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ECOLOGICAL STUDY OF THE STOMATIC DENSITY OF FOREST SPECIES

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Abstract: The Amazon concentrates the greatest diversity of forest species on the planet, among these species are the *Adenhantera pavonina* L. and the *Hevea brasiliensis* Muell Arg. Considering the scarcity of studies relating stomata with the physiology of these species in the literature, the objective of this work was to analyze the stomatal density and its relationship with the physiology of the species, as well as identify the types of stomata present in each species and classify the leaves as for the presence of stomata. The botanical material was collected in two green areas of Itacoatiara-AM, in Horto Florestal, the leaves of *A. pavonina* and in the Rubber Tree Forest, the leaves of *H. brasiliensis*. For each species, leaves from the apex, middle and base were collected, with three samples from each position of the tree from three different individuals. The leaves were preserved in 70% alcohol for further analysis. To determine the stomatal density, the dissociation of the epidermis was done with sodium hypochlorite, stained in safranin and subsequent mounting of slides for analysis under an optical microscope. The stomata had their classification confirmed by means of freehand paradermal cuts. Both species studied presented hypostomatic, homeostomatic leaves with the presence of paracytic stomata. In this study, evidence was found ($p = 4{,}4^{-46}$) to affirm that the density of H. brasiliensis differs in the different positions of the tree, and, for the *A. pavonina* ($p = 0{,}577$) no differences were found in relation to stomatal density.
Keywords: Microscopy, Dissociation, Hypostomatic leaves.

INTRODUCTION

The Amazon region is home to the greatest diversity of forest species on the planet, and these are responsible for maintaining the forest ecosystem, producing oxygen and fixing carbon (GAMBARINI, 2020). Among the various species that exist in the Amazon, there are the *Adenanthera pavonina* L. and *Hevea brasiliensis* Muell Arg., large and fast growing trees.

The anatomical study of these plants is usually associated with their wood, without highlighting the leaf, the organ responsible for carrying out photosynthesis (PES; ARENHARDT, 2015), and in which there is a greater amount of stomata. These are small holes surrounded by two guard cells that control the stomatal opening (MENDONÇA, 2016).

Basically, the stomata are responsible for communication between the interior of plants and the external environment, providing a channel that performs gas exchange and transpiration (TAIZ et al., 2017). These aspects, together with the physiology of trees, have received great attention, due to changes in the forest scenario (REINHARDT et al., 2007) and pollution in urban areas (SANTOS et al., 2019) that have affected plant development.

In general, as a survival strategy, plants tend to change their morphological and physiological characteristics when subjected to certain stress conditions, especially water. Initially, in response to low water availability, stomatal closure occurs to prevent excessive water loss through transpiration (MARTINEZ; LUCEMA; BONILLA, 2021; PIMENTEL et al., 2016).

The reduction in the volume of water lost by plants can also be associated with variations in the size and distribution of stomata (LAKE et al., 2001), with stomatal density being an important ecophysiological parameter (CAMARGO; MARENCO, 2011), since, how much the greater the density, the greater the absorption ofCO_2 and the photosynthetic rate will probably be higher, if in a suitable environment (MELO JUNIOR; GONÇALVES; JARDIM, 2019).

This study aimed to characterize the stomata and analyze the stomatal density present in the leaves of two forest species, seeking to relate these characteristics to the physiology of the species.

MATERIAL AND METHODS

The botanical material used in this study was collected in two green areas in the municipality of Itacoatiara, AM, Brazil: 1) forest garden, located in Tiradentes neighborhood, where leaves of*Adenanthera pavonia* L. were collected; and 2) rubber tree forest, located in the Santo Antônio district, where the leaves were harvested. *Hevea brasiliensis* Muell Arg. (Figure 1).

The climate type of the study area, according to Koppen's classification, is Af (equatorial or tropical hot and humid). It has an average temperature of 26 °C, with a small temperature range, and the relative humidity is always above 80%. Rainfall is high, with an annual average of 2,261 mm (CLIMATE-DATA, 2019).

BOTANICAL MATERIAL COLLECTION

The criteria used to select the two species: *Adenanthera pavonina* L. (Fabaceae) and *Hevea brasiliensis* Muell Arg. (Euphorbiaceae), were the scarcity in the literature of anatomical studies of the leaves of their stomata and the relationship of these with the physiology of the plant.

In the field, with the aid of a trimmer, adult leaves were removed and in good morphological and phytosanitary conditions from different positions of the tree: apex, middle and base. For each species, three individuals were selected. The collected plant material was preserved in 70% alcohol.

