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ORIGINAL ARTICLE

Effects of carbonization in the anatomical structure of *Alexa grandiflora*'s Ducke wood

Efeito da carbonização na estrutura anatômica da madeira de Alexa grandiflora *Ducke*

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ABSTRACT: The biodiversity of the Amazon rainforest acompanny intense rates environmental degradation. To expand the information on wood and charcoal coming from the Amazon could help in the inspection, contributing to the conservation of the local biodiversity. This research aimed to anatomical characterize the wood and charcoal made out of *Alexa grandiflora* Ducke, besides to evaluate the anatomical changes in the wood resulting from the process of carbonization. We analyze three individuals of *A. grandiflora*, as from of body proofs of 1.5 cm x 1.5 cm x 3.0 cm histological slides and charcoal were obtained. For the production of charcoal, the body proofs were submitted to a carbonization process with a heating rate of 1.66°C min^{-1}, keeping it at 450 °C. Photomicrographs were taken in the cross section, longitudinal tangential and longitudinal radial sections of the wood and charcoal, from those pictures we have made the anatomical description of both products. To evaluate the effect of the carbonization on the anatomical characteristics of the wood, the data was analyzed by using "generalized linear models – GLM – for repeated measurements". There has not been seen any alterations in the qualitative characteristics of the wood after the carbonization process. We noticed that the main characteristics for specie's identification were maintained in the charcoal, with significant increase in vessel frequency per.mm^{-2} (+88.61%), decrease of the vessel diameter (-30.6%), and of the height (-30.6%) and width (-20%) of the rays. The number of rays por.mm^{-1} did not show any significant statistical difference.

RESUMO: *A biodiversidade da floresta amazônica acompanha intensas taxas de degradação ambiental. Diante disso, ampliar as informações sobre as madeiras e carvão vegetal oriundos da Amazônia pode auxiliar a fiscalização, contribuindo para a conservação da biodiversidade local. O objetivo deste trabalho foi caracterizar a anatomia da madeira e do carvão de* Alexa grandiflora *Ducke, além de avaliar alterações anatômicas da madeira decorrentes do processo de carbonização. Três indivíduos de A. grandiflora foram analisados, a partir de corpos de prova com dimensão de 1,5 cm x 1,5 cm x 3 cm obteve-se lâminas histológicas e carvão. Para a produção do carvão, os corpos de provas foram submetidos a um processo de carbonização em uma taxa de aquecimento de 1,66°C/min, mantidas a 450°C.Obtiveram-se fotomicrografias nas seções transversal, longitudinal tangencial e longitudinal radial da madeira e do carvão vegetal, a partir destes, foi realizada a descrição anatômica dos dois materiais. Para avaliar o efeito da carbonização sobre as características anatômicas da madeira, os dados foram analisados utilizando "modelos lineares generalizados (GLM) para medidas repetidas". Não foram observadas alterações nas características qualitativas da madeira após a carbonização. Constatou-se que as características principais para identificação da espécie foram mantidas no carvão, com aumento significativo na frequência dos vasos por mm^{-2} (+ 88,61), redução do diâmetro dos vasos (-30,6%) e da altura (-20%) e largura (-39,5%) dos raios. O número de raios por mm^{-1} não apresentou diferença estatisticamente significativa.*

KEYWORDS
Tropical woods
Forest inspection
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1 Introduction

The Amazon rainforest detains the greatest extension of tropical forest in the world and it is the home of an immensity diversity of plants, although however, this great biodiversity comes together with profound rates of environmental degradation, increases in the consumption of natural resources, humanitarian crises and difficulties in promoting environmental, social and more sustainable activities, this supports that the challenges for environmental conservation are systemic (ICMBIO, 2017). The pressure that exists on the Amazon biome, directly or indirectly driven by the illegal wood exploitation has been resulting in the inclusion of many species in the list of the Brazilian flora that is under some degree of threat or extinction.

The population displacement to the Amazon, in many times attracted by big economic projects, has been systematically motivating economic and social pressures in the region. This has influence in the usage of natural resources, in this way cooperating with the illegal market of charcoal in the Amazon rainforest, which contributes to the forest degradation in the region (Sonter et al., 2017). This Amazon vulnerability points out to the necessity to provide information about the particularities of this biome, because from the knowledge of these particularities it becomes possible to act and to achieve the sustainable development of the region.

Alexa grandiflora Ducke is a tropical specie, endemic in Brazil, occurring predominantly in the phytogeography domain of the Amazon rainforest, its distribution covers mainly the states of Amazonas, Amapá and Pará (Lima, 2015). The species is known as one of the main woody species commercialized in the Amazon region (Corandin et al., 2010; PARÁ, 2016), however, the information on the effect of the carbonization in the anatomical structure of the specie is unknown.

