



ORIGINAL ARTICLE

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**PALAVRAS-CHAVE**

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## Leaf anatomy and chlorophyll content in *Psychotria vellosiana* Benth influenced by the edge and two matrices

### *Anatomia foliar e teor de clorofila em Psychotria vellosiana Benth influenciados pela matriz e pela distância da borda*

**ABSTRACT:** The distance effect of the edge and two matrices (pasture and sugar cane) on the microclimate and anatomical and physiological properties of *Psychotria vellosiana* leaves were studied in two semideciduous forest fragments in southern Minas Gerais. The plots were set at distances (0, 20, 40, 60 and 80 m) from the edge to the interior of each fragment: 0, 20 and 40 m distances were considered edge, whereas 60 and 80 m were considered interior. Microclimate data were collected in all plots and radiation data and canopy openness at 0, 40 and 80 m. Leaves were collected for analysis of their chlorophyll content and anatomical properties. No differences were found for the canopy openness and radiation between the edge and interior of the fragments. The temperature and humidity data and the soil humidity displayed no differences within or between each fragment. Only the soil temperature data differed between the fragments. The leaves displayed the same chlorophyll content for the edge and interior of the fragments and between the fragments. These results may be related to the microclimate properties found along the 80 m transect in each fragment. Some anatomical data suggested a better adaptation of *P. vellosiana* to the fragment surrounded by the pasture matrix. However, we require a more detailed analysis that includes nutrient information and water availability from the soil.

**RESUMO:** O efeito da distância da borda e de duas matrizes (pastagem e cana-de-açúcar) sobre o microclima e as características anatômicas e fisiológicas de folhas de *Psychotria vellosiana* foi estudado em dois fragmentos de floresta estacional semidecidual no sul de Minas Gerais. As parcelas foram estabelecidas em distâncias – 0, 20, 40, 60 e 80 m – a partir da borda para o interior de cada fragmento. As distâncias de 0, 20 e 40 m foram consideradas como borda e de 60 e 80 m, como interior. Em todas as parcelas, foram coletados dados microclimáticos e, nas parcelas de 0, 40 e 80 m, dados de radiação e abertura de dossel. Folhas foram coletadas para análises de teor de clorofila e características anatômicas. Não houve diferença em relação à abertura do dossel e à radiação entre borda e interior de fragmentos. Os dados de temperatura e umidade do ar, e umidade do solo não apresentaram diferenças dentro dos fragmentos e entre estes. Somente os dados de temperatura do solo variaram entre os fragmentos. As folhas apresentaram o mesmo teor de clorofila para borda e interior de fragmentos, e entre os fragmentos. Estes resultados podem estar relacionados às características microclimáticas encontradas ao longo dos 80 m em cada fragmento. Alguns dados anatômicos sugerem melhor adaptação de *P. vellosiana* no fragmento circundado por matriz de pasto. No entanto, é necessária uma análise mais detalhada, incluindo a disponibilidade de água e nutrientes do solo.

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## 1 Introduction

Forest fragmentation can be understood as a process that transforms a continuous forest by creating small remnants surrounded by non-forest habitats that cause isolation and habitat loss (FREITAS; DAMBROS; CAMARGO, 2013). The conversion of tropical forests into other land use systems (matrix) has had one of the greatest impacts on biodiversity (DEIKUMAH; MCALPINE; MARON, 2013).

The ecological qualities of such a matrix can influence the rates of animal migration between fragments, movement and quality of pollen, and extent of the edge effect within the forest fragments. Consequently, the dynamics in fragments vary depending on the types of matrices surrounding the fragments (JULES; SHAHANI, 2003).

The composition and land use around fragments (matrix) can positively or negatively influence organisms through different mechanisms, such as by influencing the quality of habitat patches and causing changes in the microclimate, nutritional status or even biotic interactions next to habitat edges (REIS et al., 2004).

The disruption of continuous natural vegetation to create new agricultural areas is reported as one of the main reasons for fragmentation and causes species to experience different environmental conditions, such as higher luminosity, temperature, and intensity of winds and lower air humidity and soil moisture, compared to the conditions found inside the forest (HOLANDA et al., 2010).

Although plants are of great importance for studies in ecology, their use in investigations of the effects of different types of matrices has been rare. One reason for this is their frequent distribution along environmental gradients, which may result in more complex responses than those of animal groups (MURPHY; LOVETT-DOUST, 2004).

*Psychotria vellosiana* Benth (Rubiaceae) is a 2.5 to 4.0 m tall shrub that is distributed in eastern Brazil from Santa Catarina to Pernambuco in shaded sites and is found in ombrophilous forests, tropical rainforests and dense “cerrado” (ARAUJO; CARDOSO, 2006).

This study aimed to compare the anatomical and physiological characteristics of *Psychotria vellosiana* individuals that grow in two semi-deciduous forest fragments surrounded by distinct matrices (pasture and sugar cane).

## 2 Materials and Methods

The study was conducted during the month of July 2011 in two semi-deciduous seasonal Atlantic forest fragments near the city of Alfenas / MG. One of the fragments was located at Monte Alegre (MA) (LS 21° 21'; LW 46° 17'); the study area was 229.6 ha, and the south face was inserted into a matrix of pasture. Another fragment was at Pousada do Porto (PP) (LS 21° 25'; LW 46° 07'); study area was 72.3 ha, and the south face was inserted into a matrix of sugarcane.