These plants were herborized and incorporated into the herbarium collection of the Center for Higher Studies of Itacoatiara of the State University of Amazonas - CESIT/UEA, receiving the number 0058 for *A. pavonina*, and, 0059 to *H. brasiliensis*.

DISSOCIATION OF FOLIAR EPIDERMIS

For epidermis dissociation, sections of the apex, median region of the rib and base of the leaf blade were removed (Figure 2A). These sections were subjected to a sodium hypochlorite (NaClO) solution, changing this material every five days (INMETRO, 2020). This process was repeated until complete dissociation of the epidermis (Figure2B).

Subsequently, the sections were cleaned with the aid of a brush number zero to remove the mesophyll and stained in safranin, with passage in an ethanol series and subsequent assembly of the semi-permanent blades (Figure 2C). Also, to confirm the classification of the stomata and their position on the leaves, paradermal cuts were made freehand (Figure 2D), clarified with NaClO and stained with safranin for mounting the slides.

The stomata were classified taking into account the position of the cells adjacent to the stomata in relation to the guard cells, as proposed by Cutler, Botha and Stevenson (2011).

The stomatal density was calculated for each species, being determined by counting stomata from 30 microscopic fields observed in the 40x objective. The number of stoma observed in each field was divided by 0.39 mm² (field area), obtaining the density in mm². Then, the average of the 30 fields was calculated to obtain the average stomatal density for each treatment. These data were subjected to analysis of variance and their means compared by Tukey test at 5% probability.

DISCUSSION RESULTS

Hevea brasiliensis MUELL ARG.

In the leaves of *H. brasiliensis*, stomata were identified only on the abaxial face, characterizing them as hypostomatic leaves. Similar results were found by Carvalho, Marenco and Neta (2011), when evaluating samples of this species, from five seedlings collected at Campus V8 of the National Institute for Amazonian Research (INPA), Manaus, AM, Brazil.

In frontal view, the leaf epidermis presented cells with irregular walls, sinuous on the adaxial face (Figure 3A) and slightly sinuous on the abaxial face, plus the presence of paracytic stomata (Figure 3B). According to Thakur and Patil (2011), the contour of epidermal cells is appointed as a taxonomic character for species of Euphorbiaceae.

Cutler, Botha e Stevenson (2011), describe the concept of paracytic stomata those that have one or more subsidiary cells positioned on each side, so that their longitudinal axis is parallel to the stomatal cleft. Studies carried out on species of the genus: *Croton*, belonging to the same family as the *H. brasiliensis*, it was possible to verify that the presence of paracytic stomata for Euphorbiaceae is common (ALVES, 2017; NOBREGA et al., 2018).

The average stomatal density for the apex, middle and base regions were, respectively, 186.3 stomata/mm², 153.5 stomata/mm² and 129.3 stomata/mm² (Figure 4). Analysis of variance (ANOVA) showed that there is evidence ($p= 4,4^{-46}$) to state that the stomatal density differs in the different positions of the tree.

Conforto, Andreoli and Cavalcante (2007), in a study with the same species, found mean values of stomatal density higher than those found in this study for the three positions. Identifying according to the ANOVA difference in the mean density of the median region, in relation to the mean of the apical and basal region, which showed no differences.

Medri e Leras (1980) observing aspects of the ecological anatomy of leaves of *H. brasiliensis* observed that the number of stomata significantly increases in relation to the height of the leaflets. Similar results can be observed in the study by Jardim, Melo Junior and Silva (2018) who found a higher density of stomata in leaves collected in the canopy in *Hyeronima alchorneoides* (Phyllanthaceae). Thus confirming the difference in stomatal density in relation to the different leaf positions.

Variations in stomatal densities in different positions of the plant reveal that the species has anatomical plasticity. Coinciding with studies by Giambelluca et al., (2016) and Kumagai et al., (2015), who found variations in regulation and stomatal conductance in *H. brasiliensis* grown in Asia. For Justo et al., (2005), the increase in stomatal density is generally related to a higher stomatal conductance, preventing photosynthesis from being limited under adverse conditions.

Nascimento, Nascimento e Gonçalves (2019) when analyzing gas exchange in young plants of *H. brasiliensis* under water stress conditions until hydrated, they found, respectively, photosynthetic rates ranging from 0,4 μmol m^{-2} s^{-1} until 8,4 μmol m^{-2} s^{-1}, showing that the amount of available water probably significantly influenced photosynthesis.

Adenanthera Pavonina L.

