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Wood functional anatomy of *Chiococca alba* Hitch. (Rubiaceae) from cerrado

Anatomia funcional da madeira de Chiococca alba *Hitch. (Rubiaceae) de cerrado*

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ABSTRACT
The wood anatomy is able to evidence systematic and ecological aspects associated with the evolution and functionality of the secondary xylem. The present study was carried out using wood of *Chiococca alba* (Rubiaceae) from *cerrado* (savannah), to describe its anatomy and to verify if the hydraulic architecture of this species corroborates the theory that postulates the functional tendency that optimizes the transport efficiency associated with safety. The anatomical analysis followed the conventional protocols of wood anatomy. Different indexes of wood hydraulics quantification were calculated, such as solitary vessels index, vessel grouping, conductivity, vessel collapse, theoretical resistance to vessel implosion and mesomorphism. The structural characteristics described for *C. alba* are in agreement with the general anatomical descriptions for the Rubiaceae family that relate the presence of exclusively solitary vessels and small diameter, simple perforation plates, alternate intervessel pits, apotracheal parenchyma in species with non-septate fibers and narrow and heterogeneous rays. The calculated indexes showed that *C. alba* is a xerophyte species with great resistance to the collapse of the vessels during the transport of water, little vulnerability to embolism and relative efficiency in the transport when compared to other species of its subfamily (Cinchonoideae) in function of the typical low water availability of the savannah soil.
Keywords: biodiversity; biodiversity conservation; ecological anatomy; hydraulic conductivity; wood anatomy.

RESUMO
A anatomia da madeira é capaz de evidenciar aspectos sistemáticos e ecológicos associados à evolução e à funcionalidade do xilema secundário. O presente estudo, realizado com madeira de *Chiococca alba* (Rubiaceae) proveniente de cerrado, objetivou descrever sua anatomia e verificar se a arquitetura hidráulica dessa espécie corrobora a teoria que postula a tendência funcional que otimiza a eficiência no transporte associada à segurança. A análise anatômica seguiu os protocolos convencionais da anatomia da madeira. Foram calculados diferentes índices de quantificação da hidráulica da madeira, tais como índice de vasos solitários, de agrupamento de vasos, de condutividade, de colapso dos vasos, de resistência teórica a implosão de vasos e de mesomorfia. As características estruturais descritas para *C. alba* estão de acordo com as descrições anatômicas gerais para a família Rubiaceae que relacionam a presença de vasos exclusivamente solitários e de pequeno diâmetro, placas de perfuração simples, pontoações intervasculares alternas, parênquima apotraqueal em espécies com fibras não septadas e raios estreitos e heterogêneos. Os índices calculados mostraram que *C. alba* é uma espécie xerófita com grande resistência ao colapso dos vasos durante o transporte de água, pouca vulnerabilidade ao embolismo e relativa eficiência no transporte em comparação a outras espécies da sua subfamília (Cinchonoideae), em função da típica baixa disponibilidade hídrica dos solos de cerrado.
Palavras-chave: anatomia do lenho, anatomia ecológica, biodiversidade, condutividade hidráulica, conservação da biodiversidade.

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INTRODUCTION

The ecological anatomy of wood has long contributed to the expansion of knowledge about the functionality of secondary xylem and the evolution of its characters (CARLQUIST, 2001). The trade-off triangle of xylem evolution has been extensively tested for species of different floras. It consists of an adaptive mechanism between safety and efficiency in water transport (BHASKAR *et al*., 2007), which implies in the satisfactory conduction of water in order to minimize the effects of mechanical stress associated with negative pressure and the formation of bubbles inside the vessels which block the transport of water (BAAS *et al*., 2004).