The fragment shape was determined using the formula described by Viana and Pinheiro (1998) using the area and perimeter of each fragment. The size (area and perimeter) of the fragments and distance to the center of the fragments were calculated with the ArcGis 10 program (Esri, Redlands, CA, USA).

In each fragment, three transects were installed from the south facing edge towards the interior. Each transect of 80 m was subdivided at right angles to the edge of the fragment every 20 m into 5 plots of 10 × 2 m (20 m<sup>2</sup>). The plots at 0, 20 and 40 m from the edge were characterized as edge, and the plots of 60 and 80 m were characterized as interior. Two fully expanded leaves were collected from the 2nd and 3rd nodes of two individuals of *Psychotria vellosiana* in each square. The species was identified by samples deposited at the Universidade Federal de Alfenas herbarium (UALF 1546).

In all plots, air temperature data were collected by means of a maximum and minimum thermometer, and the relative humidity was collected with the aid of an analogical psychrometer. A soil thermometer read the temperature 10 cm below the soil surface. A soil sample was collected from each square for moisture determination.

A spectroradiometer (model SPR-4002) was used to measure the radiation. In the plots at 0, 40 and 80 m at hourly intervals between 10:00 and 14:00 h, a 2 min measurement was performed every 15 seconds. Canopy openness values were estimated at 1.30 m (chest height) with a plant densitometer in the center of the plots at 0, 40 and 80 m away from the edges.

To determine the *a* and *b* chlorophyll content, five discs were taken from each leaf and weighed. The discs were then placed in 5 mL of dimethyl sulfoxide (neutralized in CaCO<sub>3</sub>) at a temperature of 65 °C in a water bath for three hours. Afterwards, the absorbance of the samples was determined in a spectrophotometer (Dynamica-Halo model SB-10) at 649 and 665 nm.

Throughout the chlorophyll extraction process, the environment was kept dark to prevent molecule photo-degradation. The wavelengths and equations to calculate *a* and *b* chlorophyll concentrations were based on Wellburn (1994). The calculations were expressed as mg g<sup>-1</sup> FM (fresh mass).

The leaves were fixed in 70% formaldehyde, acetic acid and ethanol (F.A.A.) for 72 h and then stored in 70% ethanol. Paradermic sections from leaves were obtained from the abaxial surface using a steel blade. The sections were clarified with 50% sodium hypochlorite, washed in distilled water, stained with 1% safranin solution, and mounted as semi-permanent slides (KRAUS; ARDUIN, 1997). Two-centimeter fragments were taken from the central midrib region of the leaves and cut in cross-sections with the aid of a microtome LPC model. The sections were clarified in sodium hypochlorite, washed in distilled water, stained with Safrablau solution (1% safranin and 0.1% astra blue in the ratio of 7:3) and mounted on a slide in 50% glycerol. The preparations/sections were photographed using a Zeiss Scope AX 10 microscope coupled to a digital Canon Powershot G10 camera.

The photomicrographs were analyzed by means of an image analysis software-imagetool UTHSCSA, and the following measures were taken: DET = abaxial epidermis thickness, BET = adaxial epidermis thickness, MPT = mesophyll thickness, CT = cuticle thickness, PP = palisade parenchyma thickness, SP = spongy parenchyma thickness, PP/SP = ratio of palisade and spongy parenchyma, NS = number of stomata per field; NC = number of epidermal cells per field; SPD = polar diameter of stomata; SED = equatorial diameter of the stomata; SD = stomatal density = (stomata per mm<sup>2</sup>); SF = stomatal

functionality (POL/EQU relation); SI = stomatal index, PL = phloem thickness, VD = diameter of metaxylem vessels, NV = number of metaxylem vessels, VI = vascular system vulnerability index (index of vulnerability by Carlquist (1975)) SD and SI were calculated according Castro, Pereira and Paiva (2009). For every feature, three fields in each photomicrograph were evaluated, for a total of 180 fields per evaluation.

The data were subjected to an analysis of variance (ANOVA), and means were compared by the Scott-Knott test at 5% ( $p < 0.05$ ). The Shapiro-Wilk test for normality and Bartlett test for homogeneity of variance were also performed.

### 3 Results

The values for air temperature, air humidity and soil moisture did not vary between the two fragments, although the soil temperature was higher in the fragment at PP (Pousada do Porto) (Table 1). Within each fragment there was no significant difference between the microclimatic data and different distances from the edge (Table 2).

The mean values for canopy openness and radiation did not differ statistically between the fragments or between the edge and the interior. The average radiation values at MA and PP were 38.03 and 125.43  $\text{mw m}^{-2} \text{nm}^{-1}$ , respectively, and the values at the edge and interior were 96.27 and 52.70  $\text{mw m}^{-2} \text{nm}^{-1}$ , respectively. The average canopy openness values at MA and PP were 22.32% and 21.3%, respectively, and the values at the edge and interior were 20.08% and 23.87%, respectively.

The 80 m transects did not reach the center of the forest fragments. At MA and PP, the transects were approximately 7%, and 13% of the distance to the center, respectively.

A higher content of chlorophyll *a* was found inside the MA fragment in relation to the edge compared to the interior (Table 3), whereas the PP fragment showed the same values of chlorophyll *a* at the edge and interior (Table 3). There was no

significant difference between the edge and interior fragments based on the levels of chlorophyll *b*, total chlorophyll and chlorophyll *a/b* ratio (Table 3).