The Anthracology is a science compromised to study the charcoal, independently of the process that gave rise to it, the principle is the characterization of the charcoal based in the wood anatomy of the specie. The anatomical analysis of the charcoal has been successfully applied for purposes of identification on what specie has originated the product (charcoal) analyzed. This is possible because the anatomical characteristics of the wood is preserved after the carbonization process (Gonçalves et al., 2016; Scheel-Ybert & Gonçalves, 2017).

Nisgoski et al. (2014) and Gasson et al. (2017) evaluating the effect of the carbonization on the anatomical structure of *Ocotea porosa* and *Croton sonderianus*, respectively, reiterate that wood anatomy, qualitatively, is conserved after the carbonization. However, in both studies it was observed important morphometric variations, which indicated that the effect of the carbonization on the cells, depend not only of the characteristics of the process, but also on the anatomical characteristics of the wood that will be carbonized.

The identification or distinction of the source material can contribute to the control of the illegal production of charcoal, this way, cooperating to the supply of information about the technologic characteristics of the charcoal (Gonçalves et al., 2014). The adversities for the inspection of wood and charcoal identification contributes to the illegal market of these products in the Amazon region.

To expand the information on the anatomical characteristics of the charcoal of different woody species from the Amazon, as well as to understand the possible changes in cellular structure between wood and charcoal, resulting from the carbonization process would help forest inspection, contributing to the control of this market and walking hand in hand with the conservation of the biodiversity of the region.

This study has aimed to characterize the anatomy of the wood and the charcoal coming from *Alexia grandiflora* Ducke, an Amazonian endemic specie, as well as to evaluate the anatomical alterations in the wood due to the process of carbonization.

2 Material and Methods

In this work we have used three individuals of *Alexa grandiflora* Ducke, which were registered in the wood collection of the "Instituto Agronômico do Norte – IAN" that belongs to the "Embrapa Amazônia Oriental". Table 1 lists the examined individuals in accordance with the wood collection number and herbarium.

The wood samples obtained from the wood collection were subdivided into two body proofs, contiguous to each other, with dimensions of 1.5 x 1.5 x 3.0 cm (in the cross section and longitudinal tangential and radial sections), one intended for the anatomical characterization of the wood and the other for the carbonization and posterior anatomical characterization of the charcoal.

As for the anatomical study of the wood, the body proofs were softened by cooking it in water and glycerin (4:1). Posteriorly, we used a slide microtome (Leitz 1208) to obtain histological cuts in the cross section and longitudinal sections (tangential and radial) with thickness varying from 16 to 20 µm. Next, we clarified the histological cuts with sodium hypochlorite 60% and stained it with hydroalcoholic safranin 50% (Sass, 1940). The histological cuts were next dehydrated (Johansen, 1940; Saas, 1940) and used to set the permanent slides, those were confectioned with synthetic resin. All counting and measurements of the wood cells were made by using a light microscope ZEISS Primo Star HAL/LED, coupled to a digital camera Opton microscopio and an image analyzing software (Image-Pro Express 6.0).

As for the analysis with charcoal, the wood samples were involved in aluminum paper and carbonized in a muffle, in a carbonization regime of 5 hours, with final temperature of 450 °C and heating range of 1.66°C min^{-1}; the samples were maintained at the final temperature for 2 hours

Table 1. List of analyzed woods in accordance with the wood collection number and herbarium

Tabela 1. Lista de madeiras analisadas de acordo com o número de coleção da xiloteca e herbário

Specie	Individual	Collection number	Herbarium number	Site
	1	7789	182782	
Alexa grandiflora	2	7790	182801	terra firme forest
	3	7774	182817	

(Muñiz et al., 2012). For the obtainment of fracture section of the charcoal fragments in all three plans of study, we used the methodology described by Scheel-Ybert (1999) that preconizes the manual fracture of the wood or done with blades, in the three wood anatomical plans. The obtained fragments of charcoal were properly prepared to be analyzed in a scanning electron microscope (SEM) and with the achieved images, we made all counting and cell measurements by using the software for image analysis (Image-Pro Express 6.0).

The anatomical description of both wood and charcoal followed the proceedings and terminology recommended by the International Association of Wood Anatomists (Iawa) (Wheeler et al., 1989). The analyzed qualitative anatomical characteristics were: vessel diameter (µm), vessel frequency per.mm^{-2}, number of rays per.mm^{-1}, ray height (µm) and ray width (µm), for each of these evaluated characteristics we did 30 counting and measurements.

