Seasonal and spatial variation of the iridoid specioside in monodominant formation of *Tabebuia aurea* in the Brazilian Pantanal wetland

Variação sazonal e espacial do especiosídeo iridóide em formação monodominante de *Tabebuia aurea* na zona húmida do Pantanal brasileiro

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ABSTRACT

It is common in the Pantanal the occurrence of monodominant formations, such as "paratudal" dominated by *Tabebuia aurea*. Although it is of high importance, little is known about how it is maintained and what factors limit its progress. We investigated the variation of secondary metabolism between individuals located on the edge and center of the formation as well as the effect of flood pulse and what part of the plant (stem bark or leaves) is there greater accumulation of iridoids glycosides, and if is there a translocation of specioside between them. We collected stem bark and leaves samples from individuals of *T. aurea* located in the center and edge of paratudal in drought and flooded season, sampled individuals were the same in both seasons. We also analyzed the topographic profile of paratudal. Ours results showed that the specioside concentration in stem bark decreased during flood season only on the edge of paratudal and this region was lower than the center. Individuals located on the edge stay more time exposed to the flooding effect. These results suggest that the flooding is the main cause of the change in the specioside concentration on monodominant formation and the topography should delimitate the monoformation.

Keywords: *Tabebuia*, Seasonal variation, Paratudal, Specioside, Flooding

RESUMO

É comum no Pantanal a ocorrência de formações monodominantes, tais como "paratudal" dominada por *Tabebuia aurea*. Embora seja de grande importância, pouco se sabe sobre como ela é mantida e quais fatores limitam a sua expansão. Investigamos a variação do metabolismo secundário entre indivíduos localizados na borda e no centro da formação, bem como o efeito do pulso de inundação e em que parte da planta (entrecasca do caule ou folhas) está havendo maior acúmulo de iridoides glicosilados, e se há uma translocação de especiosideo entre as partes da planta. Coletamos amostras de entrecasca do caule e folhas de indivíduos de *T. aurea* localizados no centro e na borda do paratudal nas estações seca e cheia, os indivíduos amostrados foram os mesmos em ambas as estações. Também analisamos o perfil topográfico do paratudal. Nossos resultados mostraram que a concentração de especiosideo na entrecasca do caule diminuiu durante a cheia somente na borda do paratudal e esta região é mais baixa do que o centro. Os indivíduos localizados na borda permanecem mais tempo expostos ao efeito da cheia. Estes resultados sugerem que a cheia é a principal causa da mudança na concentração de especiosídeo na formação monodominante e a topografia deve delimitar a monoformação.

Palavras-chave: Tabebuia, Variação sazonal, Paratudal, Especiosideo, Inundações

1 INTRODUCTION

The Pantanal wetland is characterized by distinct periods of drought and rainy, and the last regularly flooding of the low-land areas (Junk and Cunha 2005; Fernandes et al. 2010). In the Pantanal wetland is common to find some species forming groups known as monodominant formations, such as Paratudal (dominated by *Tabebuia aurea* Silva Manso Benth. and Hook. f ex. S. Moore Bignoniaceae), Carandazal (*Copernicia alba* Morong and Britton Arecaceae), Acurizal (*Attalea phalerata* Mart. ex Spreng. Arecaceae), Cambarazal (*Vochysia divergens* Pohl. Vochysiaceae) (Prance and Schaller 1982; Arieira and Nunes Da Cunha 2006), among others. The Paratudal is considered one of the main monodominant formations, which cover broad areas of this biome and are used by local population as medicinal (Soares and Oliveira 2009). It is a floodable savanna formation almost exclusively arboreal stratum of *T. aurea* and an herbaceous layer composed of grasses (Soares and Oliveira 2009; Bueno et al. 2014).