In paradermic sections, the leaves of *P. vellosiana* showed paracytic stomata (Figure 1) on only the abaxial face of the leaf blade. The leaves of individuals in the interior of the MA fragment presented a higher number of stomata than those at the edge (10.4%), whereas no difference in this parameter was found between the edge and interior in the PP fragment (Table 4).

The number of epidermal cells was 8.5% higher in leaves from individuals at the edge than the interior of the PP fragment, whereas there was no difference between the edge and interior for this parameter in the MA (Table 4). The stomatal index was 7.1% larger in the interior compared with leaves from the edge in the MA fragment, but there was no significant difference in this index between the edge and interior leaves in the PP (Table 4).

The polar diameter of the stomata was 4.5% larger in the interior compared to the edge in the PP fragment, but there was no difference between leaves for this character at the edge and interior at Monte Alegre (Table 4). There was no significant difference in the equatorial diameter and stomatal functionality between leaves of *P. vellosiana* at the edge and interior of either fragment (Table 4).

In cross-section, the palisade parenchyma, abaxial face epidermis and mesophyll of leaves were 5.8, 7.6 and 6.8% thicker, respectively, in the interior than towards the edge at MA (Table 5 and Figure 2), whereas there was no significant difference between the edge and interior for leaves at Pousada do Porto for these parameters (Table 5).

For the thickness of the cuticle, epidermis of the adaxial face, spongy parenchyma, palisade parenchyma/spongy relationship (Table 5 and Figure 2), and fiber (Table 6 and Figure 3), there were no significant differences between the leaves from the edge and interior of the fragments.

The phloem thickness was 14.3% greater in the interior compared to the edge in the MA fragment, but there were no differences in phloem in the leaves from the edge and interior at PP (Table 6). The diameter and number of vessels of the metaxylem were statistically the same between the edge and interior of both fragments (Table 6 and Figure 3). The leaves at the edge of the PP fragment showed a vulnerability index (VI) that was 14.3% higher compared to the leaves of the interior. However, this index did not vary significantly between the edge and interior at MA.

The mean values of the contents of chlorophyll *a* and *b*, total chlorophyll and the chlorophyll *a/b* ratio (Table 3) did not differ significantly between the edges of the two fragments.

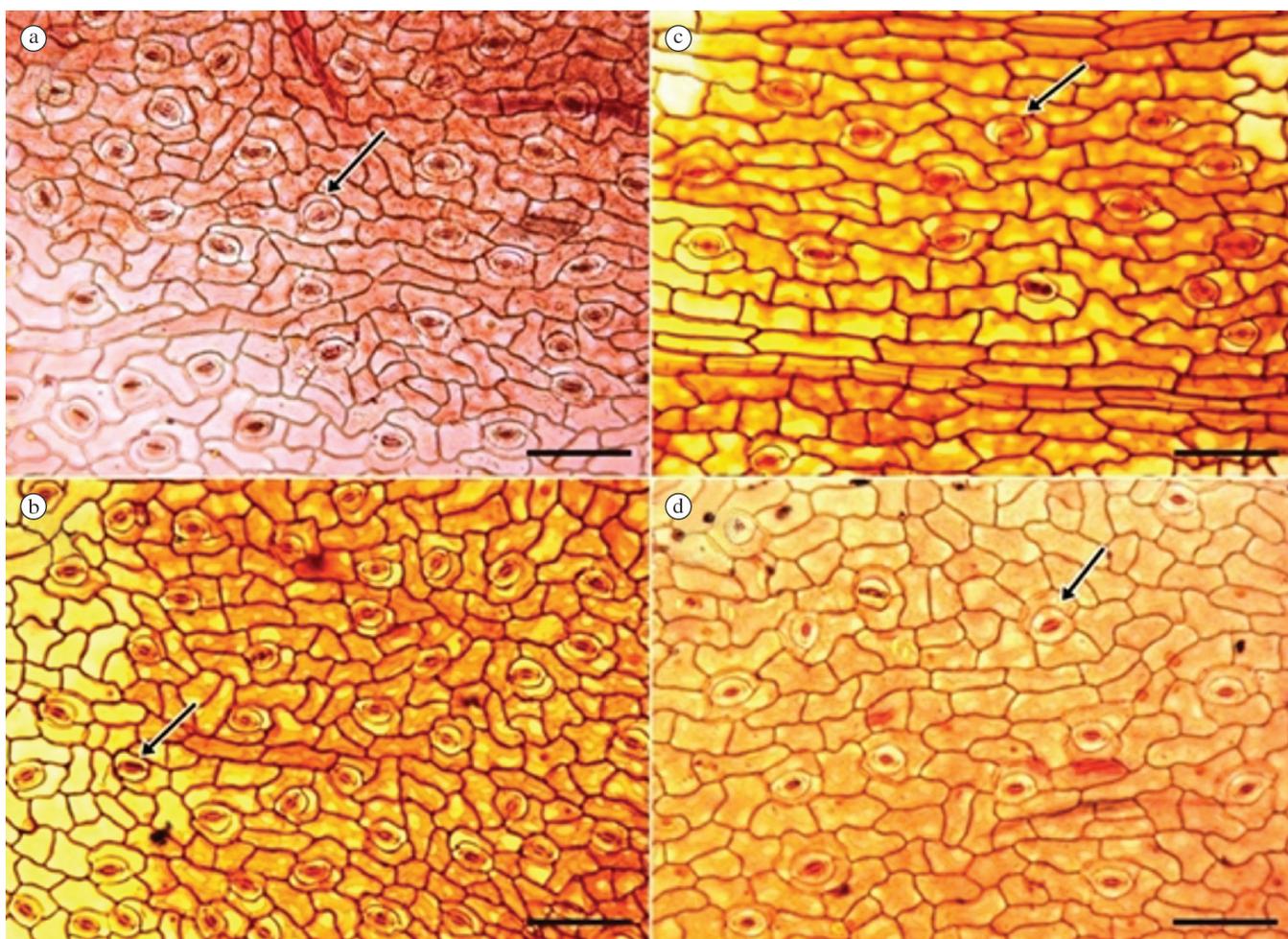
**Table 1.** Average data of air temperature and humidity and soil moisture in the Monte Alegre and Pousada do Porto fragments.

