

COMPARATIVE WOOD ANATOMY OF TWO TROPICAL SPECIES

Cassiana Alves Ferreira^{1*}, Alessandra de Oliveira Ribeiro¹, Cláudia Viana Urbinati²,
Paulo Junio Duarte³, Fábio Akira Mori⁴, Beatriz Valente Miglio⁵

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ABSTRACT - Wood Anatomy is science field very relevant to understanding environmental climate changes and important in suggested to species conservation. The present study proposed the characterization of wood anatomy of *Inga alba* and *Tapirira guianensis* from two locations in the Legal Amazon, establishing ecological relationships between both and their habitat. The species were described and characterized according to standards for wood anatomy of IAWA. The quantitative data were submitted to ANOVA and later to PCA (principal components analysis). Qualitatively, characters such as slightly different growth layers, bounded by fibrous zones, diffuse porosity, diagonal arrangement, predominantly solitary vessel, simple perforate plate, libriform and septate fibers, heterogeneous rays were common the two species independent sites following the pattern for Tropical rainforests. However, there were quantitative differences for diameter and frequency mm² of vessel, and frequency of rays per linear mm related in sites where substrates are most nutritious. As for diameter of pits, there were significant differences, however, in this case, related to species. Similarities between qualitative characters suggest that species inserted in the same environment, even if they belong to different rates, can share several adaptive characters.

Keywords: Amazon rainforest, brazilian flora, environmental wood anatomy, species conservation.

ANATOMIA COMPARADA DA MADEIRA DE DUAS ESPÉCIES TROPICAIS

RESUMO - A anatomia da madeira é um campo da ciência de grande relevância para a compreensão das mudanças climáticas e a conservação das espécies. O presente estudo propôs caracterizar a anatomia da madeira de *Inga alba* e *Tapirira guianensis* provenientes de dois locais na Amazônia Legal, estabelecendo relações ecológicas entre ambas e o seu habitat. As espécies foram descritas e caracterizadas de acordo com as normas para anatomia da madeira do comitê IAWA. Os dados quantitativos foram submetidos a ANOVA (análise de variância) e posteriormente ao PCA (análise dos componentes principais). Caracteres qualitativos comuns foram observados nas diferentes espécies, tais como camadas de crescimento distintas e delimitadas por zonas fibrosas, porosidade difusa, arranjo diagonal, elementos de vasos predominantemente solitários, placa de perfuração simples, fibras libriformes e septadas, raios heterogêneos encontradas nos dois locais de coleta, independente da espécie, sugerindo um padrão para espécies observadas em ambientes mésicos. Entretanto, houveram diferenças significativas para diâmetro e frequência por mm² dos elementos de vasos e frequência de raios por mm linear relacionados aos locais e aos nutrientes do solo. Para o diâmetro das pontuações houveram diferenças significativas, porém nesse caso relacionadas a espécie. Semelhanças entre os caracteres qualitativos sugerem que espécies inseridas em um mesmo ambiente, mesmo que pertencentes a taxos distintos, podem compartilhar diversos caracteres adaptativos.

Keywords: Floresta Amazônica, flora brasileira, anatomia ecológica do lenho, conservação de espécies.

INTRODUCTION

The tropical taxa *Inga alba* (Sw) Willd. (Fabaceae) and *Tapirira guianensis* Aublet (S) (Anarcadiaceae) are high tree species that occupy a great number of niches, therefore, species indicate everything they have great plasticity in

different environments, also being extremely used as lumber in states that integrate Amazon Basin.

Amazon forests is considered one of the most diverse planet's vegetation, sheltering thousands of tree species (TER STEEGE et al., 2016; CARDOZO et al., 2017)

¹PhD in Botany, Department of Biology of Federal University of Lavras (UFLA), University Campus, s/n, Lavras, Minas Gerais, Brazil. E-mail: cassianaaf@gmail.com, alebioribeiro@gmail.com. *Correspondence author.

²PhD in Wood Science and Technology, Wood Technology Department of State University of Pará (UEPA), Belém, Pará, Brazil. E-mail: urbinatiuepa@gmail.com.

³PhD student in Wood Science and Technology, Department of Wood Science and Technology of Federal University of Lavras (UFLA), University Campus, s/n, Lavras, Minas Gerais, Brazil. E-mail: pauloduarte.floresta@gmail.com.