Tabebuia aurea popularly known as "ipê-amarelo" or "paratudo" has broad geographic distribution in the Neotropics, especially in Brazil where it occurs in the Amazon, Caatinga, Cerrado and Pantanal (Pott and Pott 1994; Lorenzi and Matos 2008; Collevatti et al. 2015). It is used for ornamental purposes because of the exuberant beauty of its yellow flowers; furthermore, it has a wide medicinal use (Lorenzi and Matos 2008). Its bark and leaves are used for making teas, infusions, with medicinal purposes (Lorenzi and Matos 2008); such as anti-inflammatory (Reis et al. 2014) and anti-mycobacterial (Agarwal and Chauhan 2015).

Tabebuia species are recognized for the presence of iridoids glycosides (Von Poser et al. 2000; Castillo and Rossini 2010; Santos et al. 2017). Compounds of this class stand out not only for presenting relevant ecological role, acting as mediators in plant-herbivore interactions (Bowers et al. 1992; Castillo and Rossini 2010; Pellissier et al. 2014; Kelly and Bowers 2016), but also by their potential activities such as anti-allergic (Villasenor 2007), anti-inflammatory (Villasenor 2007; Viljoen et al. 2012), neuroprotective (Yang et al. 2014), antimycobacterial (Chaipukdee et al. 2016). Many studies have reported seasonal and spatial variation for diverse classes of secondary metabolites, including iridoid glycosides (e. g. Moranz and Brower 1998; Jamieson and Bowers 2010; Yang et al. 2014; Pellissier et al. 2014). Bowers et al. (1992) found seasonal and spatial variation in the concentration of iridoid glycosides aucubin and catapol of *Plantago lanceolata*, according to the season and environmental conditions. Hogedal and Molgaard (2000) found seasonal variation in the concentration of four iridoids. Several factors can influence the accumulation of secondary metabolites (Gobbo-Neto and Lopes 2007; Rodziewicz et al. 2014), as

water stress, that can change the composition of secondary metabolites (Khan et al. 2011; Verma and Shukla 2015).

Previous studies have focused on the vegetation structure of monodominant formations in the Pantanal. For example, the study of the floristic composition and structure of the population of *T. aurea* in sub-region of Miranda (Soares and Oliveira 2009); the structure of arboreal and herbaceous strata of monodominant formations of *T. aurea* (Bueno et al. 2014). Despite this, little is known about how the concentration and composition of secondary metabolites varies and how it is affected by environmental factors in monodominant formations.

In this context, we investigated the variation of specioside in leaves and stem bark of *T. aurea* in a monodominant formation on Pantanal. More precisely, we considered the following questions: (1) The concentration of specioside of *T. aurea* differ between the center and edge of monodominant formation? (2) The flood pulse of the Pantanal wetland affects the accumulation of the iridoid specioside? (3) What part of the plant (stem bark or leaves) is there greater accumulation of iridoids glycosides? (4) Is there a translocation of specioside between stem bark and leaves during the two seasons?

2 MATERIALS AND METHODS

2.1 STUDY AREA

The Pantanal wetland cover approximately 140.000 km² located in the Upper Paraguay River Basin in the Brazilian Midwest region, divided between the states of Mato Grosso and Mato Grosso do Sul. This study was conducted in a monodominant formation of *T. aurea* (henceforward "paratudal") located in the subregion Miranda of Pantanal Sul-Mato-Grossense (19°34'12.54"S, 57°00'44.15"W), on period from July 2009 to March 2010. This subregion is delimits to the west by the confluence of the rivers Aquidauana and Miranda, southwest and south by the adjacent plateau and the east and north by the river Aquidauana. Of the 14 vegetation types discriminate for this subregion, the paratudal is the predominant occupying 31.24% (Da Silva et al. 1998).

2.2 PLANT SAMPLING

It was collected stem bark and leaves of 24 individuals: 12 located at the center and 12 at the edge of paratudal in the drought (July 2009) and flood (March 2010) season to verify if the specioside concentration of *T. aurea* varies spatially, as well as between drought and flood season on paratudal. The sampled individuals were the same for both seasons. Also, during the collection in the flood season, water depth was measured on 157 points randomly on paratudal and surrounded it. The geographical coordinates of each point were recorded for obtaining a topography map. The

species was identified by Dr Arnildo Pott and the voucher specimen was deposited at the CGMS herbarium under the registration number 29578.