Variables	Fragments	
	Monte Alegre (MA)	Pousada do Porto (PP)
Air temperature (°C)	19.86 a	21.20 a
Soil temperature (°C)	14.93 b	15.80 a
Air humidity (%)	89.60 a	92.86 a
Soil moisture ( $\text{mg g}^{-1}$ )	19.15 a	19.70 a

Means followed by the same letter in the same line do not differ (Scott-Knott 5%).

**Table 2.** P Values for the following variables: fragment, distance and fragment x distance from the data of temperature and humidity of the air and soil.

Variation source	p value			
	Air temperature (°C)	Soil temperature (°C)	Air humidity (%)	Soil moisture ( $\text{mg g}^{-1}$ )
Fragment	0.12	0.01	0.28	0.39
Distance	0.98	0.09	0.56	0.77
Fragment x Distance	0.99	0.33	0.96	0.87



**Figure 1.** Paradermic section of *Psychotria vellosiana* leaves stained with safranin. Arrows indicate paracytic stomata. a = leaves from edge of Monte Alegre fragment (MA); b = leaves from interior of Monte Alegre fragment (MA); c = leaves from edge of Pousada do Porto fragment (PP); d = leaves from interior of Pousada do Porto fragment (PP) (bar = 100  $\mu$ m).

The number of stomata was higher in leaves at the edge of the MA fragment than the PP (Table 4 and Figure 1). The number of epidermal cells was higher in leaves at the edge of the MA fragment than the PP (Table 4). The leaves at the edges of both fragments showed the same stomatal density and stomatal index (Table 4).

The polar diameter of the stomata was higher in leaves from the edge at PP (Table 4). There was no significant difference regarding the equatorial diameter of the stomata and its functionality in the leaves between the edges of the fragments (Table 4).

In a cross-section of the leaves at the edge of the MA fragment a higher thickness of spongy parenchyma, mesophyll (Table 5 and Figure 2) and phloem (Table 6 and Figure 3) was shown. However, the leaves at the edges of the two fragments had the same thickness of cuticle, epidermis of the adaxial and abaxial faces, palisade parenchyma, palisade parenchyma/spongy relationship (Table 5 and Figure 2), and fibers, diameter and number of vessels of the metaxylem (Table 6 and Figure 3). However, the leaves at the edge of the PP fragment showed a higher vulnerability index (VI) compared to leaves at the edge of the MA (Table 6).

The leaves of *P. vellosiana* collected from the interior at MA had a higher chlorophyll *a* content than the leaves of the interior at PP (Table 3). The levels of chlorophyll *b*, total chlorophyll and the *a/b* ratio did not differ between the interior environments of the fragments (Table 3).

The number of stomata, stomatal density, number of epidermal cells, and stomatal index were higher in the leaves of individuals collected from the interior of the MA fragment (Table 4 and Figure 1). The polar diameter of the stomata was higher in the interior of the PP fragment, whereas no significant difference was found for the equatorial diameter and functionality between the interiors of either fragment (Table 4).

The palisade and spongy parenchyma, epidermis of abaxial face, mesophyll (Table 5 and Figure 2), and phloem (Table 6 and Figure 3) were thicker in leaves from the interior of the MA fragment.

There was no significant difference for the thickness of the cuticle, epidermis of the adaxial face, palisade parenchyma, palisade/spongy parenchyma (Table 5 and Figure 2), fiber, diameter and number of vessels of the metaxylem and vascular system vulnerability index (VI) between the interiors of the fragments (Table 6 and Figure 3).

**Table 3.** Chlorophyll content of leaves of *Psychotria vellosiana* growing at the edge (B) and interior (I) of the two fragments: Monte Alegre (MA) farm surrounded by a matrix of pasture and Pousada do Porto (PP) surrounded by a matrix of sugar cane.

		Fragments	
Chlorophyll content (mg g <sup>-1</sup> FM)		Monte Alegre (MA)	Pousada do Porto (PP)
Chlorophyll a	B	0.19 bX(±0.03)	0.18 aX(±0.02)
	I	0.21 aX(±0.02)	0.19 aZ(±0.01)
Chlorophyll b	B	0.41 aX(±0.07)	0.38 aX(±0.05)
	I	0.45 aX(±0.06)	0.41 aX(±0.03)
Total Chlorophyll a	B	0.60 aX(±0.10)	0.56 aX(±0.08)
	I	0.67 aX(±0.08)	0.60 aX(±0.05)
Chlorophyll ratio a/b	B	0.48 aX(±0.13)	0.48 aX(±0.02)
	I	0.47 aX(±0.02)	0.46 aX(±0.02)

Means followed by the same lowercase letters in the same column do not differ (Scott-Knott, 5%). Means followed by the same capital letters on the same line do not differ (Scott-Knott, 5%).

