (≤12,5)
K	46	Medium (41-70,5)	15	Very low (≤15)
Ca^{2+}	0,23	Very low (≤40)	0,18	Very low (≤40)
Mg^{2+}	0,07	Very low (≤0,15)	0,04	Very low (≤0,15)
Al^{3+}	0,88	Medium (0,51-1,00)	0,88	Medium (0,51-1,00)
H+Al	7,6	Very low (≤1,00)	4,2	Very low (≤1,00)
SB	0,42	Very low (≤0,60)	0,28	Very low (≤0,60)
(t)	1,30	Low (0,81-2,40)	1,16	Low (0,81-2,40)
(T)	8,02	Medium (4,31-8,60)	4,48	Medium (4,31-8,60)
V	5,2	Very low (≤20,00)	6,3	Very low (≤20,00)
M	67,7	Good (50,1-75,00)	75,9	Very low (≥75,00)
ISNa	0,27	Very low (≤15%)	1,76	Very low (≤15%)
P-rem	13,9	Good (11,5-17,5)	41,9	Very good (>17,5)
Zn	0,66	Low (0,5-0,9)	7,96	High (>2,2)
Fe	80,8	High (>45)	97,5	High (>45)
Mn	42,3	High (>12)	9,8	Good (9-12)
Cu	7,37	High (>0,90)	0,88	Good (0,61-0,90)

H + Al - Potential acidity / SB- Sum of exchangeable bases / t- Effective cation exchange capacity / T- Cation exchange capacity at pH 7.0 / V- Index base saturation / M- Index base saturation / ISNa-index saturation Sodium / P-rem- Remaining phosphorus / MO-Organic matter.

Table 2. Principal components of the correlation matrix for leaf parameters of the populations of *Roupala montana* and *Solanum lycocarpum* at two altitudinal levels from the Neotropical savannah. Note: Gs- Stomatal conductance; Ψ- Water potential.

Parameters	**Component 1**	**Component 2**
Leaf area	0,646676	0,376792
Stomatal density	**-0,867401**	**-0,244500**
Stomatal index	0,135116	0,379803
Area with tracheary elements	0,961048	0,162703
Area with sieve elements	0,971682	0,064041
Fiber area	**-0,883371**	**-0,318403**
Ψ (*predawn*)	**-0,484548**	0,715827
Ψ (*midday*)	0,837993	0,293210
Ψ (4-5.00 pm)	0,786138	**-0,510368**
Gs - 8.00 am	**-0,471203**	0,792834
Gs - 10.00 am	0,568859	0,213959
Gs - 12.00 pm	0,110523	**-0,012703**
Gs - 2.00 pm	**-0,223460**	0,800687
Gs - 4.00 pm	0,556099	**-0,130116**
Variation explained (%)	**45,06%**	**19,07%**

Capítulo III

Physiological responses of Neotropical savannah plants during dry and rainy seasons

Abstract

This study aims to assess the physiological similarities of the species from the Neotropical savannah which belong of the same phenological groups, both during the dry and the rainy seasons. For this, two evergreen species (*Roupala montana* and *Solanum lycocarpum*) and one brevideciduous (*Byrsonima verbascifolia*) were selected as a comparative external taxon, all of them occurring at Serra do Cipó, Brazil. Developmental, phenological and physiological parameters were analyzed during the dry and the rainy seasons. Differences in physiological responses between the dry and rainy seasons were also observed, depicting suitability to the patterns of each period. Evergreen species had several distinct physiological responses that often resembled more brevideciduous plant. Thus, the current analysis should not evidence any pattern of physiological responses for the 3 model species with the phenological groups.

Keywords: brevideciduous, Cerrado, evergreen, leaf phenology.

Introduction

The Neotropical savannah (Cerrado *sensu lato*) occurs mainly in the midwest and southeast Brazil (Eiten 1972, Klink & Machado 2005). This biome comprises a gradient of phytophysiognomies with different levels of biomass, herein cited from the lowest towards the highest: *Campo limpo* (grasslands), *Campo sujo* (shrub Savannah), *Campo cerrado* (wooded Savannah), *Cerrado sensu stricto* (short woodland) and *Cerradão* (tall woodland) (Coutinho 1978). Such phytophysiognomies are subjected to striking seasonality in terms of temperature and precipitation, defining a rainy (hot and humid - usually from October to March) and a dry season (cold and dry – usually from April to September) (Klink & Machado 2005). During the dry season, fires are frequent (Miranda *et al.* 2002) and the soil may be subjected to water deficit in the superficial layers (Franco 2002), a condition which is reversed during the rainy season.

Physiological responses such as the adjustments in water potential, stomatal conductance (Perez & Moraes 1991, Franco 2002), and maximum photosynthesis (Moraes *et al.* 1989, Medina & Francisco 1994) are adjustable to the seasonal conditions (Medina & Francisco 1994, Niinemets & Valladares 2006), and may be decreased in the dry season. On the other hand, as the water becomes available in the soil during the rainy season, these parameters are reestablished to higher levels (Perez & Moraes 1991, Franco 2002).

In the Neotropical savannah, herbs and small plants produce flowers and fruits at the end of the rainy season, while the shrubs and trees do it at the beginning (Batalha *et al.* 1997, Batalha & Mantovani 2000). Trees typically maintain green leaves throughout the year, for their underground system is deep and allow continuous water uptake to the shoots (Goldstein *et al.* 2008). Deciduous plants, on the other hand, are subject to greater water limitation during the dry season due to their more superficial underground system (Sarmiento 1984).

The phenological patterns of plants from the Neotropical savannah seem to be associated with physiological responses and also reflect the seasonality of the biome. Long-lasting leaves usually have lower contents of nitrogen (Wright *et al.* 2002), respiration rates (Reich *et al.* 1998) and maximum carboxylation (Wullschleger 1993) in contrast with deciduous leaves. These distinctive features are apparently related to leaf structure (Hanba *et al.* 2002), with thick laminas and cell walls generated in response to mechanical adjustments and herbivory pressure.

Three plant species typical from the Neotropical savannah, *Byrsonima verbascifolia*, *Roupala montana* and *Solanum lycocarpum* were chosen to check the association of their physiological responses along their phenological patterns. *B. verbascifolia* is brevideciduous (Lenza & Klink 2006), and loses its leavesduring the dry season (Sarmiento *et al.* 1985, Morais *et al.* 1995, Franco *et al.* 2005); while *R. montana* is evergreen with seasonal production of leaves (Lenza & Klink 2006), and *S. lycocarpum* is evergreen with continuous production of leaves along the year. This classification indicates that these last species present the continuous maintenance of the leaves in the canopy (Sarmiento *et al.* 1985, Oliveira 1998, Franco *et al.* 2005).

These three plant species are good models of study to check if species with different phenological patterns, i.e. evergreen and brevideciduous, should have divergent physiological responses. Also the continuous or seasonal leaf production should be consequence of physiological peculiarities to each species.