2.3 PREPARATION OF HYDROETHANOLIC EXTRACT

100g of stem bark was extract three times with 800 mL of ethanol/water 1:1 (v/v) in an ultrasonic bath for 30 min., filtrated and concentrated under reduce pressure.

The chromatography profile analysis of the stem bark and leaves of *T. aurea* was performed in a HPLC-DAD (high-performance liquid chromatography coupled to a diode array detector), Shimadzu LC-20AD model, manual injector with a 20 µL loop equipped with a Phenomenex onyx monolithic C-18 column (100 x 3.0 mm). The mobile phase used was: ultrapure water (phase A) and acetonitrile (phase B). Both phases were prepared with 1% glacial acetic. The gradient program was proceeded as follows: 0 - 3 min. 3% of **B**; 3-25 min. 3-17% of **B**; 25-28 min. 17-100% of **B** at flow rate of 1.6 mL/min. For the stem bark was observed a main compound (22.417 min.) that was isolated and identified as follows.

2.4 ISOLATION AND IDENTIFICATION OF SPECIOSIDE

The compound was isolated of hydroethanolic extract. The concentrated extract was partitioned with 160 mL of ethyl acetate, and washed with 20 mL of water. This process was repeated (± 6 times) until it was observed that extraction was no longer occurring on the acetate partition. This material was then concentrated in a rotary evaporator. The identification and purity of the compound was based on high resolution MS/MS and NMR analysis.

2.5 PREPARATION OF THE EXTRACTS FOR ANALYTICAL ANALYSIS

The leaves and stem bark of *T. aurea* samples were dried at room temperature for two weeks and pulverized in a knife mill and sieved on 24 mesh. 50 mg of dried plant material was extracted with 40 mL of water/acetone (7:3 v/v) for 10 min in an ultrasonic bath and filtered through 0.45 μ m membrane before HPLC analysis. All analyzes were done in triplicate.

2.6 ANALYSIS OF THE EXTRACTS BY HPLC

Chromatographic analyses were performed as described above. The chromatograms were monitored at 314 nm. Calibration curve of specioside was prepared in concentration range (0.015 to $500 \mu g/mL$). The concentrations of specioside are presented as mg/g dry weight.

2.7 TOPOGRAPHIC PROFILE

The topographic modeling was performed with data observed in the field. The interpolation of the altimetric information was made in Surfer (Golden 2009) using ordinary kriging. As cartographic support was used an ALOS AVNIR satellite image, with 10 m of spatial resolution on an infrared false color composition, which allow the comprehension of the local vegetal cover. This image has already come with geometric and radiometric corrections from the supplier (1B2 G level - IBGE 2009) and the conversion of the data format was realized with the ASF Software (ASF 2008). The final model shows the topography as vectorial layer over the image/rater layer.

2.8 STATISTICAL ANALYSIS

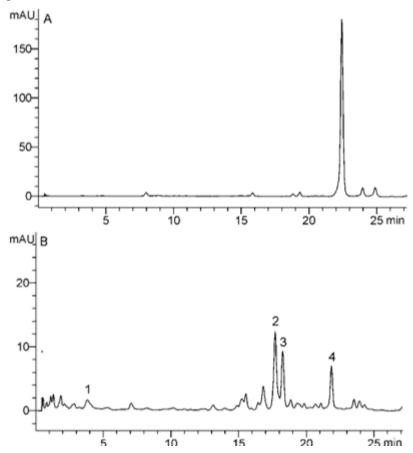
Specioside concentrations of stem bark and leaves were compared between different locations (center and edge) and seasons (drought and flood) using a repeated measures ANOVA, where the repeated factor was the same plants sampled in the drought and flood. When necessary, Tukey test was carried out to compare the levels within of the factors that showed significant differences at the level of p < 0.05.