⁴PhD in Forest Sciences, Department of Wood Science and Technology of Federal University of Lavras (UFLA), University Campus, s/n, Lavras, Minas Gerais, Brazil. E-mail: mori@ufla.br.

⁵Forest Engineer, Wood Technology Department of State University of Pará (UEPA), Belém, Pará, Brazil. E-mail: valentebeatriz06@gmail.com.

and play an important role in the global carbon cycle (FAUSET et al., 2015). Meanwhile, a strong anthropic pressure on these forests threatens their role in mitigating climate change and biodiversity loss due to increased deforestation and forest degradation (GAUI et al., 2019).

The plant species, their variations and adaptations in different habitats are currently the main studies among populations and their interactions. In researches of secondary xylem it isn't different, since the simple description of xylem tissue became, in a way, unusual. The same happens with the studies about the structure of wood, that aim at description the tissue, determining its xylem composition along an ecological gradient (BARROS et al., 2006; MELO JUNIOR et al., 2011; SONSIN et al., 2012; LONGUI et al., 2014).

In arboreal communities, such variation expressed to xylem structure in a quantitative and qualitative way (biometry of cellular elements and tissue proportions). Only the intrinsic factors do not explain this variation in tropical species, but also, latitudinal and longitudinal gradients in different macro and mesoclimates (ALVES and ANGYALOSSI-ALFONSO, 2000; ALVES; ANGYALOSSI-ALFONSO, 2002), suffers direct interference of water availability (BUCCI et al., 2004; RODRIGUEZ-ZACCARO; GROOVER, 2019), temperature and soil conditions (FONTI et al., 2012), resulting in works that have high relevance in climates and past environments,

supporting structural phylogenetic studies and research of future climate projections (BAAS, 1982).

Carlquist (2001) suggests two different approaches to correlation between ecology and xylem anatomy. The first is based on taxonomic groups and aims at the comparative analysis to species or same genus along an ecological gradient. The other part consists the comparison of small plant formations, aiming at recognition of anatomical characters common a given habitat. Therefore, is it possible that certain species do not have a phylogenetic relationship, family or gender in common, and can they present certain characteristics because they share the same environment?

In view the environmental and economic importance of species, its wide use by community, the present study aimed to characterize the structure of the secondary xylem two species from two sites belonging to the Legal Amazon, establishing ecological relations between them and their habitat.

MATERIAL AND METHODS

Collection areas

The soil characteristics and the location of the collection areas are described in Table 1 and Figure 1, respectively.

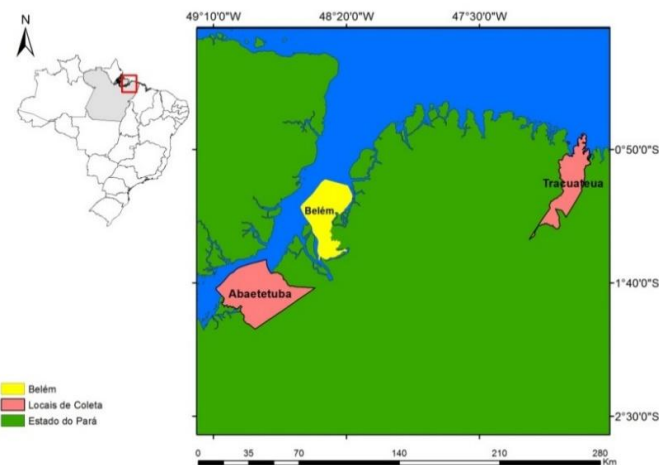


FIGURE 1 - Presents the location of collection areas in relation to Belém, state capital of Pará.

TABLE 1 - Soil-climatic data of the areas collected according to Idesp (2013) and Oliveira Júnior et al. (1999).

	Abaetetuba (PA)	Tracuateua (PA)
Rainfall /average year	2.577 mm	2.552 mm
Soils type	Yellow dystrophic Oxisols	Inseptisols Aquepts
Climate (Köppen)	<i>Am</i>	<i>Awi</i>
Geographic coordinates	1° 43' 24" S 48° 52' 54" W	1° 9' 7,50" S 46° 58' 12" W
Temperature	27°C	27°C

Am = super humid equatorial, *Awi* = humid equatorial.