Material and methods

The study was conducted in areas of *Campo sujo* (*sensu* Coutinho 1978) in the Serra do Cipó, part of a mountain chain called Cadeia do Espinhaço, in Minas Gerais state - Brazil.

Developmental, phenological and physiological parameters were analyzed in individuals of *Byrsonima verbascifolia* (L.) Rich. (Malpighiaceae), *Roupala montana* Aubl. (Proteaceae) and *Solanum lycocarpum* A. St.-Hil. (Solanaceae). Vouchers were deposited in the herbarium of the Universidade Federal de Minas Gerais (BHCB), under the registration numbers: 161584, 161585 and 161586. Climate data from October 2011 to October 2012 were obtained from the weather station of Diamantina/Minas Gerais state – Brazil, provided by the Instituto Nacional de Meteorologia (INMET 2013).

Analysis of growth and phenology

Monthly measurements of the height and the area of the canopy of the plants were taken from October 2011 to October 2012, in six individuals per species. The most apical green branch was used to measure the height of the plants. The area of the canopy was calculated by the formula of the ellipse ($A = a.b.\pi$, where a = major semi-axis and b = minor semi-axis).

Vegetative phenology was assessed qualitatively during the same period, and the following phenophases were observed: 1 - newly formed leaves, 2 – mature and green leaves and 3 - senescent leaves. The results were expressed as percentages and represented the occurrence of the phenomenon in the individuals.

Physiological analyses

The stomatal conductance (Gs), water potential (Ψw), the photosynthetic quantum yield and the production of photosynthetic pigments were measured in leaves of the 3rd node from the shoot apex in 4 individuals per species during the dry and rainy seasons. For the dry season, the data were obtained in August 2011, and for the rainy season in February 2012, on sunny and cloudless days.

The water potential was evaluated in leaves collected at *predawn*, at *midday* which is the period of greatest evaporative demand of the air, and later in the daylight, between 4:00 to 5:00 pm, using a pressure camera PMS, model 1000. The water potential of *B. verbascifolia* was not assessed because the diameter of its petiole was too big to fit the equipment. The measurements of stomatal conductance were carried out using the porometer LI-COR model LI-1600, between 8:00 am and 4:00 pm.

The potential quantum yield (Fv/Fm) was measured in leaves adapted to the dark for 30 minutes between 5:00 am and 4:00 pm, where *Fm* is the maximum fluorescence emitted after a saturating pulse of light, and $Fv=F_m-F_0$ (F_0 minimum fluorescence of leaves adapted to the dark). The effective quantum yield (ΔF/Fm') was obtained from instantaneous measurements under environmental conditions from 8:00 am to 4:00 pm, and is calculated by the equation (Fm'-F)/Fm', where *F* is the fluorescence in stationary state, and *Fm'* is the maximal fluorescence after a saturating pulse of light (Genty *et al.* 1989). Both parameters were obtained with a photosynthesis yield analyzer (MINI-PAM, Walz).

The he photosynthetic pigments were quantified using $1cm^2$ leaf discs immersed in 80% acetone for 48 hours and centrifuged at 1000rpm for 5 minutes in a centrifuge (Fanem Mod 206BL). The supernatant was analyzed in a spectrophotometer (Thermo Spectronic Mod. Genesys 10UV) at the wavelengths of 470, 646 and 663nm. The quantification of chlorophyll *a*, *b*, total chlorophylls, carotenoids and the ratio between them followed the equations proposed by Lichtenthaler and Wellburn (1983).

Statistical analyzes

Statistical analyzes were performed using JMP 5.0 software (SAS Institute). The normality and homogeneity of the data were verified by the tests of Shapiro-Wilk and Levene, respectively. ANOVA was used to evaluate the variance of parametric data, and

Kruskal-Wallis test followed by Dunnett's test for non-parametric data. All tests had 5% of significance.

Quantitative physiological parameters were used to compare the plant responses during the dry and rainy seasons, through cluster analysis and principal components analysis (PCA). Data were logarithmic transformed to attend to parametric analyses (Zar 1996). The dendrogram of similarity was calculated based on the average Euclidean distance, using the software PAST 2.17 (Hammer *et al.* 2001). The PCA was performed based on the correlation matrix of all variables using the program STATISTICA 7.

The IPDR measured the relative distance among the physiological parameters for all individuals of each area. These distances were calculated according to the formula:

$$RD_{ij\searrow i'j'} = d_{ij\searrow i'j'} / (x_{i'j'} + x_{ij})$$

Where j and j' are individuals from distinct phytophysiognomies. The IPDR varies from 0 (with no plasticity) to 1 (total plasticity), calculated according to the formula:

$$IPDR = \sum(d_{ij\searrow i'j'}) / (x_{i'j'} + x_{ij}) / n$$

where i, j and n refers to the treatments, repetitions and overall number of repetitions, respectively. The physiological results of IPDR were submitted to ANOVA test (α=0,05) to evaluate the species plasticity during seasons.

Quantitative genetic data of population were not raised, which does not allow the evaluation of phenotypic plasticity (Pil *et al.*, 2012). Therefore, the IPDR used herein estimates the physiological distance among populations by using difference on the genotype and/or phenotypic plasticity (Valladares *et al.* 2006).

Results

During the study, the difference on the precipitation well marked the dry and rainy seasons. Maximum precipitations were recorded during the months of November, December and January 2011/2012 and the minimum in June, July and August (Figure 1A).

Analysis of growth and phenology

All species presented reduced growth throughout the period of analysis (Figure 1B). *B. verbascifolia* had greater height growth (4.16%) and increased canopy area (4.75%) during the rainy season when compared to the dry season (height: 3.68% and canopy: -18.22%) (Figure 1B-C). Significant reduction in the area of the canopy was due to the loss of leaves in the dry season, while *S. lycocarpum* showed an increase in canopy area in both seasons, though slightly smaller (the rainy season: 27.71% and dry season: 18.33%) (Figure 1B-C). Conversely, *R. montana* had increased height and canopy area during the dry season (height 6.30%, and canopy 17.61%) than in the rainy season (height -0.70%, and canopy 7.23%) with higher rates of growth in September (Figure 1B-C).

B. verbascifolia emits new leaves in the rainy season (Figure 2A), keeping them for the rest of the year (Figure 2B). The most pronounced senescence begins in July, and it completely loses the leaves by early September (Figure 2C). The formation of new leaves starts in late September (Figure 2A), and the species is classified as brevideciduous. *R. montana* and *S. lycocarpum* present green leaves throughout the whole year (Figure 2B), however *R. montana* present new leaves predominantly in the late dry season and early rainy season (Figure 2A) while *S. lycocarpum* emits new leaves continuously throughout the year (Figure 2A). These two species are, thus, evergreen with seasonal production and evergreen with continuous production, respectively. The senescence of the leaves of *S. lycocarpum* is continuous and in *R. montana* it fluctuates along the year (Figure 2C).