In plants of dry environments, some anatomical patterns of wood have been portrayed in a recurrent way in literature, being the high frequency of vessels per unit area and the reduction in the tangential diameter of the vessels, the main characteristic for many shrub and tree species (ALVES & ANGYALOSSY-ALFONSO, 2000; MELO JÚNIOR *et al*., 2011, MELO JÚNIOR & BOEGER, 2017). In this environmental condition, the high density of wood is added as a physical property which tends to contribute to the reduction of the risks of vessel implosion (HACKE *et al*., 2001). Although these characteristics are observed in many tropical species, a study with 335 angiosperms showed, through low correlations between safety and efficiency, that no species had high efficiency and high security, supporting the idea of a tradeoff between safety and efficiency. In addition, species with low efficiency and low security were poorly associated with higher wood density (GLEASON *et al*., 2016). Thus, the counterpoint between safety and transport efficiency still poses a great challenge to the understanding of the evolution of the xylem. In a mega-diverse tropical flora, such as the Brazilian one, estimated at about 7,880 arboreal species (FAO, 2005) and with a more promising current estimate that brings this wealth to the plateau of 11,200 tree species only in Amazonia (HUBBELL *et al*., 2008), studies of wood anatomy and its hydraulic attributes are becoming increasingly necessary.

Among the botanical families with the greatest number of arboreal and representative species in the Brazilian flora are Fabaceae and Myrtaceae (GIULIETTI *et al*., 2005), which present a greater number of anatomical studies of the wood when compared to other families, such as Rubiaceae.

Rubiaceae is considered the fourth largest family among the angiosperms, with 611 genera and 13,000 species (GOVAERTS *et al*., 2014), with a diversity center in the humid forests of the neotropical region (DAVIS *et al*., 2009). In Brazil, it occurs in all states and presents 126 genera and 1,394 species, with 16 genera and 730 endemic species (BARBOSA *et al*., 2015) represented by several life forms, including shrubs and trees (ZAPPI *et al*., 2014). The genus *Chiococca* comprises about 20 species of vines, shrubs and trees, found from the southeastern United States to northern Argentina (LORENCE, 2012).

In Brazil, four species are recorded, *C. alba* (L.) Hitchc., *C. nitida* Benth., *C. insularis* (Ridley) CM Taylor & MR Barbosa, *C. plowmanii* Delprete, the last two being endemic to the northeast region (BARBOSA, 2015). The species *Chiococca alba* is a widely distributed native tree of the American continent (ACEVEDO-RODRÍGUEZ & STRONG, 2012), and in Brazil occurs in several phytogeographic regions of all states, from the rain, semideciduous, riparian, *cerrado* (savannah) and *restinga* (dune) forest formations (BARBOSA *et al*., 2015).

Although the anatomical characterization of the wood of Rubiaceae is well described in literature (CALLADO & SILVA NETO, 2003; POLLITO & TOMAZELLO FILHO, 2006; CASTELAR, 2014; MARQUES *et al*., 2015; BALDIN, 2015; CESAR, 2015; BALDIN *et al*., 2016) and have recognized taxonomic importance in this group (LENS *et al*., 2000; JANSEN *et al*., 2001; CALLADO *et al*., 2001), systematic or ecological work of the wood done with *C. alba* from Brazilian ecosystems is non-existent.

The present study aimed to describe the anatomy of the wood of *Chiococca alba* occurring in Brazilian flora (*cerrado* or savannah) and to verify if its hydraulic architecture corroborates the theory that postulates the functional tendency that optimizes the transport efficiency associated with safety.

MATERIAL AND METHODS

STUDY AREA

The studied botanical material comes from the *cerrado* field formation (savannah) (COUTINHO, 1978) located in the municipality of Matozinhos, Minas Gerais, under the geographical coordinates 19° 27' S and 43° 58' W (figure 1). It is located in a plateau area at an altitude of 762 m, it has an Aw climate, according to Köppen classification. The average annual temperature is around 21.6°C.

The average annual rainfall is 1321 mm, distributed seasonally (CLIMATE-DATA, 2017). The geological framework is characterized by well preserved dolomites, limestones and stromatolite (VIEIRA, 2007) pellets of the *Sete Lagoas* Formation, inserted in the *Bambuí* Group, an integral part of the *Sao Francisco* Supergroup, associated with the Neoproterozoic age (CPRM, 2010). Soil typified as dystrophic red Latosol is characterized as poor in mineral nutrients, deep and with low base saturation (V <50%) up to 1,0 m deep (EMBRAPA, 2013).