Study material

The botanical material was collected, identified and herborized according to the norms for collection and herborization proposed by Ferreira (2006). Afterwards, they were sent to the Federal Rural University of the Amazon (UFRA) Herbarium for procedures to determine and certify species.

Three individuals of each species were collected in two different locations, Abaetetuba and Tracuateua in the state of Pará, Brazil, and the distance between them is approximately 300 km, both inserted in the Legal Amazon (IBGE, 2016). The specimens were collected at a minimum distance of 100 meters between them, and diametric classes between 125,66 - 141,37 cm in DBH (diameter at breast height, at 1.30 m from the ground).

The anatomical characterization of wood was executed in the Laboratory of Wood Anatomy at the Federal University of Lavras (Minas Gerais, Brazil), in Department of Forestry Sciences, following the Standard for Anatomical description of the IAWA Committee (1989), with adaptations made by Coradin and Muniz (1992).

The specimens were made from 5 cm disks removed from the base of the individuals, in dimensions of 2x2x2 cm, oriented in three cutting planes of wood, transverse and longitudinal, tangential and radial, these were removed near the bark or vascular exchange. Subsequently the specimens were submitted to saturation in water, in a desiccator coupled to a vacuum pump. The histological sections were removed with the aid of a Leica® slide microtome, model Jung SM2000 with thickness determined between 16 and 20 µm. Subsequently, the histological sections were submitted to clarification in commercial hypochlorite, dehydrated in a hydro-alcoholic sequence (20,50,70,80,100%), stained with safranin and astra blue and later fixed in a series of ethanol-butyl acetate (3:1: 1: 1, 1:3 and 100% butyl acetate); finally, histological sections were mounted on lamina and glass slide in Entellan®.

For the histochemical tests with Sudan IV for the detection of lipid and Lugol compounds for detection of starch cells according to method (JOHANSEN, 1940). The macerated material was obtained with the aid the method of Franklin (1945), from longitudinal fragments of specimens.

The anatomical characters were measured and photographed with the aid of Wincel® software using computer coupled to an image capture camera and optical microscope. The 'n', number of observations for each anatomical character was 40 replicates, for each specimen, at different collection sites. The data were then tabulated for Excel® software to obtain the maximum, minimum, rate, standard deviation and coefficient of variation values.

The quantitative data were first submitted to analysis variance (ANOVA), submitted to Tukey test, at 5% of confidence. Subsequently, the significant variables were submitted to grouping analysis of main components (PCA) in order to determine the anatomical characteristics that

segregate the species, performed in R Studio® software (2018).

Results and Discussion

Qualitative analysis of the species studied

In general, the specimens had slightly different growth layers, delimited by the radial flatness of fiber wall forming fibrous zones, diffuse porosity in a diagonal arrangement, with circular contour, predominantly solitary, and may occur multiple (2-4), single perforate plate, intervessel pits alternate, composition rays formed by rows of square cells and body constituted by procumbent cells, forming heterogeneous wood rays. Axial parenchyma predominantly paratracheal, being abundant in *I. alba* and scarce in *T. guianensis* independent of collection site (Figure 3A and 4A). The fibers libriform and septate (Figure 3D and 4C).

The *I. alba* presented paranchyma axial paratracheal vasicentric/aliform, being able to occur confluence from short to long. Diffuse porosity, vessels with diagonal arrangement, mostly solitary 53%, occurring multiples of 2, 3 or 4. Vessels with circular outline, frequency per mm² of 6, length of 339.96 µm, diameter of 190.32 µm, small alternate intervessel pits with mean diameters of 3µm; vessel rays pits are similar to intervessel in size and shape. Mucilaginous cells associated with the axial parenchyma (Figure 2B). Libriform fibers septate (Figure 2D) of average length of 1235.06 µm, with a mean wall thickness of 5.95 µm. Predominantly homogeneous rays consisting of procumbent cells, which may be heterogeneous consisting of procumbent and upright cells, divided into two classes: larger with a height of 286.50 µm and a mean width of 40.42 µm and smaller with a mean height of 149.79 µm and average width of 18.73µm. Presence of rhombohedral crystals in fibrous chambers. Lipids present in radial cells (Figure 2B) and starch granules in fibrous chambers (Figure 4B).