**Table 4.** Anatomical characteristics in paradermic sections of leaves of *Psychotria vellosiana* growing in two distinct fragments: Monte Alegre (MA) surrounded by pasture matrix / pasture and Pousada do Porto (PP) surrounded sugar cane matrix / sugar cane.

		Fragments	
Anatomical characteristics	Local	Monte Alegre (MA)	Pousada do Porto (PP)
NS	B	32.76 bX (±9.42)	28.56 aZ(±6.39)
	I	36.18 aX (±10.81)	25.72 aZ(±6.52)
SD	B	115.86 aX (±33.31)	101.00 aZ(±22.61)
	I	127.95 aX (±38.24)	99.99 aZ(±23.07)
NC	B	287.89 aX (±52.69)	256.04 aZ(±40.00)
	I	292.9 1aX(40.39)	236.04 bZ(±32.84)
SI (%)	B	10.12 bX(±1.92)	10.08 aX(±1.89)
	I	10.84 aX(±2.13)	9.74 bZ(±1.60)
SPD(μm)	B	23.78 aZ(±2.62)	24.52 bX(±2.50)
	I	23.61 aZ(±2.93)	25.62 aX(±2.29)
SED(μm)	B	14.95 aX(±2.20)	15.45 aX(±2.08)
	I	15.14 aX(±2.36)	15.86 aX(±1.78)
SF	B	1.61 aX(±0.27)	1.61 aX(±0.26)
	I	1.58 aX(±0.24)	1.63 aX(±0.22)

Means followed by the same lowercase letters in the same column do not differ (Scott-Knott, 5%). Means followed by the same capital letters on the same line do not differ (Scott-Knott, 5%). NS = number of stomata per field; SD = Stomatal density = (stomata per mm<sup>2</sup>); NC = number of epidermal cells per field; SI = stomatal index; SPD = polar diameter of stomata; SED = equatorial diameter of the stomata; SF = stomatal functionality.

## 4 Discussion

In the two forest fragments in this study, the microclimatic data collected up to 80 m away from the edges did not vary, but the PP fragment presented a higher soil temperature than what was found at MA. Edge effects may vary according to the orientation of the edge of the forest structure, the forest's age and the characteristics of the matrix surrounding the fragment (HOFMEISTER et al., 2013).

**Table 5.** Anatomical characteristics of leaves of *Psychotria vellosiana* in a cross-section of vegetation in two distinct fragments: Monte Alegre (MA) fragment surrounded by pasture matrix/ pasture and Pousada do Porto (PP) surrounded by sugar cane matrix.

		Fragments	
Anatomical characteristic	Local	Monte Alegre (MA)	Pousada do Porto (PP)
CUT(μm)	B	2.13 aX(±0.45)	2.06 aX(±0.29)
	I	2.16 aX(±0.42)	1.93 aX(±0.24)
DET(μm)	B	42.64 aX(±4.58)	40.30 aX(±4.82)
	I	40.79 aX(±5.49)	40.70 aX(±4.99)
PP(μm)	B	29.31 bX(±5.16)	27.93 aX(±3.84)
	I	31.02 aX(±3.97)	26.45 aZ(±3.71)
SP(μm)	B	41.48 aX(±10.01)	34.77 aZ(±6.88)
	I	43.54 aX(±7.16)	32.30 aZ(±5.73)
BET(μm)	B	13.91 bX(±2.53)	13.42 aX(±1.70)
	I	14.96 aX(±2.55)	13.17 aZ(±1.59)
MP(μm)	B	69.47 bX(±13.73)	60.74 aZ(±8.76)
	I	74.20 aX(±8.67)	58.46 aZ(±7.80)
PP/SP	B	1.43 aX(±0.34)	1.25 aX(±0.26)
	I	1.42 aX(±0.28)	1.24 aX(±0.26)

