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Key anatomical attributes for occurrence of *Psychotria schlechtendaliana* (Müll.Arg.) Müll.Arg. (Rubiaceae) in different successional stages of a tropical moist forest

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ABSTRACT

We analyzed the effects of two different successional stages on leaf and wood anatomy of *Psychotria schlechtendaliana* in a tropical moist forest. Leaf and wood samples were collected from plants growing in two sites representing two successional stages: advanced and intermediate stages of forest succession (ASFS and ISFS, respectively). The leaves have typical mesomorphic anatomy. Wood exhibits growth rings slightly different. The vessels elements are solitary, arranged radial or in clusters, with diffuse porosity, simple perforation plates, septate fibers, radial parenchyma multiseriate, and heterogeneous and perforated ray cells, intervessel pits bordered, small, alternate, vestured and/or scalariform. Quantitative analyses showed significantly differences in leaf and wood anatomy. Top and base leaves on both sites differed in the thickness of cuticle, palisade parenchyma, and palisade:spongy parenchyma ratio. Plants at ISFS had leaf lamina with thinner adaxial cuticle, smaller cells on the adaxial epidermis, smaller width of palisade parenchyma, smaller palisade:spongy parenchyma ratio, and lower stomatal density than at ASFS. The wood of plants in ISFS had presented smaller diameters of the lumen of vessels and fibers, higher frequencies of vessels and rays, and fiber and rays with longer lengths than at ASFS. The differences between leaf and wood anatomy at the two sites confirm a structural adjustment in relation to forest succession for this species. The anatomical differences reflect the sunlight distribution and water availability, allowing the adjustment in photosynthetic efficiency, and safety water transport.

1. Introduction

The Brazilian Atlantic Forest is one of the most biodiverse biomes in the world with several phytophysiognomies subjected to different environmental conditions (Peixoto et al., 2002). Unfortunately, this biome has been subjected to intense anthropogenic disturbance, which has resulted in loss of much of its forest cover (Ribeiro et al., 2011). The remaining extent of Atlantic Forest is fragmented, with only 8.5% of remnants larger than 100 ha (SOS Atlantic Forest Foundation, 2014). The remaining forest remnants have experienced varying degrees of degradation and are in different stages of regeneration (Martini et al., 2007; Piotto et al., 2009). As a result, the Brazilian Atlantic Forest is being ranked among the top five world hotspots for conservation priority (Eisenlohr et al., 2015; Mittermeier et al., 2005; Myers et al., 2000).

Reducing forest cover can result in a linear loss of individuals, genera and species of understory (Andrade et al., 2015), consequently, changes the canopy structure, which severely alters the forest microclimate (Wirth et al., 2001). According to Murray-Smith et al. (2009), conservation strategies should focus on small areas representative of the diversity and endemism of the biome. Thus, the identification and study of such areas can help in conservation planning, especially in those areas that are very diverse. An example is the Serra do Conduru State Park (PESC), located in southern Bahia, which has great species

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diversity and endemism (Amorim et al., 2005; Thomas et al., 2008, 1998), and is comprised of a mosaic of forests in different stages of regeneration (Martini et al., 2007; Piotto et al., 2009).

In the PESC's region, the species of the genus *Psychotria* L. (Rubiaceae) are an important component of the forest understory, and contribute significantly to its floristic richness (Amorim et al., 2009). There are approximately 252 species of *Psychotria* in Brazil, and they include shrubs, trees, and rarely climbing (Taylor et al., 2015). Members of this genus are typically understory specialists, and exhibit conserved evolutionary traits that reflect strategies related to differences in light and moisture availability, with a structural adjustments or phenotypic plasticity capacity (Mulkey et al., 1992; Sedio et al., 2012; Sterck et al., 2013; Valladares et al., 2000). The strategies adopted for species to withstand drought can be completely different (Gullo and Salleo, 1988). Anatomy of sun-exposed and shaded leaves can to present too differences in species, as the leaf anatomy is well related to foliar physiology. (Qi et al., 2006). Tree species living in wet lowland rainforest clearly differ in drought susceptibility, suggesting that there are variable water transport strategies. The differences in some characteristics (physiological, morphological and anatomical) between and within the functional groups of trees reflect the variation in strategies of water transport and resistance to drought (Apgaua et al., 2015).

Psychotria schlechtendaliana (Müll.Arg.) Müll.Arg. is endemic to Brazil and occurs in areas of semiarid forests and Atlantic Forest in the northeast, especially in the states of Bahia, Ceará and Pernambuco (REFLORA, 2012). The studies with the species *P. schlechtendaliana* are mainly aimed at the survey and floristic composition in regions of Atlantic Forest of Northeast Brazil. (Borges and Ferreira, 2017; Coelho and Amorim, 2014; Fernandes and Queiroz, 2015; Martini et al., 2007; Nascimento et al., 2012; Piotto et al., 2009; Rodal et al., 2005). In southern Bahia, this shade-tolerant species is widely dispersed among the understory of tropical moist forests (Amorim et al., 2009; Piotto et al., 2009), at various successional stages, occurring in quite preserved areas even in much degraded areas (Andrade et al., 2015). This makes the species a suitable model for studies aimed at structural adjustments developed in different forest succession stages.