T. guianensis presented distinct growth layers, delimited by radial flattening of the fiber wall, forming fibrous zones (Figure 3C). Paratracheal axial parenchyma scarce. Diffuse porosity with vessels in diagonal arrangement, mostly solitary 55%, occurring multiples of 2 and 3. Vessels with circular outline (Figure 3E), mean frequency of mm² of 15, length of 412.74 µm, diameter of 129.95 µm, single perforate plates and small intervessel pits alternate, with a mean diameter of 5,20 µm; ray vascular pit similar to intervessel ones in size and shape. Appendices present in vessels at one end, rarely in both. Vessels obstruction by tyloses. Septate libriform fibers of mean length 1297.10 µm (Figure 3F), mean fiber wall thickness 5.34 µm. Starch grains present in fibrous chambers (Figure 4B). Heterogeneous rays, cell body consisting of procumbent cells bordered by square cells, being divided into two classes: larger with height of 395.11 µm and mean width of 46.72 µm and smaller with mean height of 188.07 µm and mean width of 19.73µm. Oval radial channels present

(Fig. 3B). Rhombohedral crystals present in radial cells (Figure 3A).

The descriptive results of the anatomical structure of the species of the present study are in agreement with

works reporting the anatomical structure of the secondary xylem, Gomes et al. (2014) working with individuals of *Inga alba* (Sw.) Willd, and Costa et al. (2014) with the species *Tapirira guianensis* Aublet (S.).

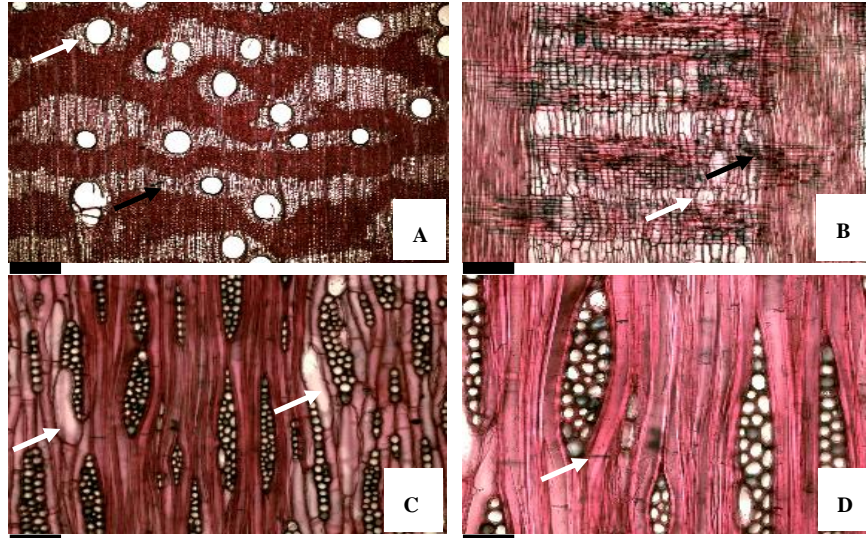


FIGURE 2 - Microscopic features of the xylem of *Inga alba* (Sw.) Will. In A) transverse plane (black arrow: parenchyma axial paratracheal confluent aliforme; and white arrow: mucilaginous cells), B) radial plane (white arrow showing mucilaginous cells and black contents in radial cells), C) tangential plane (arrow showing the presence of mucilaginous cells), D) tangential plane (arrow showing septate fibers). Bar in A at 500 μ m, B and C at 200 μ m and D 50 μ m.

Therefore, the high incidence of common anatomical characters among the studied species, independent of the collection sites, suggests an ecological standard common to this vegetation, regardless of genus and/or botanical families. According to Dickison (1989) and Wheeler and Baas (1991), Alves and Angyalossy-Alfonso (2000), Alves and Angyalossy-Alfonso (2002), this pattern follows possible ecological tendencies for tropical species that present a lower frequency of vessel with larger diameters and a higher occurrence of perforate plate simple is considered to be the most important of the plasticides found in the secondary xylem of Tropical Rainforest species, because it reduces friction and increases hydraulic efficiency, and is commonly found in tropical species, however Barros et al. (2006) working with tropical rainforest species observed the occurrence of two types of plaques for *T. guianensis*, simple and scaliform of 10 bars, being therefore a proven plastic species.