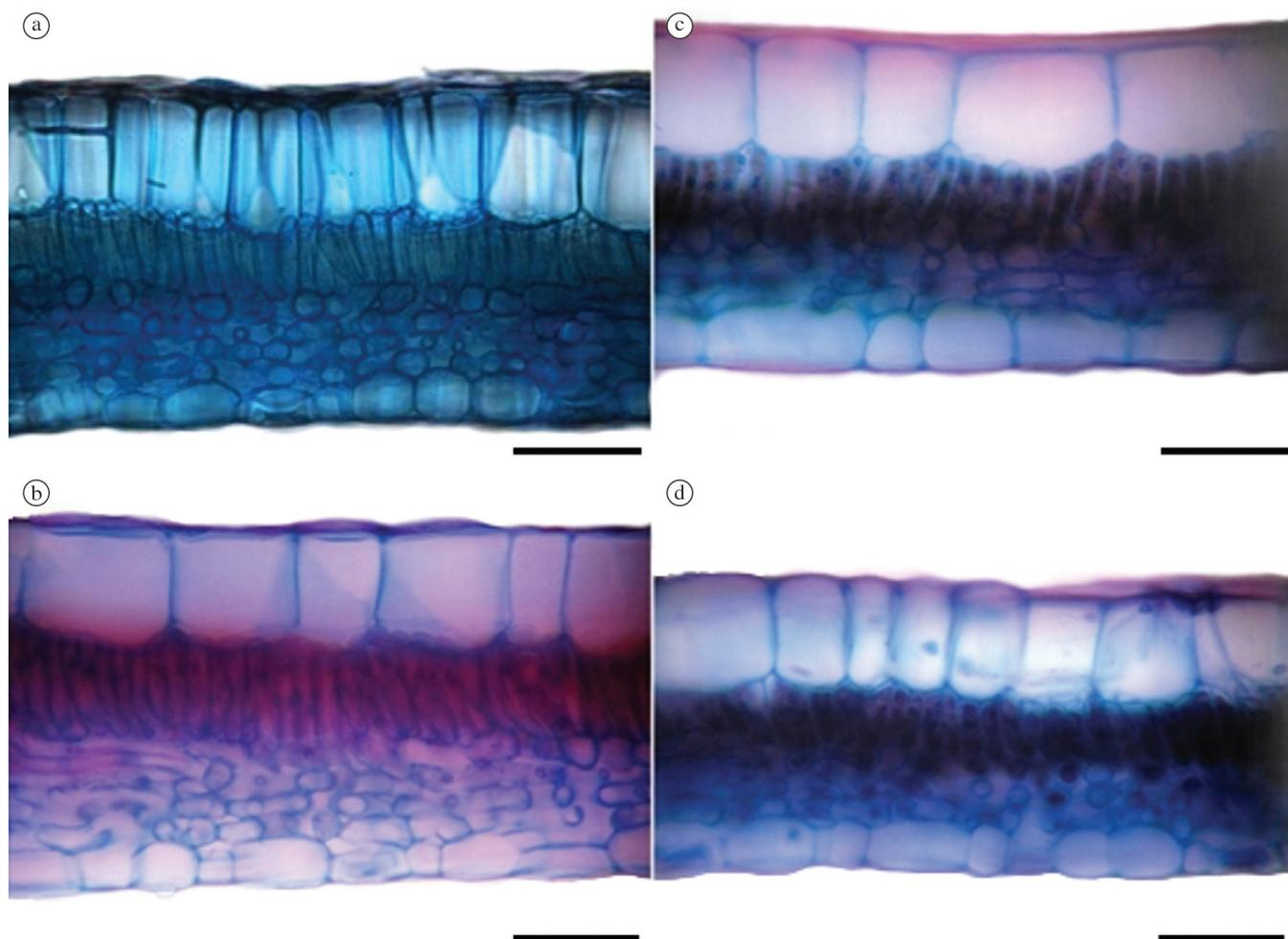
The means followed by the same lowercase letter in the same column do not differ (Scott-Knott, 5%). Means followed by the same capital letters on the same lines do not differ (Scott-Knott, 5%). CUT = thickness of cuticle; DET = thickness of epidermis of adaxial face; PP = thickness of palisade parenchyma; SP = thickness of spongy parenchyma; BET = thickness of epidermis of abaxial face; MPT = thickness of mesophyll; PP/SP = ratio of palisade and spongy parenchyma.

**Table 6.** Anatomical characteristics of leaves of *Psychotria vellosiana* in cross-sections growing in two distinct fragments: Monte Alegre (MA) surrounded by pasture matrix and Pousada do Porto (PP) surrounded by sugar cane matrix.

		Fragments	
Anatomical characteristics	Local	Monte Alegre (MA)	Pousada do Porto (PP)
FB (μm)	B	21.68 aX(±3.31)	20.51 aX(±2.97)
	I	21.84 aX(±3.48)	19.89 aX(±3.02)
PL(μm)	B	29.48 bX(±7.10)	27.23 aZ(±5.77)
	I	33.71 aX(±6.84)	26.28 aZ(±5.13)
VD(μm)	B	9.53 aX(±2.35)	9.79 aX(±1.68)
	I	9.66 aX(±1.83)	9.21 aX(±1.88)
NV	B	73.56 aX(±17.02)	62.86 aX(±13.94)
	I	78.45 aX(±21.97)	66.31 aX(±12.01)
VI	B	0.13 aZ(±0.03)	0.16 aX(±0.04)
	I	0.13 aX(±0.04)	0.14 bX(±0.03)

Mean followed by the same lowercase letters in the same column do not differ (Scott-Knott, 5%). Mean followed by the same capital letters on the same line do not differ (Scott-Knott, 5%). FB = thickness of fiber; PL = thickness of phloem; VD = diameter of metaxilem vessels; NV = number of vessels of metaxilem; VI = vulnerability index (CARLQUIST, 1975).

Both the solar radiation and canopy openness did not show differences between the two environments or the edge and interior of each fragment. In the studied fragments, both the pasture and sugar cane matrices may have had the same type of influence on the microclimate from the edge of fragments up to



**Figure 2.** Cross-section of the mesophyll of leaves of *Psychotria vellosiana*. a = leaves of the edge of the Monte Alegre fragment (MA); b = leaves of the interior of the Monte Alegre (MA); c = leaves of the edge of the Pousada do Porto fragment (PP); d = leaves of the interior of the Pousada do Porto fragment (PP) (bar = 50  $\mu$ m).