Physiological responses

In general, the species exert greater control of the stomatal opening during the dry season (Figure 3A-C). In the rainy season, the values are more variable along the day (Figure 3 A-C). Among the three species, *S. lycocarpum* presented the highest values of stomatal conductance at 8:00 am and 2:00 pm during the rainy season, showing less control of stomatal opening on this period (Figure 3C).

For the other two species, more negative water potential values were observed in the dry season and at the middle of the day (in both seasons). However, *S. lycocarpum* showed less negative values along the day when compared to *R. montana* (Figure 4A-B). At *midday*, the water potential in *R. montana* reached -3.3 MPa in the dry season, while *S. lycocarpum* reached -1.87 MPa (Figure 4A-B).

The ΔF/Fm' was similar between seasons (Figure 5A-C), but was higher in the rainy season for *R. montana* at 2:00 pm (Figure 5B), and *S. lycocarpum* in drought, at 2:00 pm and 4:00 pm (Figure 5C).

The Fv/Fm was usually higher in the rainy season, but *B. verbascifolia* presented similar values in both seasons at 10:00 am and 2:00 pm (Figure 5D). In the rainy season, *R. montana* had statistically higher values at 2:00 pm (Figure 5E) and *S. lycocarpum* at 10:00 am and 12:00 pm (Figure 5F). In the rainy season, the values of *S. lycocarpum* were around 0.8 throughout the day, as well as the ones of *R. montana* (Fig. 5E-F). In this season, *B. verbascifolia* was the only species that had values below 0.8 (Figure 5D). In the dry season, the three species showed decreased Fv/Fm during the day, with values that goes from 0.7 to 0.75 (Figure 5D-F).

Seasonal difference in the production of leaf pigments were observed mainly in *S. lycocarpum,* being higher in the rainy season, with the exception of the ratio between total chlorophylls and carotenoids, which was similar in both seasons (Table 1). *B. verbascifolia* and *R. montana* had similar responses, with higher rates of chlorophyll *a* and *b*, but lower in comparison to the dry season (Table 1).

PCA, cluster analyses and plasticity

The physiological characters separated the evaluated groups distinctly during the rainy and dry seasons (Figure 6A-D). In the rainy season, a group consists of *S. lycocarpum* and *B. verbascifolia* and another by *R. montana* (Figure 6A-B). In the dry season, *S. lycocarpum* formed a solitary group and *B. verbascifolia* and *R. montana* were clustered together (Figure 6C-D).

The first two axes of the PCA explained 56.56% and 68.22% of the total variation during the rainy and the dry season, respectively (Table 2). In both seasons, the parameters were more positively correlated to the component 1 than to the component 2, with the highest correlation occurring in the dry season (Table 2).

The IPDR varied from 0 to 1. *R. montana* presented the major plasticity, which was higher during the rainy season (Table 3). On the other hand, *B. verbascifolia* showed higher plasticity between the dry and rainy seasons, with higher values on the dry season (Table 3). Analyzing the physiological parameters during seasons, *S. lycocarpum* had lower physiological plasticity (Table 3). The stomata conductance, the relative quantum yield and the water potential (*predawn* and *midday*) were the most plastic characters (Table 3).

Discussion

The analyses of leaf phenology confirmed *S. lycocarpum* as an evergreen species with continuous leaf production. The other two species, *B. verbascifolia* and *R. montana* are brevideciduous and evergreen with seasonal leaf production, respectively, corroborating the classification of Lenza and Klink (2006).

The expected patterns of *B. verbascifolia* and *S. lycocarpum* was confirmed by the highest increase in the canopy area during the rainy season, and could be associated to the higher water availability during this period (INMET 2013). The height and canopy area also varied more over the months in *B. verbascifolia*, which may indicate that the species which produces leaves continuously are more likely to variations imposed by abiotic and biotic factors. On the other hand, the individuals of *R. montana* had greater growth in height and canopy area during the dry season, as a reflection of the leaf sprouting in September, at the end of the dry season. According to Oliveira (1998), the periodic variations in growth and reproduction patterns of plant species from the Neotropical savannah are closely related to climatic seasonality, generating adaptive strategies.

The interference of seasonality on stomatal conductance was evident, with the highest restriction in stomatal opening during the dry season, regardless of the size of the individuals. Some authors propose that trees with continuous access to the water stored in the deeper layers of the soil (Ferri 1944, Goodland & Ferri 1979), because of root systems longer than 10 meters long (Sarmiento 1983) should transpire freely throughout the year regardless of the season while others (Moraes & Prado 1998, Meinzer *et al.* 1999, Naves-Barbiero *et al.* 2000, Franco 2002, Franco 2008, Goldstein *et al.* 2008) assume that there are hydraulic limitations on the amount of water that can be extracted from the soil and, consequently, on the rate of daily transpiration (Meinzer *et al.* 1999). The stomatal conductance of *S. lycocarpum* are consistent with the studies that refute the idea that trees transpire freely throughout the year regardless of the season (Moraes & Prado 1998, Meinzer *et al.* 1999, Naves-Barbiero *et al.* 2000, Franco 2002, Franco 2008, Goldstein *et al.* 2008).

The lowest rates of stomatal conductance in the dry season were accompanied by more negative values of water potential, which represents lower water availability in that period. The evergreen species with continuous leaf production showed more negative values at *midday*, in both seasons, similarly to what has been reported for other plants from the Neotropical savannah (Perez & Moraes 1991, Meinzer *et al.* 1999, Naves-Barbiero *et al.* 2000).

The recovery of water potential values was confirmed on the *S. lycocarpum* by the measurement at the end of the daylight, which did not occur with *R. montana*, even in the rainy season. These controversal results indicate that not all the plants from the Neotropical savannah do always present the lowest values of water potential at *midday* as previously assumed. Franco (1998) reported that *R. montana*, an evergreen species with seasonal production, decreased the stomatal opening during the dry season, but the leaf water potential measured in *predawn* varied slightly, decreasing from -0.2 MPa in the rainy season to -0.4 MPa during this season. Also, values of -3.3 MPa in the dry season and -2.8 MPa in the rainy season could be found, and demonstrated the adaptability of this species to environments with less available water.

Once the values of stomatal conductance and water potential were low in the dry season, the effective quantum yield was also expected to be reduced (Moraes & Prado 1998, Paulilo & Felipe 1998, Franco 2000). However, the effective ΔF/Fm’ were similar throughout the day in both seasons, with a difference at the end of the day between *S. lycocarpum* and *R. montana*. The photosynthetic behavior of plants from the Neotropical savannah is not homogeneous. Field & Mooney (1986) reported that the deciduous species have higher rates of photosynthesis than the evergreen species, but Sobrado (1994) described lower levels for deciduous plants occurring in savannah or dry deciduous forests.