According to Alves and Angyalossy-Alfonso (2002) indistinct or even slightly different layers of growth are common in the Brazilian flora, being directly related to seasonality and temperature variation, directly affecting the exchange rate activity. The collection areas have similar characteristics, such as high temperatures and high rainfall, which may be crucial for this constancy of the cells, however, it is more noticeable in *T. guianensis*.

Parenchyma cells generally perform storage and transport of substances along the tree in both the axial and radial directions. Both species presented paratracheal axial parenchyma and heterogeneous rays, in other words, associated with the vessels, however, the specie *I. alba* presented abundance of this tissue, being a striking and identifiable characteristic of its wood. Alves and Angyalossy-Alfonso (2002) emphasize the occurrence of paratracheal axial parenchyma aliform, forming bands and/or confluences the tropical floras. *T. guianensis* presented the characteristic radial channel belonging to the family Anacardiaceae.

Alves and Angyalossy-Alfonso (2002) observed that in Brazilian species the axial parenchyma is more abundant in smaller latitudes (up to 23°), paratracheal parenchyma associated with warmer climates and apotracheal with higher latitudes and, consequently, colder climate. In the present study the abundant presence of paratracheal axial parenchyma aliform forming long confluences in *I. alba*, being an essential characteristic in the identification of the species and still associated with mucilaginous cells. However, for *T. guianensis*, this tissue is scarce, being observed in the tangential planes associated to the vascular lines and still obstructing the elements of vessels, forming tyloses.

Metcalf and Chalk (1989) mentions that the presence of septate fibers in the wood of tropical species is

directly related to the storage of substances such as water and nutrients. Other authors emphasize the function of the septate fibers as structures similar to the axial parenchyma

(CARLQUIST, 2001; ALVES; ANGYALOSSY-ALFONSO, 2002), being commonly found in tree species of the subfamily Mimosoideae (Fabaceae).

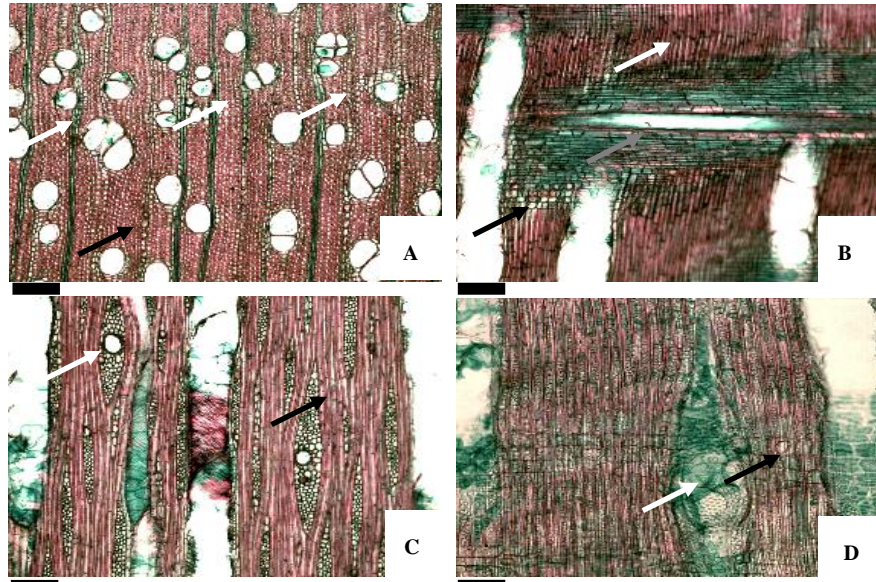


FIGURE 3 - Microscopic characteristics of the specie *Tapirira guianensis* Aubl. In A) transverse plane (white arrow: growth layers formed by fibrous zones and black arrow: crystals present in radial cells); B) radial plane (gray arrow: radial channels, black arrow square cells involving rays, white: septate fibers); C) Tangential plane (white arrow: radial channels) and D) radial plane (arrow indicates the presence of crystals in radial cells, white arrow: simple perforate plate). Bar in A at 500 μm , B and C at 200 μm and D 50 μm .