3 RESULTS

3.1 CHROMATOGRAPHY PROFILE OF STEM BARK AND LEAVES OF T. AUREA

HPLC analysis revealed a simple profile of the stem bark, with the presence of a major compound visualized at the retention time of 22.417, in high concentration (Figure 1A). On the other hand, leaves presented a complex chromatographic profile compared to stem bark (Figure 1B). Inspection of the UV spectra revealed typical profile of phenylpropanoids, exhibiting two major absorption bands in the 260-340 nm range. By coinjection, the compounds obtained at retention times 3.829; 17.697; 18.252 and 21.855 were identified as chlorogenic acid, isoquercetrine, a phenylpropanoic derivative and specioside respectively.

Figure 1. Chromatographic profile of extract stem bark (A) and leaves (B) of *T. aurea* monitored at 314 nm wavelength. The numbers indicates the compounds identified as chlorogenic acid (1), isoquercetrine (2), a phenylpropanoic derivative (3) and specioside (4).



The calibration curves of stem bark and leaves specioside show good linearity in the range from 3.9 to 250 μ g/mL and from 0.20 to 16 μ g/mL respectively, and a correlation coefficient above of 0.999.

3.2 PURIFICATION AND IDENTIFICATION OF SPECIOSIDE

The extraction yield from the crude extract was 312 mg pure compound. In the mass spectrum in negative mode was observed an ion at m/z: 507.1523 (M-H), compatible with the molecular formula $C_{24}H_{27}O_{12}$ (507.1508).

Analysis of the ¹H and ¹³C NMR spectrum (Table 1) and comparison with the literature data allowed to confirm the identification of the compound as the 6-OEp-cumaroylcatalpol, known as specioside. Its chemical structure consists of an iridoid nucleus with a coumaric acid attached at carbon 6 and a glucose attached to carbon 1 (Figure 2). This compound was used as the basis for the quantification and observations of possible variations in secondary metabolism.

Position	$^{\delta}$ H (J in Hz)	δC
1	5.09 d (8.9)	93.0
3	6.42 dl (6.1)	141.2
4	4.94 dd (5.9, 4.0)	101.7
5	2.49 m	35.1
6	5.00 dl (7.7)	79.2
7	3.64 sl	58.2
8		65.7
9	2.46 m	41.8
10a	3.92 d (13.1)	58.5
10b	3.71 dl (12.0)	
1'	4.62 d (7.9)	97.9
2'	3.0-3.24 m	73.4
3'	3.0-3.24 m	77.5
4'	3.0-3.24 m	70.3
5'	3.0-3.24 m	76.4
6'a	3.70 dl (10.9)	61.4
6'b	3.43 dd (10.9, 6.5)	
1"		125.0
2"	7.59 d (8.4)	130.6
3"	6.80 d (8.5)	115.8
4"		160.1
5"	6.80 d (8.5)	115.8
6"	7.59 d (8.4)	130.6
7"	7.63 d (15.6)	145.7
8"	6.47 d (16.0)	113.6
9"	• • •	166.7

Table 1. ¹H (DMSO, 300 MHz) and ¹³C (DMSO, 75 MHz) NMR data of specioside.

Figure 2. Structure chemical of 6-O-E-p-cumaroilcatalpol (specioside)

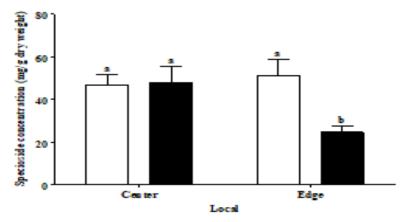
3.3 VARIATION IN THE SPECIOSIDE CONCENTRATION

Among the sampled individuals in the two seasons and locality, the specioside concentration varied in the range of 0.8 - 9.0% dry weight of the stem bark of *T. aurea*.

The specioside concentration in the stem bark did not differ significantly between the sites (repeated measures ANOVA, $F_{1,22} = 2.177$, P = 0.154). However, there was significant differences between the seasons ($F_{1,22} = 5.686$, P = 0.026). There was a significant interaction between the site and the period of collection ($F_{1,22} = 6.891$, P = 0.015). The concentration of specioside of the stem

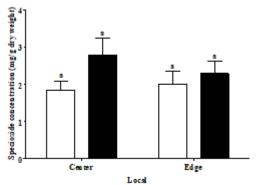
bark decreased from drought to flood on the edge of paratudal (Tukey test, P = 0.009); 51 mg/g in the drought for 24 mg/g in flood (Figure 3).

