

Effect of carbonization on wood anatomy of three Fabaceae species from an Araucaria forest stand in Southern Brazil

Efecto de la carbonización en la anatomía de la madera de tres especies de Fabaceae del Bosque de Araucaria, sur de Brasil

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SUMMARY

The aim of this study was to measure and compare some anatomical elements of wood and charcoal of *Inga vera*, *Machaerium paraguariense* and *Muelleria campestris* to support identification of the materials seized by regulatory authorities. For each species, three trees were analyzed. After wood evaluation, all samples (moisture content of 12 ± 1 %) were wrapped in aluminum foil and carbonized in a muffle furnace for 8 hours with final temperature of 450 °C and a heating rate of 1.66 °C/min. The number of measurements was based on 30 readings regarding tangential diameter and vessel density, along with frequency, height and width of rays (in micrometers). After carbonization, changes in cell dimensions and different behavior were observed in the Fabaceae species evaluated. In all species, vessel diameter declined; vessel density decreased in *Inga vera* and increased in the other species; ray height decreased in *Inga vera* and *Muelleria campestris*, and increased in *Machaerium paraguariense*; and ray width and frequency increased in all species. We concluded that due to the conservation of wood anatomical structures after carbonization, the inclusion of this species in a database would be effective to support efforts to control deforestation in the south of Brazil.

Key words: *Inga vera*, *Machaerium paraguariense*, *Muelleria campestris*, anatomical characteristics, charcoal.

RESUMEN

El objetivo de este estudio fue medir y comparar algunos elementos anatómicos de madera y carbón vegetal de *Inga vera*, *Machaerium paraguariense* y *Muelleria campestris* con el fin de apoyar la identificación de los materiales incautados por las autoridades reguladoras. Para cada especie, se analizaron tres árboles. Después de la evaluación de la madera, todas las muestras (contenido de humedad de 12 ± 1 %) se envolvieron en papel de aluminio y se carbonizaron en un horno de mufla durante 8 horas con una temperatura final de 450 °C y una velocidad de calentamiento de 1,66 °C/min. El número de mediciones se basó en 30 lecturas con respecto al diámetro tangencial y densidad de los vasos, junto con frecuencia, altura y ancho de los radios (en micrómetros). Después de la carbonización, se observaron cambios en las dimensiones celulares y diferentes comportamientos en las especies de Fabaceae evaluadas. En todas las especies, el diámetro del vaso disminuyó; la frecuencia de los vasos disminuyó en *Inga vera* y aumentó en otras especies; la altura de los radios disminuyó en *Inga vera* y *Muelleria campestris*, y aumentó en *Machaerium paraguariense*; y el ancho y la frecuencia de los radios aumentaron en todas las especies. Concluimos que debido a la conservación de las estructuras anatómicas de la madera después de la carbonización, la inclusión de esta especie en una base de datos sería efectiva para apoyar los esfuerzos para controlar la deforestación en el sur de Brasil.

Palabras clave: *Inga vera*, *Machaerium paraguariense*, *Muelleria campestris*, características anatómicas, carbón vegetal.

INTRODUCTION

Charcoal is an important raw material for iron and steel making. A potential problem is identification of its origin,

which can be from planted forests or illegal wood from native forests (Gonçalves and Schell-Ybert 2012). Sometimes illegal logging is hidden by making charcoal because carbonization makes it harder to identify species. Additiona-

lly, charcoal from native wood is often mixed, for example, with charcoal from *Eucalyptus* L'Hér., a species planted for charcoal production. On this basis, it is necessary to know which anatomical changes that occur during carbonization are related to each species. In general, qualitative characteristics remain unchanged, while some variations occur in dimensions and frequency of vessels and rays (Gonçalves *et al.* 2012, Gonçalves and Scheel-Ybert 2016). Similarly, Osterkamp *et al.* (2018) reported that during carbonization, chemical and physical processes occur that result in changes in wood cell dimensions, although general anatomical characteristics remain without major alterations. Therefore, based on wood anatomy, it is possible to distinguish most species after burning (Muñiz *et al.* 2012b).