In this study, however on the other hand, the ΔF/Fm’ was similar between the three model species, with the greatest difference observed for *S. lycocarpum* in the afternoon.

In the study conducted by Lüttge *et al.* (1998) with plants from the Neotropical savannah, leaves that had values of Fv/Fm below 0.8 after being adapted to the dark for 30 minutes were considered photoinhibited. Among the three model species, the photoinhibition was more evident during the dry season, following an increasing in the sense: from *B. verbascifolia*, to *R. montana*, and to *S. lycocarpum*. Franco *et al.* (2007) also observed that the photoprotective capacity was temporarily reduced with the increasing irradiation after the sunrise for two species from the Neotropical savannah. However, for these two species the photoinhibitory effect was reversed during the diurnal cycle, as evidenced for the *S. lycocarpum* and *R. montana* in both seasons, but not for the *B. verbascifolia*.

A seasonal production of pigments was evidenced similarly for *B. verbascifolia* and *R. montana*, i.e., they presented a higher amount of chlorophyll *b* in the dry season. This increasing in chlorophyll *b* production can increase the absorption of energy and enable higher photosynthetic rates (Lüttge 1997). Carvalho *et. al* (2007) showed the influence of the phytophysiognomies and the months of analysis over the concentration of photosynthetic pigments in five plant species from the Neotropical savannah, but unrelated to leaf phenology. In this study, similar responses among species phenological group next were not found.

The higher IPDR of *R. montana* contrasts the studies of Valladares *et al.* (2000) and Givnish (2002) that found lower values for evergreen species of Neotropical savannah. The result showed are not constant-response (Markesteijn *et al.*, 2007). The low IPDR usually is associated to dry season in Neotropical savannah because the high water restriction limits plant growth (Franco, 2002), and may consequently influence the plastic plant responses (Valladares et al., 2007). *R. montana* presented the expected pattern, with the higher plasticity in the rainy season, and restrict responses in the dry season (Bedetti *et al.*, 2011). *B. verbascifolia*, on the other hand, presented opposite patters of responses, which indicates a high and complex dynamic of plasticity among the plants of the Neotropical savannah during dry and rainy seasons.

The low plasticity of *S. lycocarpum* was atypical, for this species has wide occurence in Neotropical savannah (Felfili *et al.*, 1992). For Pigliucci (2001), the most elevated the plasticity is, more possibilities of colonization of heterogeneous habitats. As *S. lycocarpum* occurs commonly in open areas (Oliveira-Filho & Oliveira, 1988), this low plasticity should be associated to the species dependence of high light incidence. On the contrary, *B. verbascifolia* and *R. montana* colonize wider vegetational gradients of the Neotropical savannah (Ratter *et al.*, 1996), which is consequence of their higher morphophysiological plasticity.

The cluster analysis grouped *B. verbascifolia* and *S. lycocarpum*, during the rainy season, while in the dry season, *B. verbascifolia* were grouped with *R. montana*, which was also evidenced by the PCA. These analyses have shown that despite the *R. montana* and the *S. lycocarpum* were gathered in the same leaf phenology group, they have distinct physiological responses in the seasons. The *B. verbascifolia* had the most variable responses between seasons, which was responsible for the alternate pattern of grouping between the two seasons.

Evergreen species (*R. montana* e *S. lycocarpum*) had several distinct physiological responses that often resembled more brevideciduous plant (*B. verbacifolia*). Thus, the current analysis should not evidence any pattern of physiological responses for the 3 model species with the phenological groups. Differences in physiological responses between the dry and rainy seasons were also observed, depicting suitability to the patterns of each period.

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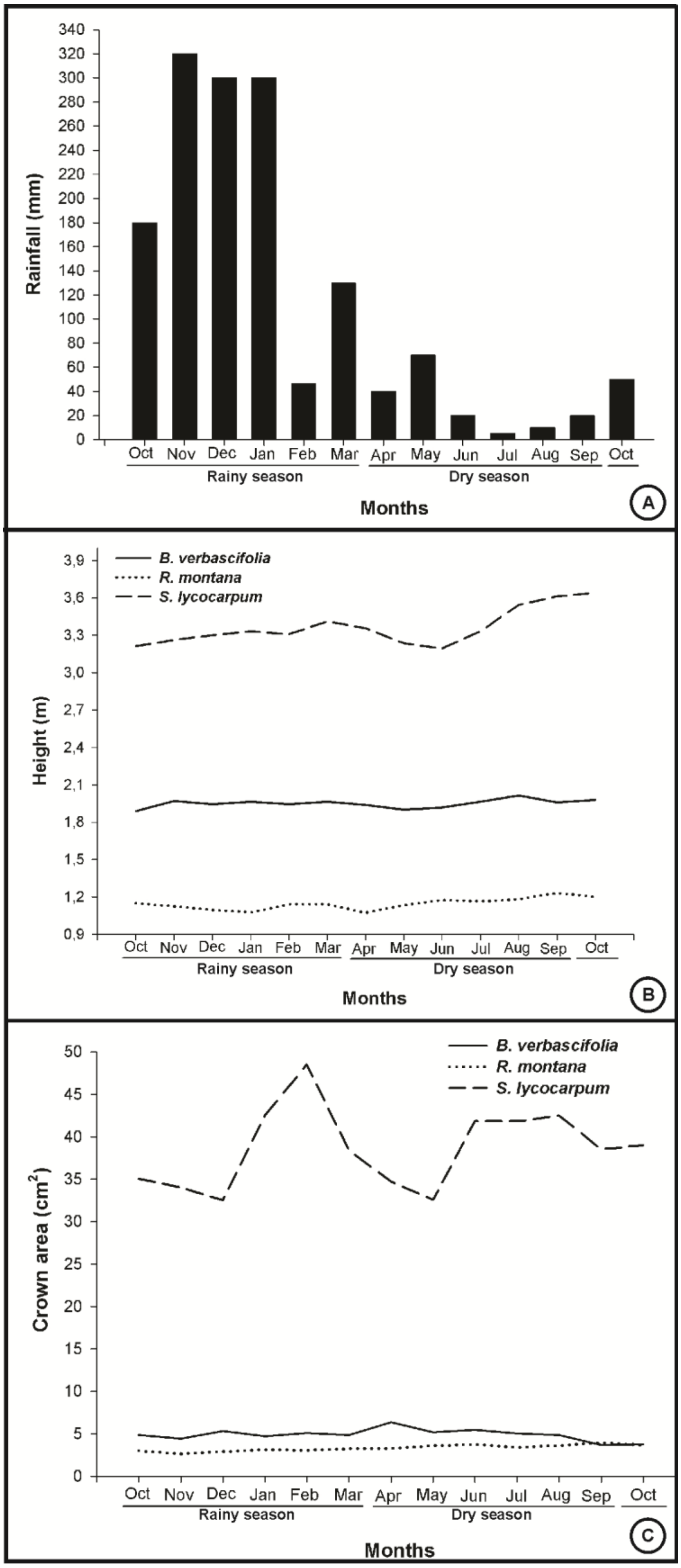

Figure 1. Rainfall and developmental analyzes of three species along the dry and rainy seasons of Neotropical savannah. A- Rainfall indices, B- Height growth; C- Growth canopy area.