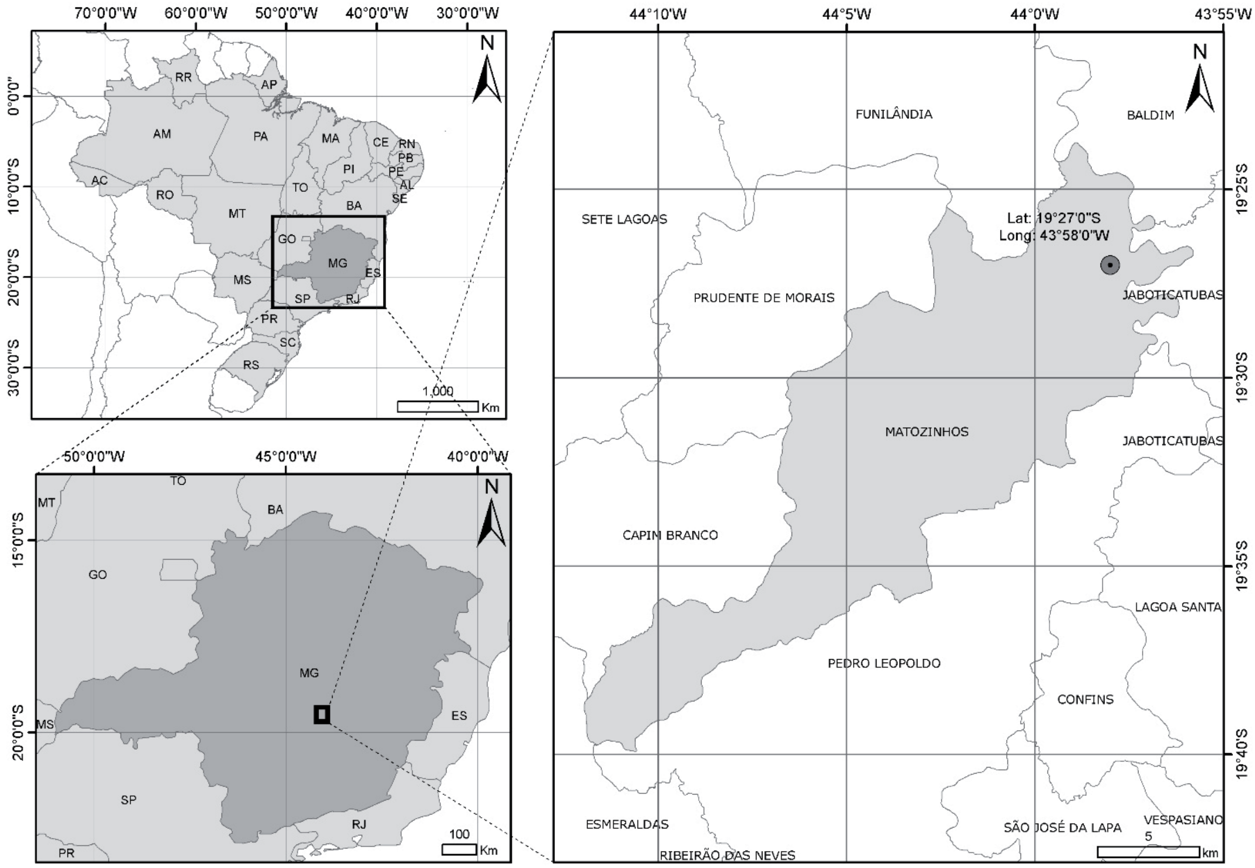

Figure 1 – Spatial localization of the sampling area of *Chiococca alba* (Rubiaceae) in cerrado (savannah) environment in the municipality of Matozinhos, Minas Gerais, Brazil.