Literature reports wood and charcoal anatomy for some species in Brazil, such as macro or microscopic description of *Copaifera cf. langsdorfii* Desf. and *Dipteryx odorata* (Aubl.) Willd (Nisgoski *et al.* 2012); *Cedrelinga cateniformis* (Ducke) Ducke and *Enterolobium schomburgkii* (Benth.) Benth. (Muñiz *et al.* 2012a); *Pouteria macrophylla* (Lam.) Eyma and *Micropholis guianensis* (A.DC.) Pierre (Muñiz *et al.* 2013); *Byrsonima spicata* (Cav.) DC., *Calophyllum brasiliense* Cambess., *Cecropia sciadophylla* Mart., *Cochlospermum orinocense* (Kunth) Steud. and *Schefflera morototoni* (Aubl.) Maguire *et al.* (Nisgoski *et al.* 2014); *Brosimum acutifolium* Huber, *Ficus citrifolia* Mill., *Hyeronima laxiflora* (Tul.) Müll. Arg. and *Sapium glandulatum* (Vell.) Pax. (Nisgoski *et al.* 2015); Anacardiaceae species (Gonçalves and Scheel-Ybert 2016); *Mimosa scabrella* Benth., *Miconia cinnamomifolia* (DC.) Naudin, *Cecropia glaziovii* Snethl, *Hyeronima alchorneoides* Allemão and *Pera glabrata* (Schott) Baill. (Carvalho *et al.* 2017); and angelim species (Muñiz *et al.* 2016); besides 80 species described in an anthracology atlas (Scheel-Ybert and Gonçalves 2017).

The Araucaria forest stands in Santa Catarina State, Brazil, are composed of approximately 925 species, from 439 genera in 116 botanical families (Gasper *et al.* 2013). One of the most important is the Fabaceae family, with 58 species recorded (Gasper *et al.* 2013), some with high commercial importance, such as *Inga vera* Willd. and *Muelleria campestris* (Mart. ex Benth) M. J. Silva et A. M. G. Azevedo (Richter and Dallwitz 2000), while others are listed by the Environmental Ministry as endangered in Brazil, such as 12 species from the genus *Inga* Mill. and one from *Machaerium* Pers. (MMA 2014).

Considering the extraordinary biological diversity in Brazil, as well as the difficulties related to the inspection of environmental crimes, a reference collection and database with a large number of samples is necessary to facilitate efficient identification of wood and charcoal by regulatory authorities. The aim of this study was to verify wood and charcoal anatomical characteristics in different radial positions of three Fabaceae species (*Inga vera*, *Machaerium paraguariense* Hassl and *Muelleria campestris*) to contribute with information to a database for these species evaluation.

METHODS

Wood samples from *Inga vera*, *Machaerium paraguariense* and *Muelleria campestris* were obtained from trees cut by Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) in a region that is now inundated by a reservoir (San Roque hydroelectric plant) in Santa Catarina State. For each species, three trees were collected. Specimens of the botanical material were deposited at Lages Herbarium of Santa Catarina State University – LUSC (table 1). The access to the botanical material was registered under the code AF3EDDC with the Genetic Heritage Management Council (CGEN/SISGEN).

For each tree, we selected a disc with no defects at breast height (DBH) for evaluation and wedge obtainability. The material was divided into three samples oriented in anatomical sections (transversal, longitudinal radial, longitudinal tangential), with dimensions of 2 x 2 x 2 cm, codified as near pith (next to the pith, though not including it), intermediate (exactly halfway between near bark and near pith) and near bark (in the outermost part of the disc) (figure 1). Radial sampling was done to cover most anatomical variation that can interfere in wood identification, since results will be added to a database for posterior practical application in forest supervision. Samples were from heartwood, transition and sapwood, varying depending on tree diameter. For characterization, samples were smoothed with #1200 sandpaper.

Wood and charcoal analyses were performed on the same samples. After wood evaluation, all samples (moisture content of 12 ± 1 %) were wrapped in aluminum foil and carbonized in a muffle furnace, as described by Muñiz *et al.* (2012b), with final temperature of 450 °C (two hours at final temperature) and a heating rate of 1.66 °C min⁻¹, totaling eight hours. Description of wood and charcoal was based on images obtained with a Discovery V12 stereomicroscope (Zeiss) with the Axio Vision Rel. 4.7 software. Charcoal details were observed with a Hitachi

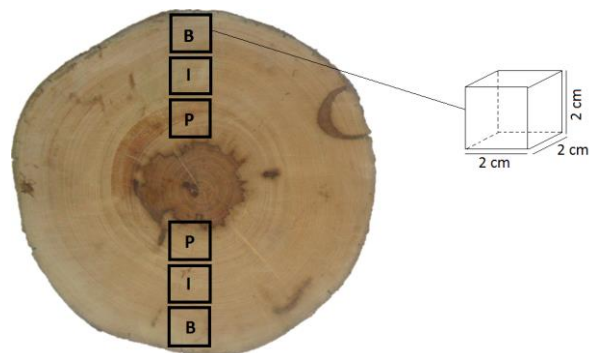


Figure 1. Illustration of sampling disk diagram of *Muelleria campestris*, where B: near bark, I: intermediate, P: near pith.

Diagrama de muestreo en disco de *Muelleria campestris*, donde: B: próximo a la corteza; I: intermedio; P: próximo a la médula.

Table 1. Species and record numbers.
 Especies y números de registro.