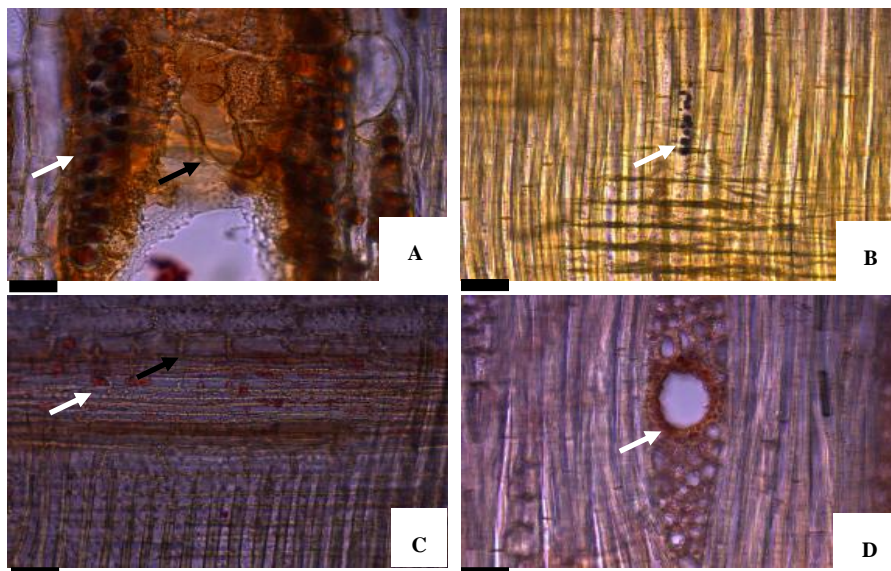


FIGURE 4 - Histochemistry of *I. alba* and *T. guianensis* wood. In A, C and D the test with Sudan IV evidencing the presence of lipids. In A and B, *I. alba*, transversal plane and tangential, respectively, white arrow evidencing the presence of lipids inside the rays and black arrow inside the vessels. In C and D, *T. guianensis*, histochemical test evidencing the presence of lipids (white arrow in C and D); radial channels characteristic of the Anacardiaceae family with lipid molecules present inside the channels (white arrows) and epithelial cells (black arrow). 50 μm bars.

Histochemical tests with Lugol and Sudan IV evidenced the presence of starch in fibrous chambers for all individuals of *I. alba* (Figure 2B) and the presence of lipid molecules in the characteristic radial channels of the family Anacardiaceae, both intracellular (Figure 2C and 2D). Lipid molecules were evidenced by Sudan IV in *I. alba* in their greater amount of vascular lines (Figure 2A) associated with radial, axial parenchyma and still mucilaginous cells in the axial parenchyma (Figure 3C).

Comparative anatomy of wood between collection sites Quantitative analysis

The analysis of variance for the quantitative traits in relation to the collection sites as well as the species are presented in Table 2. There were significant differences between species and collection sites for vessel diameter and frequency per mm², pits diameter, ray frequency per linear mm by the Tukey test at 95% probability. There were also differences between *I. alba* specimens for diameter and frequency per mm² of vessel and ray frequency per linear mm.

TABLE 2 - Biometry of the cellular elements of the studied species in function of the collection sites.