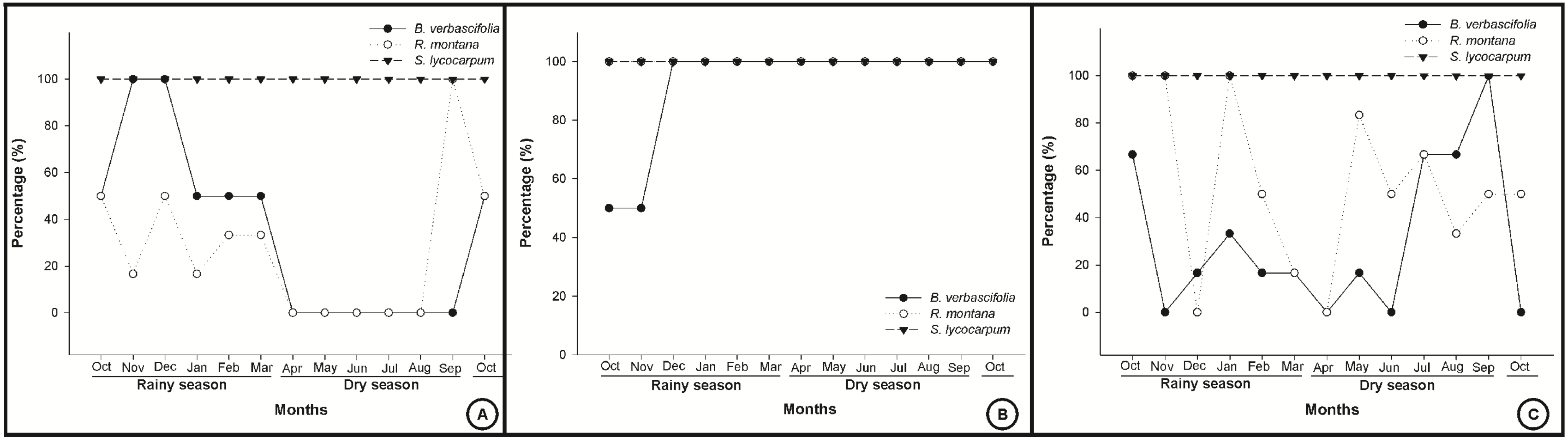

Figure 2. Leaf phenology of three species along the dry and rainy seasons of Neotropical savannah. A- New leaves; B- Mature leaves; C- Senescent leaves.

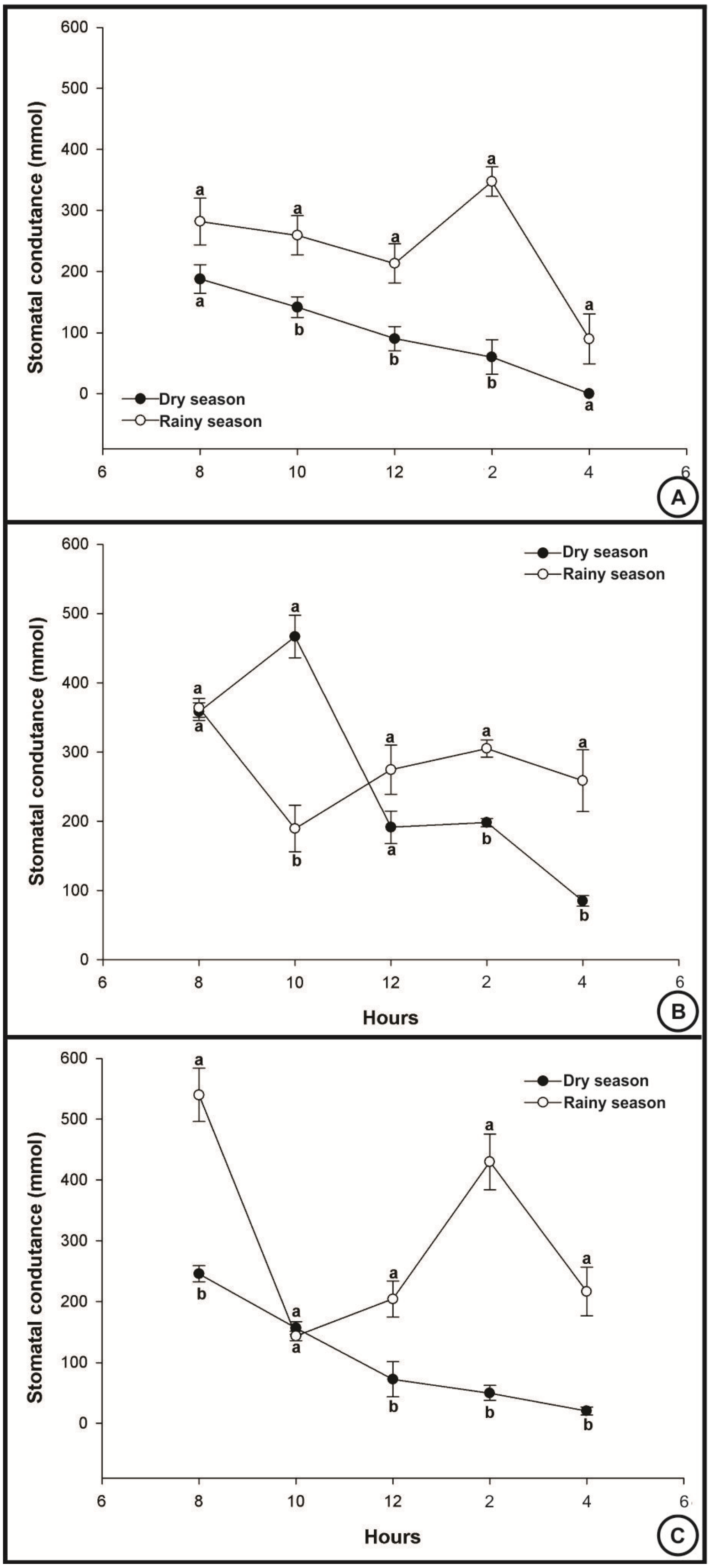

Figure 3. Average of stomatal conductance ± SE of three plant species during the rainy and dry seasons of Neotropical savannah. A- *Byrsonima verbascifolia;* B- *Roupala montana*; C-*Solanum lycocarpum*. Note: Means followed by the same letter do not differ statistically.