THE STUDIED SPECIES

The species *Chiococca alba* presents a shrub or arboreal habitus, with erect or scandent branches, striated, puberulent to glabrescent. Petiole leaves (petiole 1.4-2.6 mm length); *stipula* broadly triangular, 1,5-1,7 × 2,1-2,5 mm, puberulent; elliptic-oval blade, acute apex, cuneate base, 3.3-4.0 × 1.2-2.4 cm, smooth, chartaceous, discolor, glabrous. Pedunculate inflorescence (peduncle 0.6-1.0 cm length); narrowly triangular bracts, *ca.* 3.0 × 0.5 mm, creamy-rusty, setose. Pedicellate flowers (pedicel 2,0-2,5 mm length); 5-lobed calyx, calyx wolves deltoid, *ca*. 0,5 × 0,5 mm, vinous, glabrous; corolla 5-lobed, campanulate, 4.0-4.5 mm long, tube 2.0-2.5 mm long, pinkish, glabrous, deltoid lobes, acute apex, creamy, glabrous; anthers *ca.* 2.5 × 0.4 mm, yellow, glabrous; stiletto *ca.* 3.0 mm length, creamy, stigma captioned *ca.* 3.0 mm, papillose. Immature fruit, 3.5-4.0 × 4.0-4.1 mm, glabrous. Seed ellipsoid, plane, *ca.* 3.0 × 2.0 mm, brown, glabrous (ACEVEDO-RODRÍGUEZ & STRONG, 2012).

SAMPLING AND ANALYSIS OF BOTANIC MATERIAL

The samples of wood of *C. alba* for the preparation of permanent histological slides are from the collection of timber of JOIw from Univille and are recorded as JOIw 53, JOIw 104, JOIw 390 and JOIw 415. The specimens were prepared for cooking in glycerine water and subsequent drying on a Zeiss slide microtome and type C razor in the radial, longitudinal and tangential longitudinal planes (JOHANSEN, 1940; SASS, 1951). Afterwards, the cuts were clarified in sodium hypochlorite, washed in distilled water, stained with safrablau, dehydrated in a growing ethyl series (KRAUS & ARDUIN, 1997) and mounted on synthetic resin of stained glass varnish type (PAIVA *et al*., 2006). Dissociated material was obtained by immersion of specimens in Franklin solution heated at 60ºC (KRAUS & ARDUIN, 1997), for later biometry of vessel elements (length and tangential diameter) and fibers (length and wall thickness), with N = 30. The microphotographs were captured with Olympus CX-31 photomicroscope. Measurements were made using Dino Eye 2.0 software. The anatomical characterization was based on the terminology of the IAWA Committee (1989).

The hydraulic conductivity of the species was measured using different indices. They are: solitary vessel index (VS) = Nvs / Nvm (ratio between solitary and multiple vessels) (SCHOLZ *et al*., 2013); vessels group index (VG) = Ntv / Nvm (ratio of the total number of vessels to the total number of multiple vessels) (SCHOLZ *et al*., 2013); conductivity index (CI) = r^4 / F (ray in the fourth power divided by mean vessel frequency) (ZIMMERMANN, 1983); vulnerability index (IV) = D / F (mean tangential diameter of vessels divided by mean vessel frequency); vascular collapse index (ICv) = Epv / D (ratio of vessel wall thickness to tangential vessel diameter) (HACKE *et al*., 2001); theoretical resistance to vessel implosion (RTIV) = $(Edp / Dmax)^2$ (ratio of double wall thickness of contiguous vessels to maximal squared diameter of vessels, powered squared) (PITTERMANN *et al*., 2006); and index of mesomorphy (IM) = IVxC (vulnerability index multiplied by the mean length of vessel elements) (CARLQUIST, 1977; 2001).

RESULTS

The anatomy of the wood of *C. alba,* shown in figure 2, is characterized by the presence of distinct growth layers demarcated by the thickening of the fiber walls with slight distinction between late and early wood.

The vessels have diffuse porosity and have no definite arrangement. They are mostly solitary (>90%) and rare multiple radial of 2-3. The tangential diameter of the vessels varies between 17.48-46.99 μm (34.46 ± 5.36), the frequency varies from 64-128 vessels/ mm^2 (90.96 ± 14.60) and the length of 305, 28-491.17 μm (377.41 ± 41.62). The perforate plate is simple. The intervessel spots are alternating and minute, whereas the radio-vascular pits have very small to seemingly simple borders. The fibers do not have septa, their walls vary from thin to thick, they have pits with distinct edges in tangential longitudinal view and their length turns around 767,63-1144,39 μm (939,05 ± 91,31). The axial parenchyma is the diffuse apotracheal type. The rays are exclusively uniseriate and heterogeneous, with a body formed by procumbent cells and with 2-4 marginal layers of square and upright cells. Its width varies from 14.02-26.60 μm (20.27 ± 2.72), while the height varies from 239.17-505.86 (370.02 ± 60.47) and from 15-28 cells (20.66 ± 3.47).