	<i>Inga alba</i> (Sw.) Willd		<i>Tapirira guianensis</i> Aubl.	
	Abaetetuba	Tracuateua	Abaetetuba	Tracuateua
1. Fiber				
1a. Length (µm)	1140.83a ± 40.76 (1106.62 – 1185.94)	1329.30a ± 223.41 (1126.86 – 1569.01)	1408.85a ± 138.24 (1249.57 - 1497.83)	1185.35a ± 115.10 (1061.71 – 1289.42)
1b. Wall thickness (µm)	5.89a ± 0.75 (5.33 – 6.75)	6.02a ± 1.10 (4.79 – 6.93)	5.42a ± 0.23 (5.24 – 5.68)	5.28a ± 0.63 (4.54 – 5.66)
2. Vessels				
2a. Length (µm)	318.15a ± 42.65 (269.03 – 345.98)	361.77a ± 87.07 (297.08 – 460.77)	446.59a ± 36.86 (405.08 – 475.51)	378.89a ± 25.87 (349.15 – 396.26)
2b. Diameter (µm)	145.72b ± 27.39 (117.56 – 172.27)	234.91a ± 13.65 (224.14 – 250.27)	124.44b ± 29.63 (90.21 – 141.75)	135.47b ± 7.85 (126.56 – 141.37)
2c. Frequency (mm ²)	6ab ± 1 (5 - 7)	4b ± 1 (3 - 4)	15a ± 6 (11 - 22)	14a ± 3 (11 - 18)
2d. Pits (µm)	2.99b ± 0.34 (2.59 – 3.24)	3.01b ± 0.72 (2.18 – 3.51)	4.74a ± 0.67 (3.96 – 5.17)	5.68a ± 0.12 (5.53 – 5.74)
3. Ray				
3a. Highest height (µm)	274.41a ± 5.38 (269.57 – 280.21)	298.60a ± 60.99 (237.90 – 359.88)	426.66a ± 154.63 (273.85 – 583.06)	363.57a ± 55.44 (310.73 – 421.29)
3b. Larger width (µm)	38.56a ± 3.06 (36.34 – 42.05)	42.30a ± 1.81 (41.10 – 44.38)	44.49a ± 7.28 (37.43 – 51.99)	48.96a ± 2.59 (47.30 – 51.95)
3d. Smallest height (µm)	149.71a ± 9.39 (140.33 – 159.13)	149.87a ± 6.41 (142.56 – 154.56)	205.23a ± 43.43 (156.87 – 240.91)	170.91a ± 21.54 (146.87 – 188.46)
3e. Smallest width (µm)	17.66a ± 2.18 (15.14 – 19.10)	19.80a ± 3.67 (16.80 – 23.92)	18.77a ± 4.27 (15.83 – 23.67)	20.70a ± 1.83 (19.15 – 22.72)
4. Linear frequency (mm)	12.54a ± 1 (11 - 14)	10ab ± 1 (9 - 11)	7bc ± 1 (6 - 8)	7c ± 1 (6 - 8)

*Values followed by the same letter do not differ from each other by the Tukey test at 95% probability.

The characters that presented significant differences are those most susceptible to environmental interferences in the exchange activity such as soil fertility and water conductivity, being these directly related to the translocation of substances by the body of the vegetable, either in the axial or radial direction.

According to Table 1, Tracueteua has more fertile soil characteristics, and has hydromorphic characteristics (inceptisols aquepts), when compared to Abaetetuba, which has soils of the Dystrophic Yellow Oxisols type, that is saturated with bases. The soil factor may have been preponderant in the development of the characters that obtained significant differences, such as the diameter of the vessel and of the points being superior in Tracueteua, for both species when compared to Abaetetuba. While the

frequency mm^2 of vessel and frequency of rays per linear mm were higher in Abaetetuba. These xylem characteristics are explained by the so-called ecological anatomy of the secondary xylem, where one feature compensates for the other, as the larger diameter of the vessel causes less frequency per unit area. However, these relationships are not well established between the diameter of the intervacular pits and the frequency of the rays per linear mm, but may be related to the translocation of metabolites.

The anatomical features where there were differences between them, were submitted to principal component analysis (PCA), they are: diameter and frequency per mm^2 of vessels, diameter of pits and frequency of rays per linear mm, together they were responsible for 93.44% of the total variance and are presented in Figure 5.