The obtained results from the quantitative anatomical parameters for both wood and charcoal, were statistically compared as an approach to identify significant variations due to the carbonization process. The data was analyzed by using "Generalized Linear Models – GLM, for all repeated measurements". The anatomical parameters that showed values of contiguous nature (all measurements) were adjusted to the GLM by assuming a Gaussian distribution (in cases where the variables have passed the Shapiro-Wilk normality test at 5% of significance) or Gamma distribution (in cases of failure on passing the Shapiro-Wilk normality test). The anatomical parameters that have specifically showed discreet values (all counting) were adjusted to the GLM by using the Poisson distribution. The mean values (averages) were then compared by contrast of models through the LSMeans Test for multiple comparisons. All GLMs were submitted to a residual analysis, as a way to evaluate the adequacy of the error distribution (Crawley, 2002).

3 Results and discussion

There has not been seen qualitative differences in the wood anatomy, as well as alterations in the pattern, disposition of cell type, as an effect of the carbonization process, that being said, the wood anatomical description also applies to the charcoal (Figure 1 A-F). **Growth Rings:** the growth ring's boundaries are distinct, demarked by thin marginal parenchyma lines or simulating marginal lines (Figure 2 A, B). **Vessels:** wood with diffuse porosity; vessels are predominantly solitary (90% or more) and sometimes multiples in up to 3; partially obstructed by oil-resins; intervascular pits are alternate, not garnished, circular, simple perforated plates; radiovascular pits are similar to intervascular in shape and size (Figure 1 A-F). **Axial Parenchyma:** paratracheal, paratracheal losangular aliform; or confluent in short stretches or oblique; or confluent in long stretches tending to form short bands or lines with up to three cells of width; 3-4 cells by parenchyma strand (Figure 1 A, D). **Rays:** 1 to 3 cells wide, <1mm of height, 4-12 rays by millimeter, heterocellular, formed by 1 to 2 rows of square shaped and upright marginal cells with procumbent body (Figure 1 B, C, E, F). **Fibers:** libriform; not septate; wall thickness varying from thin to thick. **Stratified structure:** absent. **Secretory elements:** absent. **Mineral inclusions:** prismatic crystals are present inside chambers in the axial parenchyma (Figure 2 D).

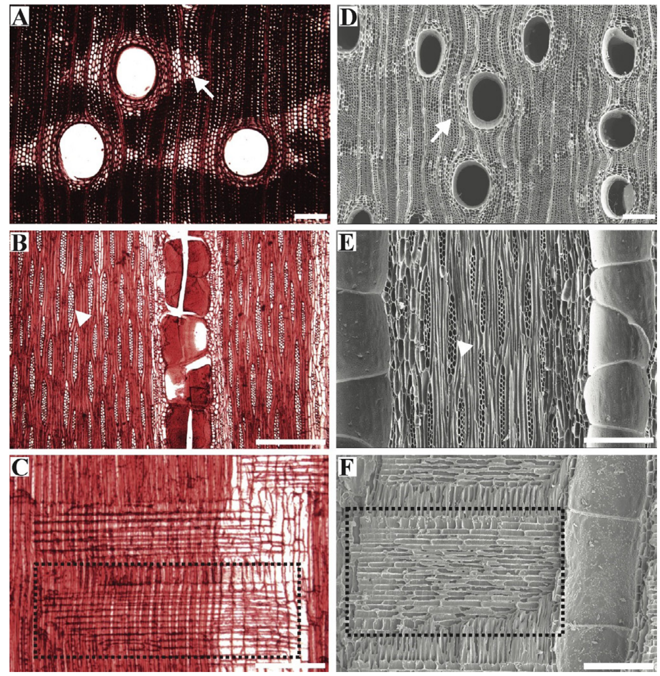

Figure 1. *Alexa grandiflora*'s wood photomicrograph (A-C) and charcoal (D-F). Cross section (A, D) arrow indicates axial parenchyma paratracheal losangular. Longitudinal tangential plan (B, F) arrow's head indicates rays with 1 to 3 cells wide. Longitudinal radial plan (C, F) dashed rectangle indicates heterocellular rays. Scale bar: 200 (µm)

Figura 1. *Alexa grandiflora* micrografias da madeira (acima A-C) e do carvão vegetal (abaixo D-F). Plano transversal (A, D), a seta indica parênquima axial paratraqueal losangular. Plano longitudinal tangencial (B, F), a cabeça da seta indica raios 1 a 3 células de largura. Plano longitudinal radial (C, F), o retângulo tracejado indica raios heterocelulares. Barra de escala: 200 µm.