80 m within the interior. With the exception of soil temperature, none of the microclimatic characteristics, i.e., air temperature, humidity and solar radiation, showed any differences at the edge or interior of the two fragments studied.

Because the chlorophyll content is directly influenced by the availability of light in the environment and radiation data did not present any differences between the edge and interior environments in either fragment, it was expected that no difference in the chlorophyll content would be found between the edge and interior in either fragment or between the interiors of the fragments. However, a larger palisade parenchyma thickness in the interior of the MA fragment was observed and can be directly related to higher levels of chlorophyll *a* in the leaves of *P. vellosiana* in this environment.

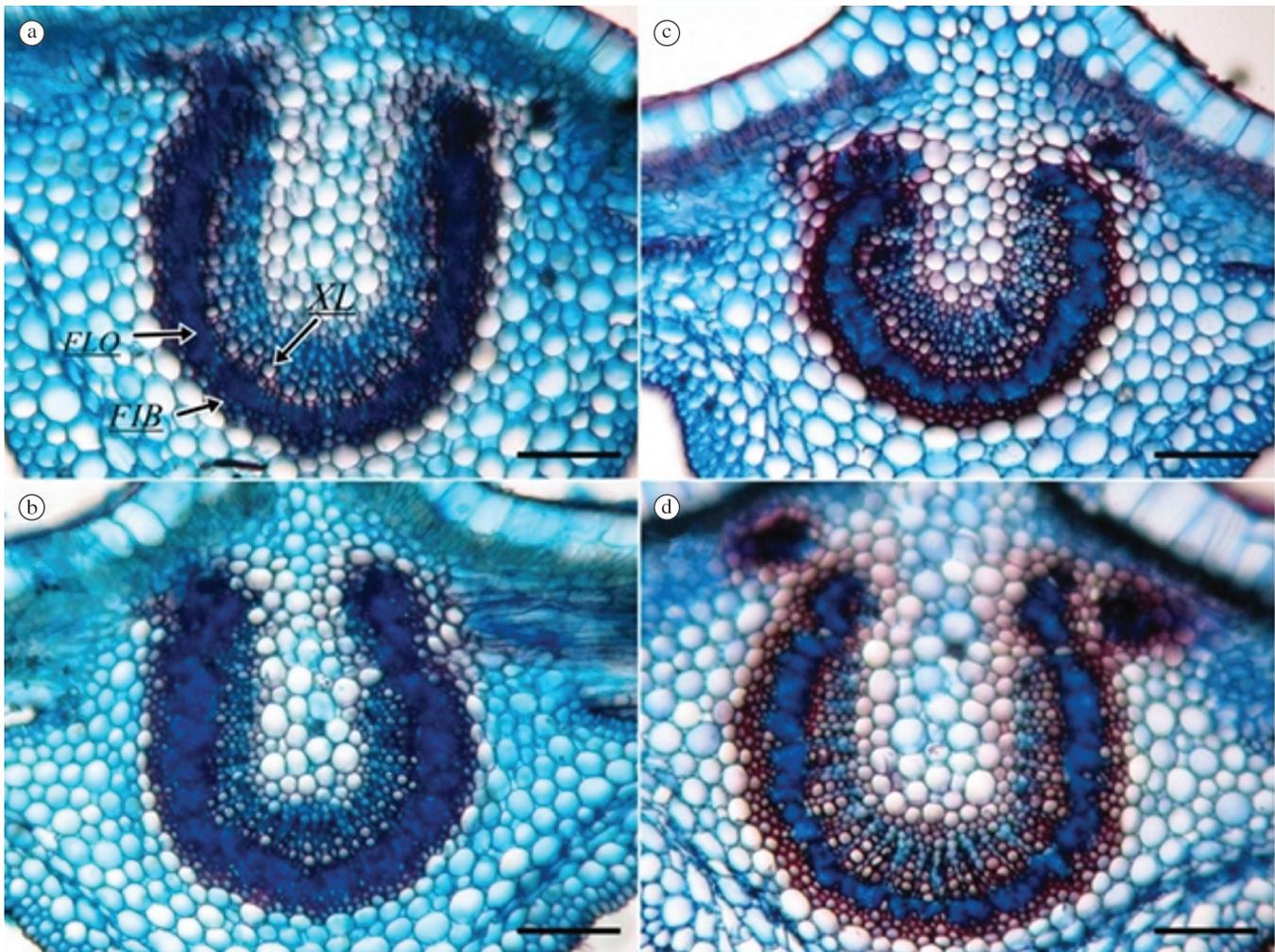
The relationship between the growth and adaptation of plants in different environmental conditions is closely linked to the efficiency of the photosynthetic process, which in turn is linked to the content of chlorophyll. External and internal factors can alter the biosynthesis of this pigment, and its leaf content can present significant variation (ALMEIDA et al., 2004) In paradermic sections, the leaves of *P. vellosiana* only showed paracytic stomata (Figure 1) on the abaxial leaf

surface, i.e., hypostomatic, which corroborated the results found by Moraes et al. (2011).

There was no linearity in the results found for the anatomical characteristics related to the number of stomata and epidermal cells, stomatal index and polar diameter of the stomata. However, the stomata had the same equatorial diameter and maintained its functionality to allow a proper flow of CO<sub>2</sub>. Santiago et al. (2001), who studied the leaf anatomy of *Piper hispidinervium* under different light conditions, did not find significant differences for the polar and equatorial diameter of stomata, which indicated that these variables were not influenced by light.