Table 1 summarizes the results obtained for all the quantitative indices of conductivity of the wood of *C. alba.*

Table 1 – Quantitative indices of the mensuration of the condutivity of the wood of *Chiococca alba* (Rubiaceae) sampled in *cerrado* grassland (savannah), Matozinhos, Minas Gerais, Brasil.

Solitary vessels index (IVS)	32,46
Vessels grouping index (IVM)	33,46
Conductivity index (IC)	1270,00
Vulnerability index (IV)	0,43
Vessels colapses index (ICV)	0,12
Theorical resistance to vessels implosion (RTIV)	0,02
ÍMesomorphy index (IM)	163,31

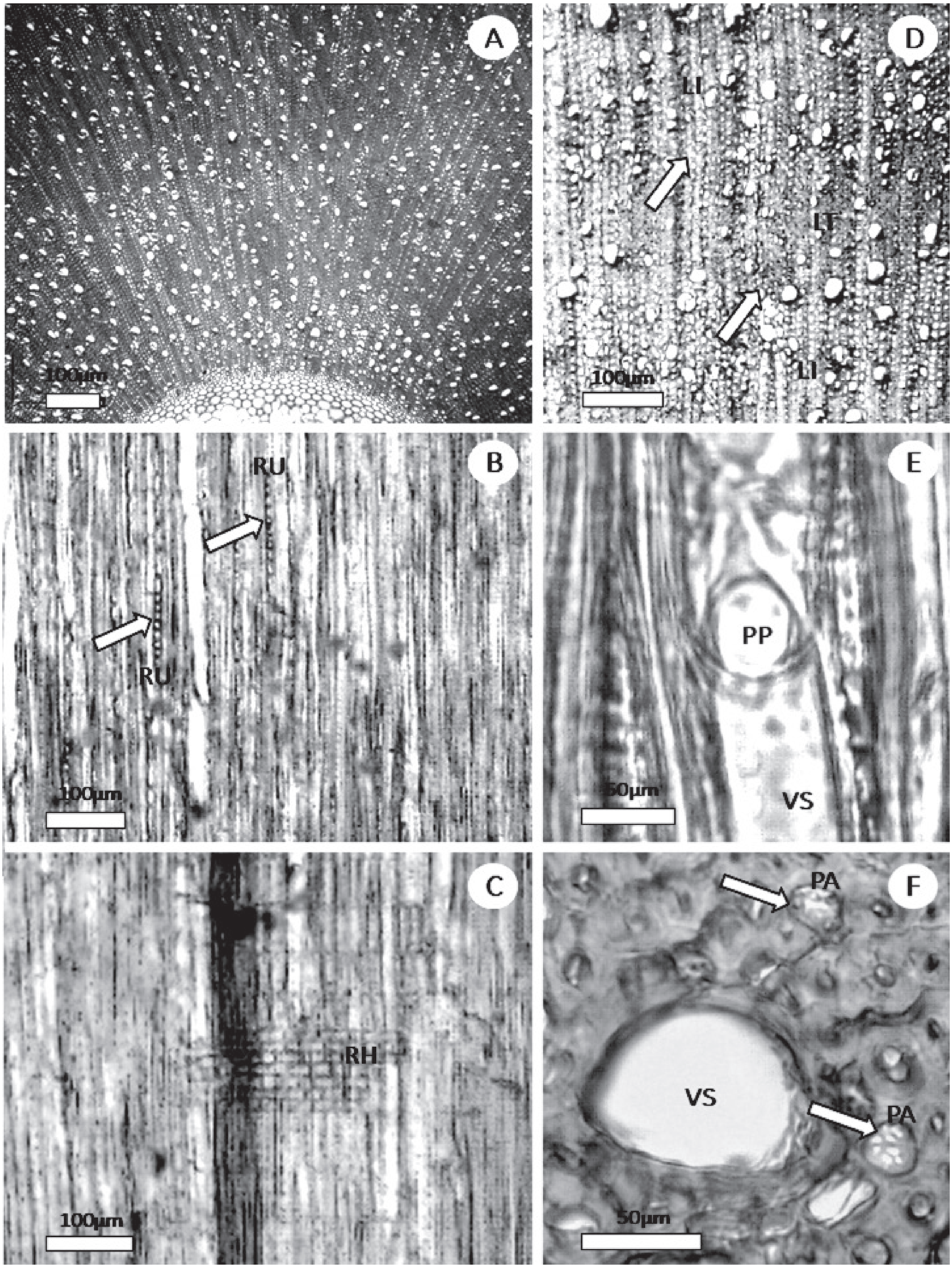

Figure 2 – Anatomy of the wood of the stem of *Chiococca alba* (Rubiaceae). A: diffuse porosity in transversal section. B: exclusively unisseriate rays tangential longitudinal section (arrows). C: heterogeneous ray in radial longitudinal section. D: growth layers (limit, arrows). E: Simple perforation plate. F: Diffuse apotracheal parenchyma (arrows). Legend: LI – Initial timber, LT – late timber, PA – Axial parenchyma, PP – perforation plate, RU – Unisseriate ray, RH – Heterogeneous ray and VS – vessel.

DISCUSSION

The structural characteristics described for *C. alba* are in accordance with the general anatomical descriptions for the Rubiaceae family, that is, the presence of exclusively solitary vessels with small diameter, simple perforation plates, alternate interveinal pits, apotracheal parenchyma in species with non-septate fibers and narrow and heterogeneous rays (METCALFE & CHALK, 1972). A study of 41 species of Rubiaceae, including 13 species of the subfamily that circumscribe the genus *Chiococca,* mentions the high frequency of vessels per unit area (40 to 100 vessels / mm²) in 48.8% of the species and the presence of short fibers (900-1600 µm) in 70.7% of the species (BALDIN *et al*., 2016) as prevalent characters, also diagnosed *in C. alba* in this study.

Variations in the structure of the secondary xylem are associated with the influence exerted by abiotic factors (BAAS *et al*., 2004). Attributes directly related to hydraulic conductivity, such as vessel frequency and diameter and vessel element length, can be influenced by several environmental factors, such as water availability in the soil (COSTA *et al*., 2009), which in the *cerrado* environment (savannah) is admittedly low due to the seasonal