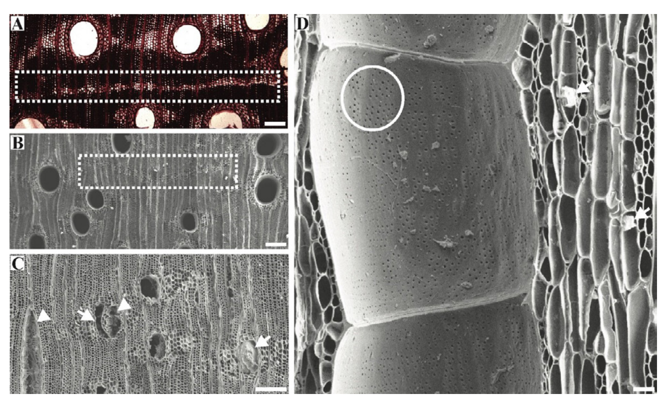

Figure 2. Photomicrographs of the anatomical changes in the wood of *Alexa grandiflora* after the carbonization process. A. Wood and B. charcoal: dashed rectangle indicates the distinct growth rings in narrow bands of marginal parenchyma or simulating marginal bands. C. charcoal: arrows indicates reduction and/or deformation of the vessels and its depositions; arrow's heads indicates rupture of the rays and axial parenchyma. D. charcoal: circle indicates pits' chambers with indistinct limits in the charcoal

Figura 2. Micrografias das mudanças anatômicas na madeira de *Alexa grandiflora* após carbonização. A. madeira e B. carvão: retângulo tracejado indica anéis de crescimento distintos por faixas estreitas de parênquima marginal ou simulando faixas marginais. C. carvão: seta indica redução e/ou deformação dos vasos e depósitos nos vasos; cabeça da seta indica ruptura dos raios e do parênquima axial. D. carvão: círculo indica câmaras das pontuações com limites indistintos no carvão.

The observed anatomical characteristics in the wood and charcoal are similar to those noticed by Fedalto et al. (1989) excepting the presence of distinct growth rings for the specie. Corandi et al. (2010), in the other hand, have classified the growth rings for the specie as indistinct or poorly distinguishable, with ring limits many times distinct by the presence of narrow bands of marginal parenchyma or simulating marginal bands, as well as seen in the specie studied in this research.

According to Alves & Angyalossy-Alfonso (2000), the presence of axial parenchyma in marginal bands demarking the growth layers is a very common characteristic in tropical and subtropical arboreal species in Brazil. This anatomical characteristic is however, strongly dependent on the climatic variables, which are, in the tropics, mainly induced by a dry season, with periods of 2-3 months and precipitation inferior to 60 mm (Worbes, 1995) and thus, it can vary depending on the original location of the individuals.

From the analysis of the charcoal's photomicrograph, we noticed that the carbonization process has led the rays and axial parenchyma to rupture, tending to form big cavities in some areas of the body proofs (Figure 2 C). We have also noticed an accentuated contraction of the vessel diameter, many times followed by distortions in the cell wall (Figure 2 C). The vessel's obstruction (Figure 2 C), as well as the crystals presence in the cells of the axial parenchyma have not changed after the carbonization process, they were kept distinct (Figure 2 D), the limits of the pits' chambers of the vessel elements became indistinct after the process of carbonization (Figure 2 D).

The accentuated contraction of the vessel diameter can be explained by the wood's anisotropy, the coefficient of retratibility varies in accordance with the anatomical wood direction, in the tangential plane occurs the biggest dimensional alteration (Kwon et al., 2009). The rupture of rays and axial parenchyma cells happens with regularity because these cells (axial and radial parenchyma cells) have thin walls.

The presence of crystals, probably of calcium oxalate, in the parenchymatic cells, even after the carbonization process has also been seen by Gonçalves et al. (2012) for many woody species in the Cerrado biome, and recently by Gasson et al. (2017) for *Croton sonderianus* under different temperatures during the carbonization. The presence of crystals is a particularity of taxonomic importance. However, it is frequently influenced by intrinsically factors to the plant, specially in cases where the vegetal is trying to protect itself from herbivory (Franceschi & Nakata, 2005).

With the exception of the number of rays by linear millimeter, all other evaluated anatomical parameters showed statistical significant differences ($p<0.05$) between wood and charcoal (Table 2).