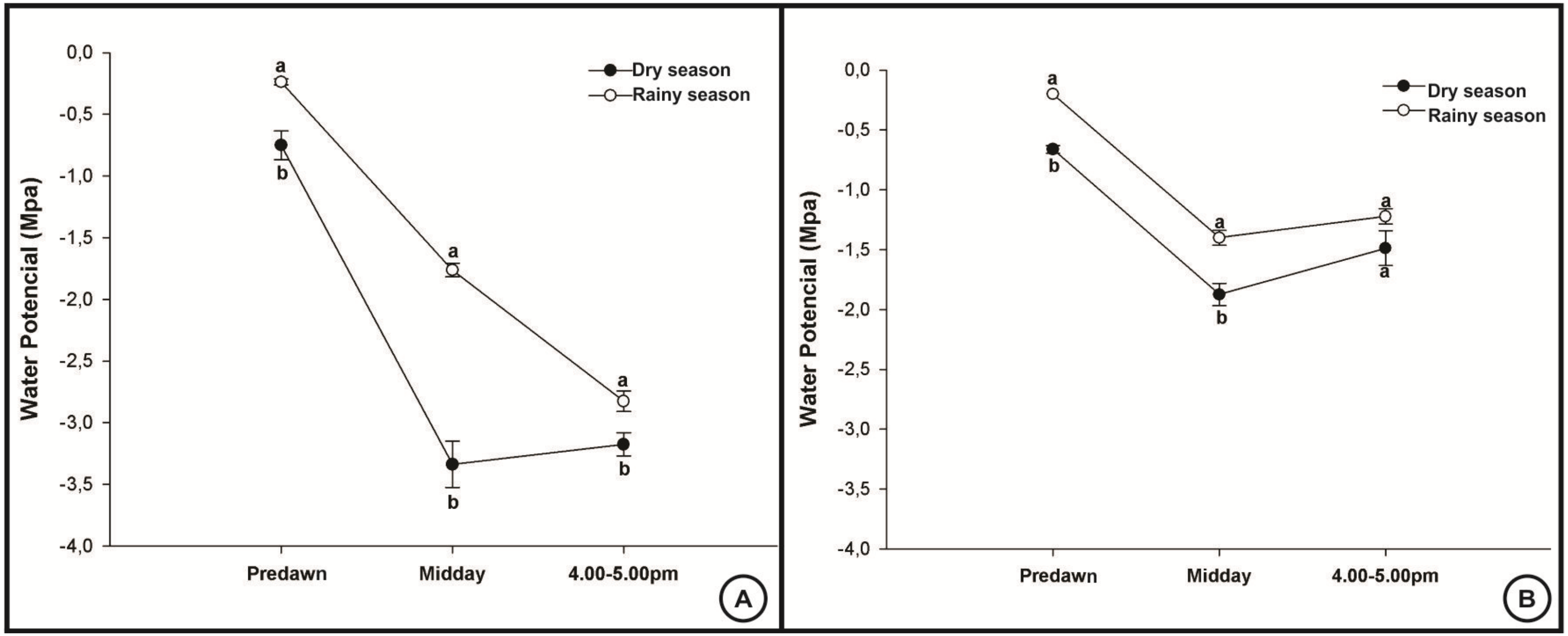

Figure 4. Water potential ± SE (D-E) of two plant species during the rainy and dry seasons of Neotropical savannah. A- *Roupala montana;* B- *Solanum lycocarpum*. Note: Means followed by the same letter do not differ statistically.

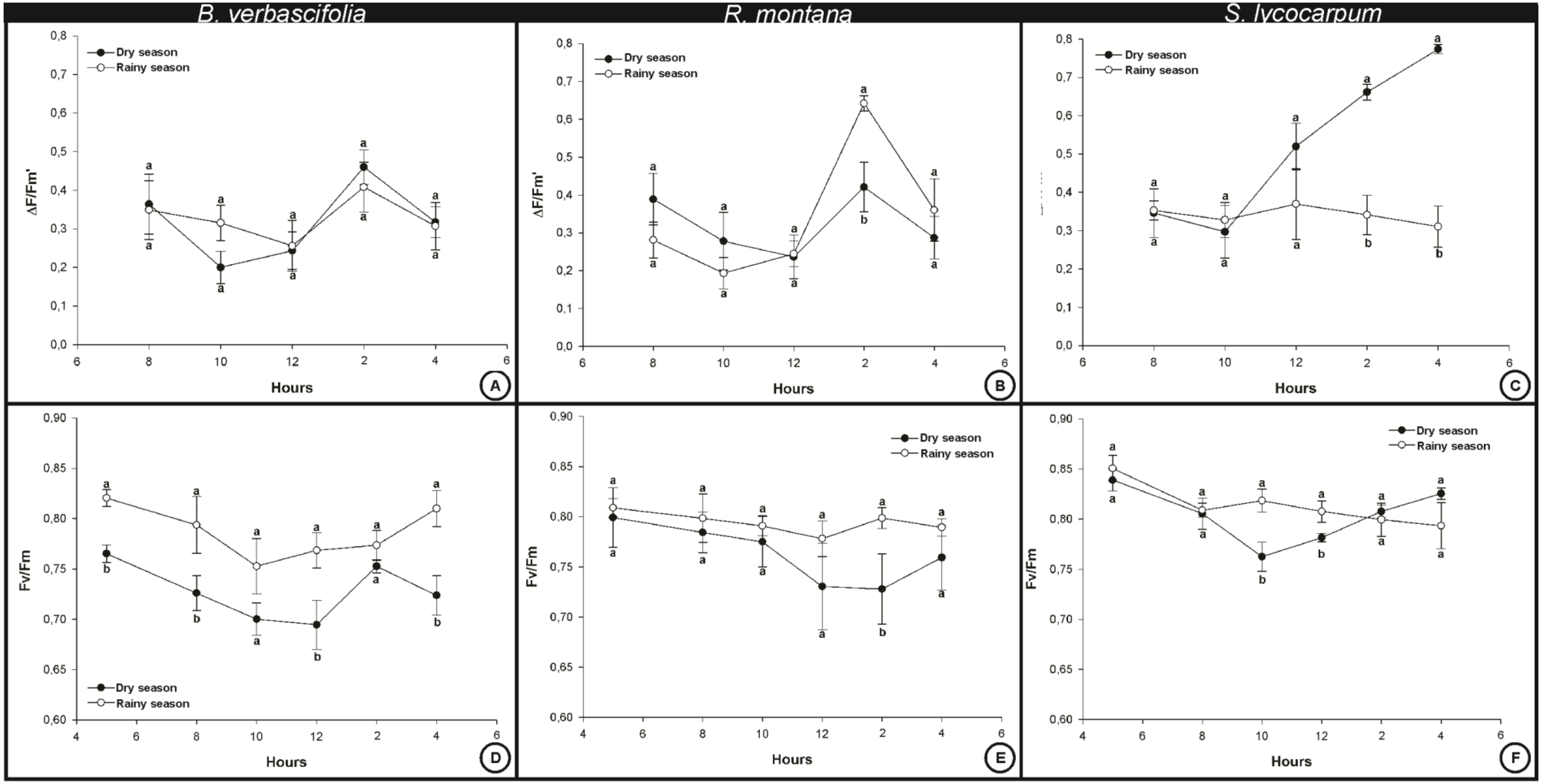

Figure 5. Averages of the effective (ΔF/Fm’) (A-C) and potential (Fv/Fm) quantum yield (D-F) ± SE of *Byrsonima verbascifolia* (A, D), *Roupala montana* (B, E) and *Solanum lycocarpum* (C, F) during the rainy and dry seasons of Neotropical savannah. Note: Means followed by the same letter do not differ statistically.