The species: *A. pavonina* has hypostomatic leaves with paracytic-type stomata located at the same level as the epidermal cells (Figure 5B). Similar results were observed in other species of the same family (SILVA; REIS; PONTES-PIRES, 2012), includingossui folhas hipoestomáticas com stomata do tipo paracíticos localizados no mesmo nível das células epidérmicas (Figura 5B). Resultados semelhantes foram observados em outras espécies da mesma família (SILVA; REIS; PONTES-PIRES, 2012), podendo citar *Hymenaea courbaril* (TIAGO et al., 2020) and *Dalberiga brasiliensis* (DALARMI, 2017). Evidencing to be a constant feature for this family.

The leaf of *A. pavonina*, in front view, it presents cells with sinuous walls, of irregular sizes and shapes on both sides (Figure 5A, 5B), but only the adaxial one presents cells with smooth walls. This description is similar to that of Tiago et al., (2020) in a study with H. *courbaril* (Fabaceae), where he observed that the anticlinal walls of epidermal cells are sinuous on both sides. However, no cells with smooth walls on the adaxial face.

The *A. pavonina* has the presence of unicellular tector trichomes, which can be observed throughout the leaf blade (Figure 5C). The presence of trichomes on the surface is usually related to the leaf water balance (CUTLER; BOTHA; STEVENSON, 2011). According to Melo Junior, Gonçalves and Jardim (2019), the increased density of these structures allows plants to adapt when subjected to water deficit and high light, since these structures have a direct effect on the reduction of transpiration under intense light conditions. (BITTENCOURT; SILVA, 2018).

The average stomatal density for the apex, middle and base were, respectively, 67.4 stomata/mm², 69.1 stomata /mm² and 67.9 stomata/mm² (Figure 6). The analysis of variance showed that there is no evidence ($p = 0.577$) to state that stomatal density differs in tree positions.

Unlike expected, as in *H. brasiliensis*, no significant differences were observed in stomatal density between tree positions. Cunha and Santos (2010) when studying the stomatal variation of the leaf epidermis of *Acacia mangium* Willd, (Fabaceae), also observed that there was no significant variation in stomatal density in the epidermis of different regions of the crown, with an average density of 54.85 stomata /mm².

Studies show a positive correlation between increased luminosity and stomatal density (NERY et al., 2007), since luminosity is one of the main factors influencing the structure and functioning of the plant (JARDIM; MELO JUNIOR; SILVA, 2018). However, other authors claim that there is no direct response of stomatal density due to different shading levels (ARAÚJO et al., 2016), as occurred in *A. pavonina*. In some cases, the plasticity of a plant is more evident in morphological aspects than in anatomical attributes. (MELO JUNIOR; BOEGER, 2016).

Hevea brasiliensis Muell Arg. x *Adenanthera pavonina* L.

Both species analyzed presented hypostomatic and homostomatic leaves. For Camargo (2009) there is a predominance of hypostomatic leaves in tree species in Central Amazonia. This absence of stomata on the adaxial face is characteristic of xeromorphic plants, they allow great water savings, by reducing the transpiration rate, due to less exposure to direct sunlight (POLESI; RODRIGUES; ALMEIDA, 2011).

As a common characteristic of the species, sinuosity in the epidermis walls was also observed. According to Glória and Guerreiro (2006), the sinuosities presented in the cell walls are probably due to the tensions that occurred in the leaf and the hardening of the cuticle during cell differentiation.

The species: *H. brasiliensis* had higher mean stomatal density (156,4 stomata/mm²) comparing to *A. pavonia* (68,1 stomata/mm²).

According to Bittencourt and Silva (2018) the leaves that present an increase in stomatal density, with a decrease in the size of the stomata, are usually developed during periods of water stress, being an adaptation of the plant that contributes to a better control of the transpiration rate.

According to Pimentel et al., (2016) tolerance to conditions of low water availability can vary even between species that belong to the same genus, as plants have different response mechanisms (BITTENCOURT; SILVA, 2018), being the economy of water a factor that directly affects the anatomical structure of the leaf (MELO JUNIOR; GONÇALVES; JARDIM, 2019). In general, the leaves increase their stomatal density, as this allows the plant to increase the conductance of gases, thus preventing photosynthesis from being limited under different environmental conditions. (LIMA et al., 2006),

FINAL CONSIDERATIONS

The species studied proved to be adapted to the dry environment, presenting structures that save water, such as hypostomatic leaves and sinuosity in the walls of the epidermis cells.

The fact that both species have homostomatic leaves opens the possibility of using the characteristics of the stomatal complexes in taxonomic work involving forest species from the Amazon.

In this study, evidence was found to state that the density of *H. brasiliensis* differs in the different positions of the tree, and for the *A. pavonina,* no differences were found regarding stomatal density.

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