Table 2. Effect of the carbonization on the quantitative anatomical characteristics of *Alexa grandiflora*

Tabela 2. Efeito da carbonização nas características anatômicas quantitativas de *Alexa grandiflora*

Anatomical characteristics	Average values	Alterations %	Standard deviation	Default error	F/χ2	p-value
Vessel frequency (by mm^{-2})						
Wood	2.3^{a}	+ 88.61	1.5	0.28	19.7*	9.2 e-^{06}
Charcoal	4.5^{b}		1.4	0.26		
Vessel diameter (μm)						
Wood	270.5^{b}	- 30.58	47.1	8.6	63.6	6.8 e-^{11}
Charcoal	187.8^{a}		31.8	5.8		
Number of rays (by mm^{-1})						
Wood	7.03^{a}	+ 15.65	1.06	0.19	2.4*	0.1217
Charcoal	8.13^{a}		1.70	0.31		
Ray width (μm)						
Wood	40.6^{b}	- 39.48	7.72	1.41	89.3	2.4 e-^{13}
Charcoal	24.6^{a}		5.14	0.94		
Ray height (μm)						
Wood	306.5^{b}	- 20.00	39.08	7.13	31.2	6.5 e-^{07}
Charcoal	245.2^{a}		45.64	8.33		

*Average values with different letter between the lines of the same anatomical characteristic indicated significant differences at 5% of probability of error. F = indicates values where the GLMs were made by the assumption of a Gaussian distribution of probability. χ2 = represents the values (*) in which the GLMs were made by the assumption of a Gamma or Poisson distribution of probability, depending if the values were contiguous or discreet.

Among the anatomical parameters the vessel frequency by mm^2 was the most affected characteristic by the carbonization process (Table 2). It has been noticed a significant increase in vessel frequency after carbonization, highlighting with the greatest mean percentage variation, +88% (Table 2). Followed by the increase in vessel frequency, we have observed a significant decrease in the vessel diameter (-30.6%), height (-20%) and width (-39.5%) of the rays

(Table 2). Although the number of rays by linear millimeter has increased (+15.6%) after carbonization, this effect was not statistically significant.

The frequency and cell's dimension had demonstrated opposite patterns after carbonization, with the increase of frequency of the cellular elements as the dimension of the cells have shown a decrease. The reduction of cellular dimension, mainly of the tangential vessel diameter, has been a pattern commonly described after wood carbonization (Gonçalves et al., 2012; Nisgoski et al., 2014; Gasson et al., 2017). The amount of axial paratracheal parenchyma may have influence on these dimensional variations, since this group of cells has thin cell's wall which makes the internal tensions, generated by the carbonization process, to retract more easily in the anatomical structure.

Gonçalves et al. (2012) report a more accentuated reduction, after the carbonization process, in vessel diameter of the species that had axial paratracheal parenchyma in abundance, while those with diffuse axial parenchyma had little or none dimensional variation in their cells. As to the alterations in the quantity of cells per area, it is possible that the anisotropic contractions due carbonization, result in volume reduction, which makes the cells to be more close one to another and consequently increases the vessel frequency.

The fact that no qualitative alteration has been noticed between wood and charcoal (Figure 1) indicates that the preservation of the anatomical structure in the charcoal contributes to the distinction of the material, helping in the inspection and environmental conservation (Gonçalves et al., 2016). The charcoal electromicrographs (Figures 1 D, E, F) can make the identification of the charcoal's anatomical structure more easy. The axial parenchyma seen in the charcoal of *Alexa grandiflora* is described as aliform losangular, sometimes forming confluences, and in marginal bands or simulating marginal bands, the pores (vessels) are solitary and multiples in a diffuse arrangement; the growth rings are distinct; there has not been seen any stratified structures in the specie's charcoal. Those characteristics can many times be observed through a commonly used 10x magnifying glass, whereas the pattern and visibility remains the same for the specie.

4 Conclusion

From the anatomical analysis on wood and charcoal of *Alexa grandiflora* Ducke, it was possible to verify that the anatomical structure was maintained after the carbonization process at 450 °C. The observations on growth rings, axial parenchyma, rays, vessels, mineral inclusions, as well as the absence of stratified and secretory structures, both in the wood and charcoal, make the taxonomic determination possible from the carbonized material.

Some morphometric modifications were observed in the anatomical structure of *A. grandiflora* after the carbonization process at 450 °C:

- The vessel frequency by mm^2 was the most affected anatomical characteristic (+ 88.61).
- The vessel diameter had a significant reduction of -30.6%.
- The height (-20%) and width (-39.5%) of the rays had shown significant reduction.
- The number of rays by linear millimeter did not had significant statistical differences.

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