Figure 3. Concentration of specioside (mean \pm standard error) of the stem bark of *Tabebuia aurea* drought (white bars) and flood (black bars) season on the center and edge of the monodominant formation. Different letters above of bars denote significant differences (P < 0,05).



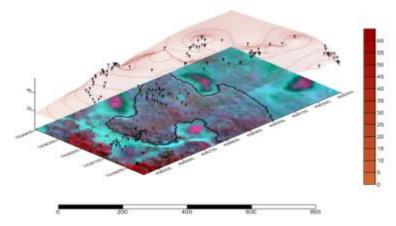
As for the concentration of specioside in leaves, there was no significant differences between center and edge (repeated measures ANOVA, $F_{1,22} = 0.207$, P = 0.654), and between the drought and flood period ($F_{1,22} = 2.859$, P = 0.105) and no interaction between the two factors ($F_{1,22} = 0.828$, P = 0.373) (Figure 4). The concentration of specioside in the central part of the paratudal in the drought season was 1.84 ± 0.25 mg/g and 2.78 ± 0.46 mg/g in flood, while that in the edge, in the drought period, was 2.01 ± 0.34 mg/g and 2.29 ± 0.33 mg/g in flood (Figure 4).

Figure 4. Concentration of specioside (mean \pm standard error) of the leaves of *Tabebuia aurea* drought (white bars) and flood (black bars) season on the center and edge of the monodominant formation. Different letters above of bars denote significant differences (P < 0,05).



The concentration of specioside in the stem bark was about 20 times higher than the leaves. Regarding the topography, it was observed that the concentrations at the edges are lower than those at the center of paratudal (Figure 5).

Figure 5. Topography of the paratudal landscape. The red lines are the vectorial representation of the topography over an ALOS AVNIR satellite image (RGB = NIR, R, G).



4 DISCUSSION

The specioside iridoid was isolated for the first time from leaves of *Catalpa specious* Warder (Bignoniaceae) by El-Naggar and Doskotch (1980). Since then, specioside has been reported in a restricted number of species such as *Veronica peregrina* (Scrophulariaceae) (Kwak et al. 2009), *Stereospermum personatum* (Bignoniaceae) (Kumar et al. 2005), *Kigelia pinnata* (Bignoniaceae) (Bharti et al. 2006), *Veronica linariifolia* (Scrophulariaceae) (Hong et al. 2010), *Heterophragma sulfureum* (Bignoniaceae) (Kaewkongpan et al. 2015). However, to the best of our knowledge, this is the first study to quantify specioside in the paratudal and to report its variation correlated with an abiotic stress.

We found high specioside concentration in the stem bark of *T. aurea*, varying from 0.8 - 9.0 % in dry weight. This concentration is relatively high compared to others natural compounds, such as harpagoside that showed a concentration in ranging from 0.5 to 1.6% in *Harpagophytum procumbens* (Pedaliaceae) (Villasenor 2007). Often, natural compounds are found in low concentration, less than 1% in dry weight (Koehn and Carter 2005; Ramakrisna 2011). As a result, the low concentration and the need to perform several procedures to obtain a pure compound are the main factors that limit the natural product drug discovery (Koehn and Carter 2005). Since its discovery, specioside has showed bioactivities as antioxidant (Kwak et al. 2009) and antiamoebic,

comparable with metronidazole (Bharti et al. 2006). These evidences indicate that specioside shown potential as a source of new drugs.