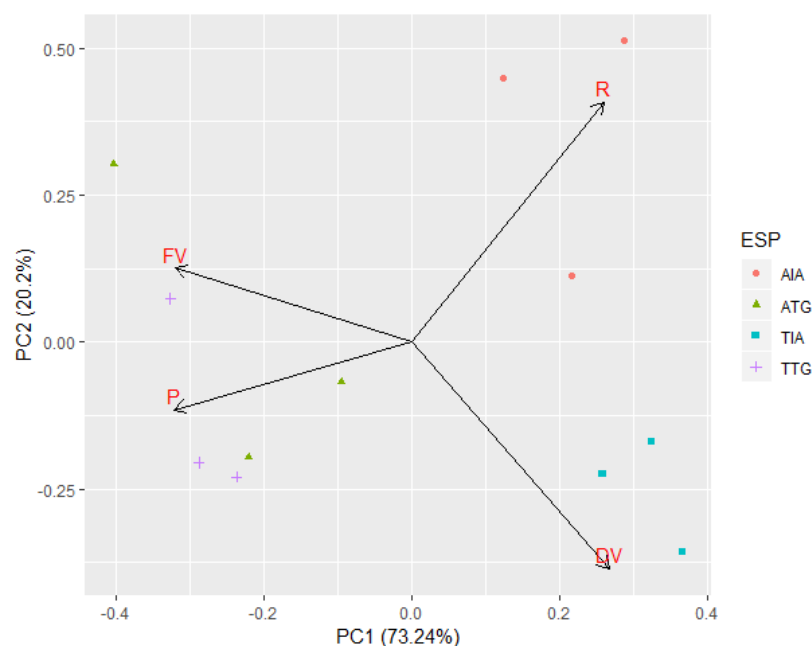


FIGURE 5 - Principal component analysis of the *I. alba* and *T. guianensis* species at the two collection sites. Where: FV = vessel frequency (mm^2), DV = vessel diameter, P = pits, R = rays per linear (mm), ESP. = specie, AIA = Abaetetuba (*I. alba*), ATG = Abaetetuba (*T. guianensis*), TIA = Tracueteua (*I. alba*), TTG = Tracueteua (*T. guianensis*).

According to the PCA, the tropical specie *I. alba* presented great plasticity in the anatomical features, the high tree of Abaetetuba (AIA) was highly influenced by the frequency of rays per linear mm, while this same taxon in another region (TIA) was mainly explained by increased diameter of vessels. Such comparative evaluation between wood allows us to infer that the *I. alba* under different soil and climate conditions may alter its cellular structures, either for higher conductivity in sapwood or for better efficiency in radial transport and storage of substances.

In *T. guianensis*, it was observed in principal component analysis that the morphological characteristics of the vessels varied in the secondary xylem and better clustered the trees of *T. guianensis*. For different sites, both

the vessel frequency and the pits that responded to this taxon, this may indicate that taxa has some adaptability and efficiency in the active transport of the sapwood.

Ecological studies emphasize that the water availability and dimensions of vessels as well as their arrangement of the wood are directly linked (CARQUIST, 2001; ALVES; ANGYALOSSY-ALFONSO, 2000; BAAS et al., 2004). Although, recent ecological studies associate these dimensions and arrangements of vessels with tree height, trunk diameter and distance at which water will travel to the apex (OLSON; ROSELL, 2013; ANFODILLO et al., 2006, 2013; LONGUI et al., 2014). Figure 5 clearly shows that, for this study, the diameter of vessels was related to the

individuals of *I. alba* in Tracuateua, probably due to nutrient characteristics and water conductivity.

Therefore, the frequency of vessel is also related to the diameter of these elements and to the conditions of nutrition and water conductivity. Barros et al. (2006) point out that, for tropical forests, the anatomical structure of xylem is characterized by a lower frequency of elements of larger vessels and simple perforate plate, in other words, such characters provide better and more efficient conduction of water by the plant. Concerning PCA (Figure 5), the frequency of vessel elements is in an opposite quadrant of the diameter, demonstrating this inverse relationship.

The radial parenchyma of the specimens was classified as two distinct classes, for height and width, however, the frequency per linear mm that obtained significant differences. Alves and Angyalossy-Alfonso (2002) point out that wider rays may be associated with a better transport of metabolites between xylem and phloem and exchange supply in favorable times of growth. However, the relation with the frequency of this per linear mm does not seem to be clear, thus requiring detailed studies. Similar groupings were obtained for ray frequencies per linear mm with the specimens of *I. alba* in Abaetetuba, probably related to conductions in the radial direction of the plant.

This work points out that some peculiarities, in the xylemic structure, show that the plasticity found in tropical species can vary according to the types of soils and the environmental variations. Therefore, it's likely that certain taxa that do not have phylogenetic relationships in common, may share adaptive characteristics because they share the same environment.

CONCLUSION

In the present study, a series of common anatomical features were observed, independent the collection sites such as slightly different growth layers, simple drill plates, predominantly solitary vessels elements, in a diagonal arrangement, infrequent, diffuse porosity, fibers and septate fibers, suggesting an ecological tendency for tropical tree species.

Characteristics such as diameter and frequency of vessels elements per mm² and frequency rays were related to collection sites, being higher among the individuals collected in Tracuateua, with more fertile soils. As for diameter of pits, this was directly related to genetic factors, in other words, they were related to the species independent of the sites.

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