rainfall regime and the great depth of the water table in the latosols (UHLMANN *et al*., 1997; 1998). The growth layers, when present in the species' timber, result from the influence of temporal variations on environmental conditions (BOTOSSO, 2011) and are observed in 48% of Brazilian flora (ALVES & ANGYALOSSY-ALFONSO, 2000). Water availability is mentioned as one of the factors involved in vascular exchange activity (KRAMER, 1964), favoring the formation of annual growth layers in tropical species of sites with defined dry periods (WORBES, 1989). The Rubiaceae family is represented by species that present both indistinct and distinct growth layers (RECORD & HESS, 1949), the latter being observed in about 25% of the species (BEECKMAN & JANSEN, 1995). The presence of layers of growth in *C. alba*, observed in this study, follows the pattern of the tribe Chiococceae, which is represented by some genera with this characteristic, such as *Alibertia* (LARA, 2012), *Alseis* (CAMPBELL *et al*., 2016) and *Tocoyena* (DÓRIA *et al*., 2016).

The remarkable presence of solitary vessels, evidenced by both microscopy and solitary vessel index (IVS), corroborates the data of Détienne & Jacquet (1983), who verified this characteristic in species of 10 genera of the Rubiaceae family of the Amazon Forest. The direct proportional relationship between the mean tangential diameter of the vessels and the water availability of the environment (CARLQUIST, 1966) was verified in *C. alba* as a function of the presence of small diameter vessels associated with the drier environment of the *cerrado* (savannah). The scarcity of multiple vessels in the wood of *C. alba* wood, commonly reported for environments with low water availability (PELLEGRINO, 2012), was evidenced by the low index of multiple vessels (IVM) and compensated by the high frequency of solitary vessels. Directly proportional relation is given between the conductivity index (CI) and the water transport efficiency, with the highest CI values being indicators of higher efficiency (CARLQUIST, 2001). The CI value obtained for *C. alba* suggests that the xylem of the species under study has lower efficiency in water transport, however, when compared to other species of the subfamily Cinchonoideae, it is equivalent to semideciduous and rain forest species (table 2).