Anatomical investigations can be a useful approach for understanding structural changes in the plants subjected to environmental changes in their natural habitats (*e.g.* Callado et al., 1997; Dickison, 2000; Juno et al., 2013; Mantuano et al., 2006). Analyses of intraspecific variation in leaf anatomy have shown that differences in the leaf blade thickness, palisade parenchyma, amount of fibers and the density and location of stomata are related to abiotic factors such as light intensity and water availability (Esau, 2000; Pireda, 2013; Rabelo et al., 2012; Rôças et al., 2001). The study of the structure and function of wood, especially in the hydraulics of trees, has also evolved as one of the most interesting fields of plant biology and evolution and forest ecology (Hacke, 2015). Studies of leaf and wood anatomy can be integrated to further characterize a species and identify those characteristics that are influenced by environmental variation. In this context, this work aimed to perform a comparative analysis of the anatomical structures of leaf and wood of *P. schlechtendaliana* occurring in two different successional stages within a tropical moist forest, seeking to associate the effect of microclimate on the structural adjustments of the species, and from this, to identify possible adaptive strategies that the species presents to survive in its natural habitat, but with different environmental changes.

2. Material and methods

The study was conducted in southern Bahia, Brazil, in the Serra do Conduru State Park (PESC) (14°20′ – 14°30′ S, 39°02′ – 39°08′ W, at altitudes from 130 m). The vegetation of PESC is classified as tropical moist forest (Gouvêa et al., 1976), with a uniform canopy of more than 25 m in height, a dense understory and numerous epiphytes and large lianas (Jardim, 2003). The park comprises a mosaic of forests in different stages of succession, including highly conserved mature forests (Martini et al., 2007). The climate is hot and wet, with average temperatures of 24 °C. November to March is the driest period and July to August is the coldest period. Relative humidity is often above 80%, with well-distributed annual rainfall averaging 2000 mm (Landau, 2003; Sá et al., 1982). The soil types in this area include yellow and red–yellow latosols, yellow and red–yellow podzols, as well as alluvial and sandy sections (Governo do Estado da Bahia, 1998).

Plant material was collected within PESC at two sites in two different successional stages (Martini et al., 2007). An advanced stage of forest succession (ASFS) that consists of a forest that has been regenerating from deforestation for nearly 30 years, but remains surrounded by large forest blocks with minor disturbances. The other an intermediate stage of forest succession (ISFS) that consists of a forest that has been regenerating from deforestation for approximately seven years, and is surrounded by small forest fragments in different successional stages or small grazing areas and agricultural land, which intensify the edge effect on forests. To distinguish sites we analyzed the soil and the canopy openness (CO). The CO was analyzed using hemispheric images captured with a fisheye lens (180°) attached to a digital camera (Coolpix 4300, NIKON, Japan). The camera was mounted at 1.40 m above the soil level on a tripod, each photograph having a range radius of approximately 10 m. The images were analyzed using the software Gap Light Analyzer 2.0 (Frazer et al., 1999). Both areas are located in a portion of dystroferric yellow latosol soil (IBGE, 2018). But for a better characterization of the soils, three samples were taken within a 1 m radius of the individuals at a depth of 20 cm, totaling 15 simple samples per area. Each set formed a composite sample of each area (Catani et al., 1954; Oliveira et al., 2007). The two composite soil samples were analyzed in the Soil Laboratory of the Centro de Pesquisas do Cacau (CEPEC/CEPLAC), Ilhéus, Brazil.

Five individual trees with cylindrical, straight trunks and without bifurcations or defects were identified in each site. The trees had height of about 6 m and diameter at breast height of 5 cm. Fully expanded leaves were collected from different vertical strata of each study trees, with five leaves collected at the highest stratum of the tree canopy (top leaves) and five leaves collected at the lower stratum of the tree canopy next to the forest understory (base leaves). Wood samples were collected from the same ten study trees in a non-destructive manner using a Pressler probe (Increment Borer, SUUNTO, USA) at 1.30 m above the soil, with two samples by individual. The plant material was vouchered in the reference collection of the Universidade Estadual de Santa Cruz Herbarium (HUESC nº 16,193), Universidade Estadual do Norte Fluminense Herbarium (HUENF nº 1,923) and Xylotheque Dra. Cecília Gonçalves Costa (HUENFw nº 202 until 211) of the last one institution.

2.1. Light microscopy

Fully expanded leaves were fixed in a solution of 2.5% glutaraldehyde, 2.0% formaldehyde and sodium cacodylate buffer at 0.05 M pH 7.3 (Karnovsky, 1965 modified by Da Cunha et al., 2000). Transverse sections were made from the middle-third of the leaves by freehand cut and stained with Safranin-Astra blue (Kraus and Arduin, 1997) for the manufacture of semi-permanent slides. For a paradermic view surface of epidermis and to determine stomata density, other leaf fragments were subjected to dissociation both epidermis surfaces (Franklin, 1945). The leaf fragments were placed in an oven at 60 °C for about 24 h until they became transparent. The fragments were washed and stained with 1% aqueous Safranin. Adaxial and abaxial surfaces of the epidermis were separated and mounted on different semi-permanent slides.

The parameters used for comparing the leaves from the two sites and from the different strata included: leaf blade thickness, thickness of palisade and spongy parenchyma, thickness of the adaxial and abaxial surfaces of the epidermis, thickness of adaxial and abaxial cuticles, the ratio between the thickness of the palisade and spongy parenchyma,