The outcomes showed that the specioside concentration varies spatial and seasonally on paratudal, and the main factor that modulate this discrepancy is the flood, whose intensity varies according to the topography. Although *T. aurea* is considered flooding tolerant (Damasceno-Junior et al. 2005; Bueno et al. 2014), this feature has a high cost. In Paratudal, individuals located on the edge showed a low specioside concentration in the stem bark in flood season, indicating that the flood affected secondary metabolism of *T. aurea*. One of the main flooding effects is oxygen deficiency or its absence in the soil (Lobo and Joly 1998). Under these conditions, plant needs more energy for surviving the flood waters, resulting in a decrease of biosynthesis of secondary metabolites (Irfan et al. 2010; Ferner et al. 2012). This idea is supported by a recent study, where it was observed that plants of *T. aurea* subjected to flooding conditions reduce their physiological activities (Oliveira and Gualtieri 2016).

Variation in the secondary metabolites has important implications for plant performance, because low concentration results in greater susceptibility to herbivory (Pellissier et al. 2014). Iridoids are known to play important role in the plant defense (Bowers et al. 1992; Alba et al. 2013; Pellissier et al. 2014). In particular, *T. aurea* showed high concentration of iridoids. Ribeiro and Brown (1999) showed that individuals of *T. aurea* are more attacked by herbivorous insects in the Cerrado than in the Pantanal. This difference could be a result of the high concentration of iridoids found in the individuals of Pantanal. Although *T. aurea* have showed relatively high concentration of iridoids, however, due to flooding stress, the individuals located on the edge are more susceptible to herbivores since they present lower concentrations than those on the center. Unfortunately, there are not studies of the herbivore attack on *T. aurea* in the different seasons of the year in the Pantanal.

Accordingly, the analysis of the topography profile revealed that the concentration of iridoids at the center is higher than the edge of Paratudal. This result suggests that a topography variation acts as a modulator of the intensity of the stress caused by flooding and interferes with the distribution of individuals on paratudal. According to Abdon et al. (1998) the Pantanal is a very complex diversity of vegetation type, where a small variation of feet in altitude and meters in the horizontal direction, cause significant variations in vegetation. Additionally, on the basis of this result, we can infer that plants located in higher regions of the Pantanal presented higher concentrations of secondary metabolites.

Regarding to the analyzed plant parts, the stem bark showed a concentration of specioside about 20 times higher than the leaves. Other studies have also reported variation of the concentration

of iridoid glycosides between plant parts (Jamienson and Bowers 2010; Sertić et al. 2015). For example, Jamienson and Bowers (2010) verified that the concentration of glycosylated iridoids, in the flowers and leaves, was more than the double of that found in the stem of *Linaria dalmatica* (Plantaginaceae). Sertić et al. (2015) found higher iridoid contents in flowers of *Globularia* species compared with others plant parts. The difference of plant part with higher concentration of iridoids between the species has components of the biotic and abiotic factors that each are exposed, as well as the exclusive genetic profile defined along the evolutionary timeline.

Plants synthesize and accumulate secondary metabolites due to abiotic and biotic stresses and can translocate them for more vulnerable and valuable parts (Rodziewicz et al. 2014). For example, the alkaloid nicotine is synthesized in the root of *Nicotiana* species (Solanaceae) and translocated to the leaves (Hashimoto and Yamada 2003). In this work, it was not observed translocation of metabolites from stem bark to leaves. The specioside concentration of leaves did not vary significantly between site and seasons. If translocation occurs, we would expect to find a relative increase in the specioside concentration in the leaves associated with the decrease in stem bark. Possibly, a cleavage might have occurred in the phenylpropanoid group of the molecule, releasing the catalpol which cannot be detected in the UV region, within the limits of the used detector.

An alternative explanation is that the specioside accumulated in the stem bark were being used to ameliorate the damage caused by reactive oxygen species, which are produced when plants are subjected to hypoxia (Irfan 2010). Such hypothesis is based on the antioxidant potential of compounds of the classes of the iridoid glycosides (Kwak 2009). These results reveal the importance of the correlation of variation in the secondary metabolism of plants with topography for the understanding of the maintenance and progress of monodominant formations in relation to flooding effect, which plays crucial role in the vegetation dynamics of Pantanal. Moreover, this work suggests that specioside can be a flooding tolerance biomarker and contribute to strategies of plants to survive to flooding.

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