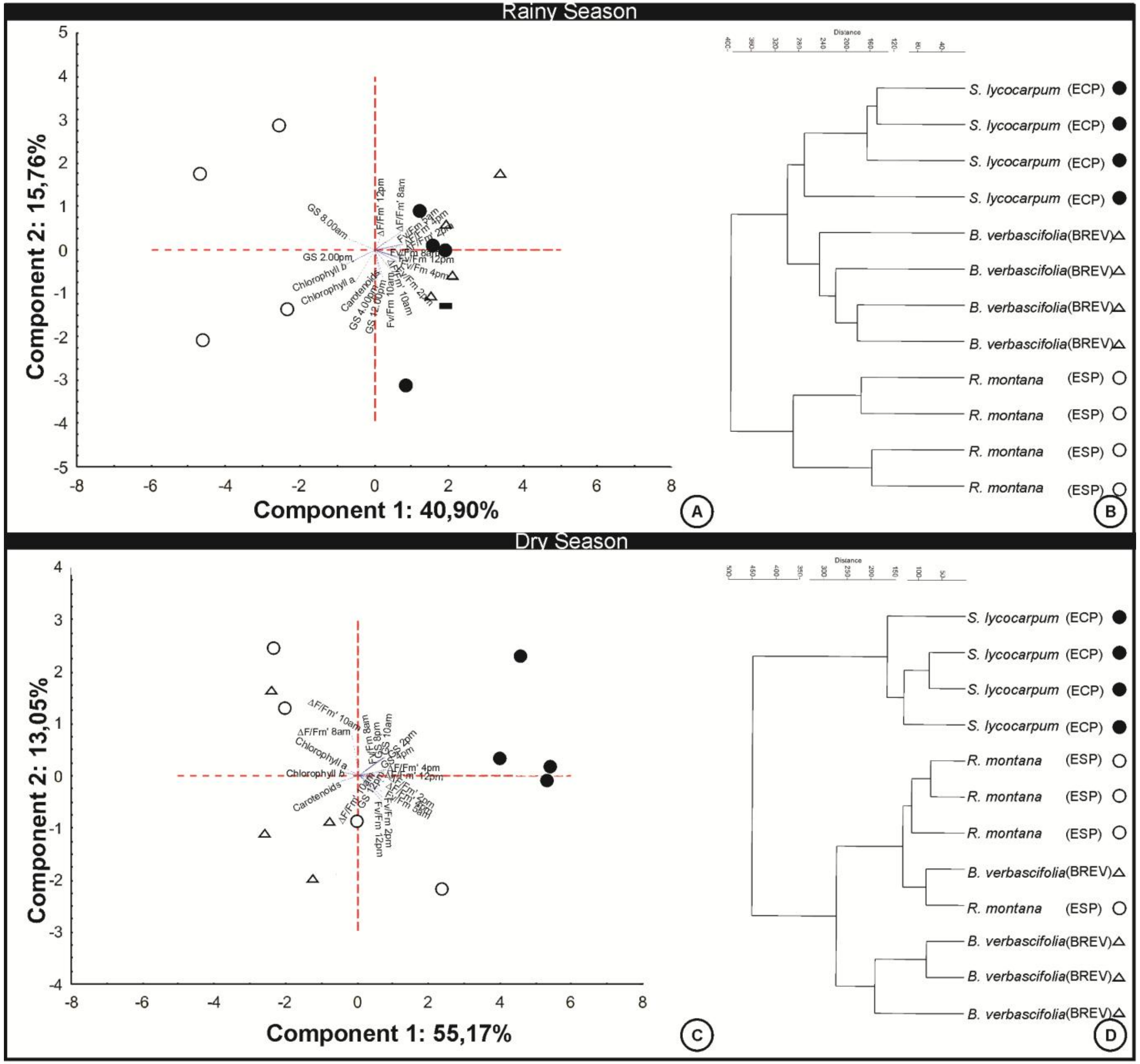

Figure 6. Scores relation of the first two axes of the principal component (A, C) and cluster analysis (B, D) obtained from leaf physiological traits of three species along the rainy (A, B) and dry (C, D) seasons of Neotropical savannah.

Table 1. Photosynthetic pigments of *Byrsonima verbascifolia*, *Roupala montana* and *Solanum lycocarpum* along the rainy and dry seasons of Neotropical savannah. Note: Means followed by the same letter do not differ statistically.

Species	**Season**											
	Dry	**Rainy**	**Dry**	**Rainy**	**Dry**	**Rainy**	**Rainy**	**Rainy**	**Dry**	**Rainy**	**Dry**	**Rainy**
	Clor. *a*		Clor. *b*		Carot.		Total Clor.		Clor. *a*/ Clor. *b*		Total Clor. / Carot.	
Byrsonima verbascifolia	8,87 ± 1,93 **a**	7,71 ± 0,45 **a**	9,42 ± 1,63 **a**	4,44 ± 0,37 **b**	551,44 ±69,2 **a**	490,55 ± 24,51 **a**	569,73 ±72,3 **a**	502.72 ± 24,65 **a**	0,92 ± 0,05 **a**	1,75 ± 0,11 **b**	1,03 ± 0,003 **a**	1,02 ± 0,002 **a**
Roupala montana	7,69 ± 0,09 **a**	10,02 ± 0,84 **a**	10,32 ± 1,22 **a**	6,86 ± 0,68 **b**	380,64± 23,40 **a**	427,76 ± 41,12 **a**	398,66 ±25,39 **a**	444,65 ± 42,25 **a**	0,74 ± 0,037 **a**	1,47 ± 0,10 **b**	1,04 ± 0,003 **a**	1,03 ± 0,002 **a**
Solanum lycocarpum	5,60 ± 1,36 **a**	10,56 ± 0,91 **b**	6,50 ± 1,09 **a**	7,76 ± 0,39 **b**	324,91 ± 27,95 **a**	538,38 ± 50,36 **b**	337,02 ± 29,87 **a**	556,71 ± 51,65 **b**	0,82 ± 0,07 **a**	1,35 ± 0,053 **b**	1,03 ± 0,005 **a**	1,03 ± 0 **a**

Table 2. Main components of the populations of *Byrsonima verbascifolia*, *Roupala montana* and *Solanum lycocarpum* obtained from the correlation matrix of leaf parameters along the rainy and dry seasons of Neotropical savannah. Note: Gs- Stomatal conductance; ΔF/Fm' - Effective quantum yield; Fv/Fm- Quantum yield potential.