According to Zimmermann (1982), narrower vessel elements (smaller tangential diameter), as observed in *C. alba*, are a common feature of most Rubiaceae (JANSEN *et al*., 2002), which functionally reduce the risk and vulnerability of xylem to the formation of embolism, but are less efficient in water transport (CARLQUIST, 1975; BAAS *et al*., 2004). The vulnerability index shows the relation between the vessels and the environment, being values below 1 indicative of greater safety in water transport and less susceptibility to cavitation at high negative pressures (CARLQUIST, 1977), as observed in *C. alba* when compared to other species of its subfamily (table 2). This safety in transport is reinforced by the very low propensity to fall from the water column at the time of vessel rupture shown by the theoretical resistance index to vessel implosion (RTIV).

Table 2 – Conductivity index (IC) and vulnerability index (IV) of species of the subfamily Cinchonoideae, family Rubiaceae, ocurring in different forests environments of the biomes Mata Atlantica, Amazonia and Cerrado.

Species	IC	IV	Environment	Reference
Alibertia concolor	1272,66	2,96	Semideciduous forest	Lara, 2012
Alseis pickelii	1009,00	0,33	Rain forest	Campbell et al., 2015
Tocoyena formosa	2987,59	0,60	Cerrado	Dória et al., 2016
Tocoyena aurea	748797,42	10,3	Cerrado	Dória et al., 2016
Cephalanthus glabratus	4316,50	0,37	Riparian forest	Siegloch et al., 2011
Uncaria guianensis	113957767,01	61,8	Amazon rain forest	Pollito & Tomazello, 2006
Uncaria tomentosa	155559264,2	66,8	Amazon rain forest	Pollito & Tomazello, 2006

Vessel frequency is an important anatomical aspect to indicate xeromorphism and mesomorphism. Carlquist (2001) considered frequencies around 100 vessels / mm² as high and, according to the author, the high frequency of vessel elements is typical of more xeric environments, which is corroborated by the high vessel frequency recorded in *C. alba* in association with the low value obtained for the mesomorph index, indicating that this species is xeromorphic.

The low value of the vessel collapse index presented by *C. alba* suggests its great resistance to the rupture of the vessels against the negative pressure inside, which is, according to Hacke *et al*. (2001), accentuated in xeric environments. According to the same authors, the safety in water transport is given by increasing the wood density in a way proportional to the resistance to cavitation.

In most tropical environments the occurrence of vessel elements with simple perforation plates is predominant (SCHMID & BAAS, 1984), although scalariform plates are also reported for Rubiaceae species (DÉTIENNE & JACQUET, 1983). The occurrence of simple drilling plates in *C. alba* may be associated to the more restrictive environment in terms of water availability. The elimination of the scalariform perforation plate in xeric environments, such as *cerrado* (savannah), is due to the high transpiration rate, which requires a higher conductivity rate, a condition for which simple drilling plates are adapted (WHEELER & BAAS, 1991).

In general, few relationships are known between the dimensions of the rays (height and width) and the development environment, and ecological interpretations are often speculative, although Alves and Angyalossy-Alfonso (2002) and Lima *et al*. (2009) state that such a characteristic cannot always be related to the environment. The structure of rays in species of the Rubiaceae family is quite variable (METCALFE & CHALK, 1972) and this variation is also accompanied by the subfamilies Cinchonoideae, Ixoroideae and Rubioideae (RECORD & HESS, 1949). The rays observed in this study contrast Outer & Veenendaal (1976), who observed rays of greater width in association with soils with mineral deficiency. However, they corroborate the studies of Barajas-Morales (1985) that observed lower rays in drier environments. It is concluded in this study that *C. alba* occurring in a *cerrado* (savannah) environment, typically reported as an ecosystem subject to seasonal rainfall that implies low water availability for most of the year, has wood with well defined structural characters within its family circumscription, but also has xeromorphic functional attributes that allow it to balance the safety and efficiency relationships in water transportation. The calculated indexes showed that *C. alba* is a xerophytic species with great resistance to vessel collapse during water transport, low vulnerability to embolism and relative transport efficiency when compared to other species of its subfamily (Cinchonoideae) due to the typical low water availability of *cerrado* (savannah) soils.

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