The responses of stomata to abiotic factors are more relevant to the stomatal conductance than its number (KOZŁOWSKI; KRAMER; PALLARDY, 1991). In this sense, the matrices of pasture and sugar cane and the microclimatic conditions within each fragment did not affect differently the working capacity of the stomata.

The epidermis of the adaxial face of leaves from *P. vellosiana* from the edge and interior of these fragments did not show any differences in thickness. The epidermis is a tissue that responds to environmental changes. The adaxial epidermis



**Figure 3.** Cross section of the midrib of leaves of *Psychotria vellosiana* stained with Safrablau. a = leaves of the edge of the Monte Alegre fragment (MA); b = leaves of the interior of the Monte Alegre (MA); c = leaves of the edge of the Pousada do Porto fragment (PP); d = leaves of the interior of the Pousada do Porto fragment (PP) (bar = 100  $\mu$ m).

of the face is subjected to higher temperatures, whereas on the abaxial surface, the humidity is higher compared to the adaxial surface (BOEGER; WISNIEWSKI, 2003).

Apparently, the pasture and sugar cane matrices that surrounded these fragments did not have a differential effect on the microclimate present along the 80 m of transects within the fragments that might explain the variation in thickness of the epidermis. Even the higher soil temperatures in the PP fragment were considered very close to the values between them and may not have been sufficient to cause changes in the epidermis.

The larger thicknesses of the spongy parenchyma observed in relation to the palisade parenchyma and the spongy parenchyma cells with irregular and intercellular spaces favor the diffusion light (ESPINDOLA JUNIOR et al., 2009). The spongy parenchyma of the leaves of *P. vellosiana* generated increased amounts of diffuse light and increased its absorption by making the light distribution more efficient within the leaf (VOGELMANN; NISHIO; SMITH, 1996).

The palisade parenchyma/spongy relationship may represent an adaptive factor of the plant to environmental conditions (CASTRO; PEREIRA; PAIVA, 2009) and perhaps

indicates that the microclimatic conditions in these fragments and the influence of the type of matrix that surrounds them may be causing similar responses in *P. vellosiana*.

The phloem is directly related to the transport of photosynthates from the leaves to sinks (RIBEIRO et al., 2012). The high values of phloem thickness may reflect a greater potential for translocation and consequently promote the development of individuals of *P. vellosiana*. Of the individuals sampled in this study, a thicker phloem was found in the leaves of individuals at MA. This may be an indication that these individuals in MA are better adapted, and consequently the matrix of pasture, which is in contact with this fragment, might not have a negative influence on the growth of *P. vellosiana*.

According to the results of the vulnerability index (VI), the leaves of *P. vellosiana* present at the edge of PP have a higher VI and may have less potential for the conductivity of water and nutrients because the vascular system is less protected against embolisms (CARLQUIST, 1975). The VI was lower in leaves of individuals at MA, which reinforces the hypothesis/possibility that the matrix of pasture may not cause harm to the growth of individuals of *P. vellosiana*.

The studied fragments were considered elongated (form factor < 0.6) with more irregular formats as the total of the fragment edges increased (VIANA; PINHEIRO, 1998). The minimum size for a fragment to maintain their functions and diversity of species was approximately 100 ha (LOMOLINO; WEISER, 2001). According to this criterion, the fragment at PP would be considered small with 72.3 ha, whereas the MA with a studied area of 229.6 ha would be considered large.

According to the information gathered on the fragments, which are located in rural properties, there is an indication that the smaller fragment (PP) survived from secondary woodland, and in this fragment, the trees were below 40 cm DBH. The MA fragment was derived from primary woodland with few changes to its original structure and characterized by species with DBH of approximately 40 to 50 cm (data not shown). Despite the size of the fragments, *P. vellosiana* was a fairly common species in the two study areas.

The 80 m length of the transects that were fixed on the south side of the fragment-matrix borders was not sufficient to reach the center of either fragment. This distance can be related to the similarity found in microclimatic data along the edge towards the interior. Genetic biodiversity of *Clusia sphaerocarpa*, *Clusia lechleri* (QUEVEDO et al., 2013) and *Psychotria tenuinervis* (RAMOS et al., 2010) species did not display any differences between plants in the edge and interior of forest. The edge effects can still be noticed 80 m away towards the center of the fragments, and for these fragments, the distinct matrices did not influence the leaf characteristics of species.

The matrix type influences the biodiversity, but these effects are minor when compared to the size of the remnants and their isolation (PREVEDELLO; VIEIRA, 2010). However, it should be noted that in this study, only two fragments were taken into account, i.e., a matrix surrounded by pasture and another surrounded by sugarcane, so the results are most likely inconclusive for the greater or lesser adaptability of the species in an environment where there may be interference from such matrices.

## 5 Conclusions

There does not seem to be a clear pattern of the extent to which the matrices of pasture and sugar cane surrounding the fragments can affect the growth and development of *Psychotria vellosiana* growing at the edge or 80 m within the fragments. According to certain analyzed parameters (chlorophyll *a*, spongy parenchyma, phloem and vulnerability index), this species may be better adapted to the MA fragment, which is surrounded by pasture. A detailed analysis is required that includes parameters that were not considered in this study, such as water availability and soil nutrient parameters.

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