Parameters	**Rainy season**		**Dry season**	
	Component 1	**Component 2**	**Component 1**	**Component 2**
GS 8am	-0,798735	0,248229	0,869714	0,338319
GS 10am	0,334903	0,192888	0,888304	0,339612
GS 12pm	0,157464	-0,551881	0,862334	0,070731
GS 2pm	-0,702281	-0,273016	0,813055	0,323482
GS 4pm	0,067760	-0,795379	0,861602	0,333704
ΔF/Fm' 8am	0,753692	0,398631	-0,290204	0,825526
ΔF/Fm' 10am	0,663265	-0,261520	-0,063377	0,830634
ΔF/Fm' 12pm	0,176114	0,297297	0,855528	-0,041740
ΔF/Fm' 2pm	0,765978	0,077227	0,863499	-0,115699
ΔF/Fm' 4pm	0,785637	0,115386	0,945443	0,111111
Fv/Fm 5am	0,834884	0,132416	0,803989	-0,306955
Fv/Fm 8am	0,613765	-0,140229	0,685964	0,288914
Fv/Fm 10am	0,570712	-0,538878	0,538526	0,009608
Fv/Fm 12pm	0,755239	-0,180006	0,802939	-0,486224
Fv/Fm 2pm	0,639429	-0,257934	0,819460	-0,412923
Fv/Fm 4pm	0,859672	-0,223898	0,866072	-0,115635
Chlorophyll a	-0,573583	-0,719419	-0,459310	0,128883
Chlorophyll b	-0,865006	-0,327215	-0,492308	0,049446
Carotenoids	0,247579	-0,669246	-0,635488	-0,112100
Variation explained (%)	**40,80%**	**15,76%**	**55,17%**	**13,05%**

Table 3. Plasticity index of the relative distance of leaf physiological characteristics of three species in two areas of Neotropical savannah during the dry and rainy seasons. Note: Fv/Fm- Quantum yield potential; Gs- Stomatal conductance; ΔF/Fm' - Effective quantum yield; Ψ- Water potential.

	B. verbascifolia		***R. montana***		***S. lycocarpum***	
	Rainy	**Dry**	**Rainy**	**Dry**	**Rainy**	**Dry**
Carotenoids	0,02**a**	0,07**b**	0,04**a**	0,00**a**	0,00**a**	0,01**a**
Chlorophyll a	0,01**a**	0,16**b**	0,05**a**	0,07**b**	0,02**a**	0,07**a**
Chlorophyll b	0,02**a**	0,16**b**	0,01**a**	0,04**a**	0,02**a**	0,06**a**
Fv/Fm 5am	0,00**a**	0,01**b**	0,03**a**	0,01**a**	0,00**a**	0,00**a**
Fv/Fm 8am	0,01**a**	0,00**a**	0,10**a**	0,06**a**	0,02**a**	0,00**a**
Fv/Fm 10am	0,04**a**	0,00**b**	0,20**a**	0,07**b**	0,00**a**	0,00**a**
Fv/Fm 12pm	0,01**a**	0,01**a**	0,14**a**	0,00**b**	0,00**a**	0,00**a**
Fv/Fm 2pm	0,01**a**	0,05**b**	0,11**a**	0,02**b**	0,01**a**	0,00**a**
Fv/Fm 4pm	0,01**a**	0,02**a**	0,10**a**	0,03**b**	0,01**a**	0,00**a**
Gs 8am	0,27**a**	0,22**b**	0,72**a**	0,10**b**	0,01**a**	0,01**a**
Gs 10am	0,20**a**	0,24**b**	0,08**a**	0,05**a**	0,01**a**	0,04**a**
Gs 12pm	0,05**a**	0,10**b**	0,09**a**	0,27**b**	0,02**a**	0,05**a**
Gs 2pm	0,24**a**	0,47**b**	0,55**a**	0,53**b**	0,01**a**	0,01**a**
Gs 4pm	0,52**a**	1**b**	0,04**a**	0,55**b**	0,09**a**	0,02**a**
ΔF/Fm' 8am	0,07**a**	0,11**b**	0,28**a**	0,05**b**	0,02**a**	0,00**a**
ΔF/Fm' 10am	0,20**a**	0,39**b**	0,10**a**	0,00**a**	0,02**a**	0,07**a**
ΔF/Fm' 12pm	0,03**a**	0,03**a**	0,06**a**	0,03**a**	0,14**a**	0,06**a**
ΔF/Fm' 2pm	0,01**a**	0,03**a**	0,65**a**	0,57**b**	0,00**a**	0,00**a**
ΔF/Fm' 4pm	0,08**a**	0,06**a**	0,30**a**	0,17**a**	0,01**a**	0,01**a**
Ψ (*predawn*)	-	-	0,36**a**	0,29**a**	0,00**a**	0,02**a**
Ψ (*midday*)	-	-	0,06**a**	0,28**b**	0,01**a**	0,01**a**
Ψ (4-5pm)	-	-	0,44**a**	0,05**b**	0,01**a**	0,02**a**

Considerações finais

Esta tese trouxe dados inéditos sobre alterações morfofisiológicas em plantas das Savanas neotropicais, considerando diferentes vertentes. A utilização de duas áreas de campo sujo, uma a 1.400 metros de altitude, sobre o campo rupestre, e outra a 700 metros em um contínuo de cerrado típico, serviu como um excelente modelo para avaliar respostas das plantas perante as condições abióticas. Os dados do campo sujo em campo rupestre evidenciaram habitat menos estressante do que era esperado, com elevada similaridade na radiação, menor temperatura e maior umidade do ar, em comparação com o campo sujo típico. Houve elevadas alterações morfofisiológicas nas espécies modelos no comparativo entre as duas áreas, porém com diferentes estratégias e, consequentemente, as plantas estudadas não apresentaram padrões similares de respostas.

Quanto as respostas relacionadas a fotossíntese, os caracteres anatômicos foliares foram os que mais variaram com a diferença altitudinal e podem ser responsáveis pela baixa alteração fisiológica entre os ambientes.

Byrsonima verbascifolia, Roupala montana e *Solanum lycocarpum* foram escolhidas para esse estudo por ocorrem nas duas áreas em estudo, apresentarem número amostral adequado e porte similar. O fato de *R. montana* e *S. lycocarpum* serem enquadradas no mesmo grupo fenológico foliar (sempreverde), diferente de *B. verbascifolia* (brevidecídua), foi fundamental para fortalecer a escolha e auxiliar a responder a questão: espécies de um mesmo grupo fenológico foliar apresentam respostas fisiológicas semelhantes durante as estações seca e chuvosa?. Contrariamente ao esperado, não encontramos padrões de respostas entre as plantas de mesma fenologia foliar.

Obrigado!