Species/Record number	DBH (cm)	Coordinates (WGS84)	Altitude (m)
<i>Inga vera</i>			
LUSC 6225	26.0	lat: -27.484728 long: -50.805003	701
LUSC 6226	18.0	lat: -27.484378 long: -50.805603	851
LUSC 6227	17.5	lat: -27.484228 long: -50.805753	731
<i>Machaerium paraguariense</i>			
LUSC 6243	18.3	lat: -27.489997 long: -50.805392	734
LUSC 6244	12.9	lat: -27.489997 long: -50.805417	734
LUSC 6245	11.0	lat: -27.490006 long: -50.805433	734
<i>Muelleria campestris</i>			
LUSC 6237	31.2	lat: -27.496892 long: -50.810606	692
LUSC 6238	25.1	lat: -27.497081 long: -50.810536	696
LUSC 6239	15.0	lat: -27.483572 long: -50.808342	740

TM-1000 tabletop scanning electron microscope (SEM) directly from the material, without coating. The description of the anatomical elements of wood and charcoal samples followed procedures from International Association of Wood Anatomists (IAWA, 1989) and 30 measurements were made for tangential diameter and vessel density, as well as frequency, height and width of rays (dimensions in micrometers).

A statistical analysis was performed considering the kind of material (wood or charcoal) and different positions in the disk (near bark, intermediate, near pith), resulting in a 2 x 3 factorial analysis. Differences in data between wood and charcoal were evaluated by the Scott-Knott test at 95% probability, using the Sisvar software.

RESULTS

Qualitative characteristics. *Inga vera*. In wood samples, growth ring boundaries were distinguished by differences in fiber zones. Vessels: diffuse porosity, solitary vessels (68 %) and in radial multiples 2-5 (14 %, 9 %, 2 %, 7 %, respectively for multiples of 2, 3, 4 and more than 4) (figure 2A), simple perforate plates (figure 2H), intervessel pits alternate, gums and other deposits present (figure 2A). Axial parenchyma: lozenge-aliform and confluent (figure 2A). Rays homogeneous, all ray cells procumbent, 1-3 cells wide, not storied (figure 2B). After carbonization, most qualitative characteristics remained intact (figure 2C) and it was also possible to observe not storied rays (figure 2D), some ray cells with ruptures (figure 2E) and cells in uniseriate and multiseriate rays (figure 2F). The presence of crystals in axial parenchyma cells was verified (figure 2G, H).

***Machaerium paraguariense*.** In wood samples, growth ring boundaries were little distinguished by fiber zones (figure 3A). Vessels: diffuse porous, mostly solitary vessels (84 %) (figure 3A), in radial multiples 2-4 present, simple perforate plate (figure 3H), alternate intervessel pits. Axial parenchyma: winged-aliform and confluent; diffuse-in-aggregates. Rays: heterogeneous, with procumbent body ray cell and 1-2 rows of square marginal cells, 1-3 cells wide, storied (figure 3B). In charcoal, qualitative characteristics remained, such as solitary vessels (figure 3C), storied rays (figure 3D), aliform axial parenchyma (figure 3E), heterogeneous rays (figure 3G) and simple perforate plate and alternate intervessel pits (figure 3H) and crystals in axial parenchyma cells were also observed (figure 3F).

***Muelleria campestris*.** In wood samples, growth ring boundaries were distinct, marked by thin lines from marginal parenchyma (figure 4A). Vessels: diffuse porous with irregular distribution, solitary vessels (62 %) or in radial multiples 2-4 (24 %, 9 %, 2 %, respectively for multiples of 2, 3 and 4), simple perforate plate, alternate intervessel pits, gums and other deposits present. Axial parenchyma: lozenge-aliform and confluent, vasicentric, unilateral present (figure 4A). Rays: homogeneous, multiseriate and storied (figure 4B). Carbonization resulted in more distinct vessels (figure 4C), more evident irregular distribution, and some contrast in storied rays (figure 4D). It was possible to observe more contrast of axial parenchyma in more detailed images (figure 4E), presence of crystals in axial parenchyma cells (figure 4F), homogeneous rays (figure 4G), simple perforate plate (figure 4H) and alternate intervessel pits (figure 4F).

Quantitative characteristics. In wood samples, increase in vessel diameter and decrease in vessel density from near pith to near bark (table 2) were observed. Ray dimensions showed no linear tendency of variation and ray frequency was not influenced by radial position in trunk (table 3). In charcoal, changes in structural dimensions were different depending on species characteristics (tables 2 and 3)

DISCUSSION

Tangential diameter of vessels. In the wood of all species, we observed an increase in vessel diameter from the region of near pith towards near bark, being more accentuated in *Muelleria campestris*. In *Inga vera* wood, mean vessel diameter was 129 μm , similar to all species of the genus *Inga*

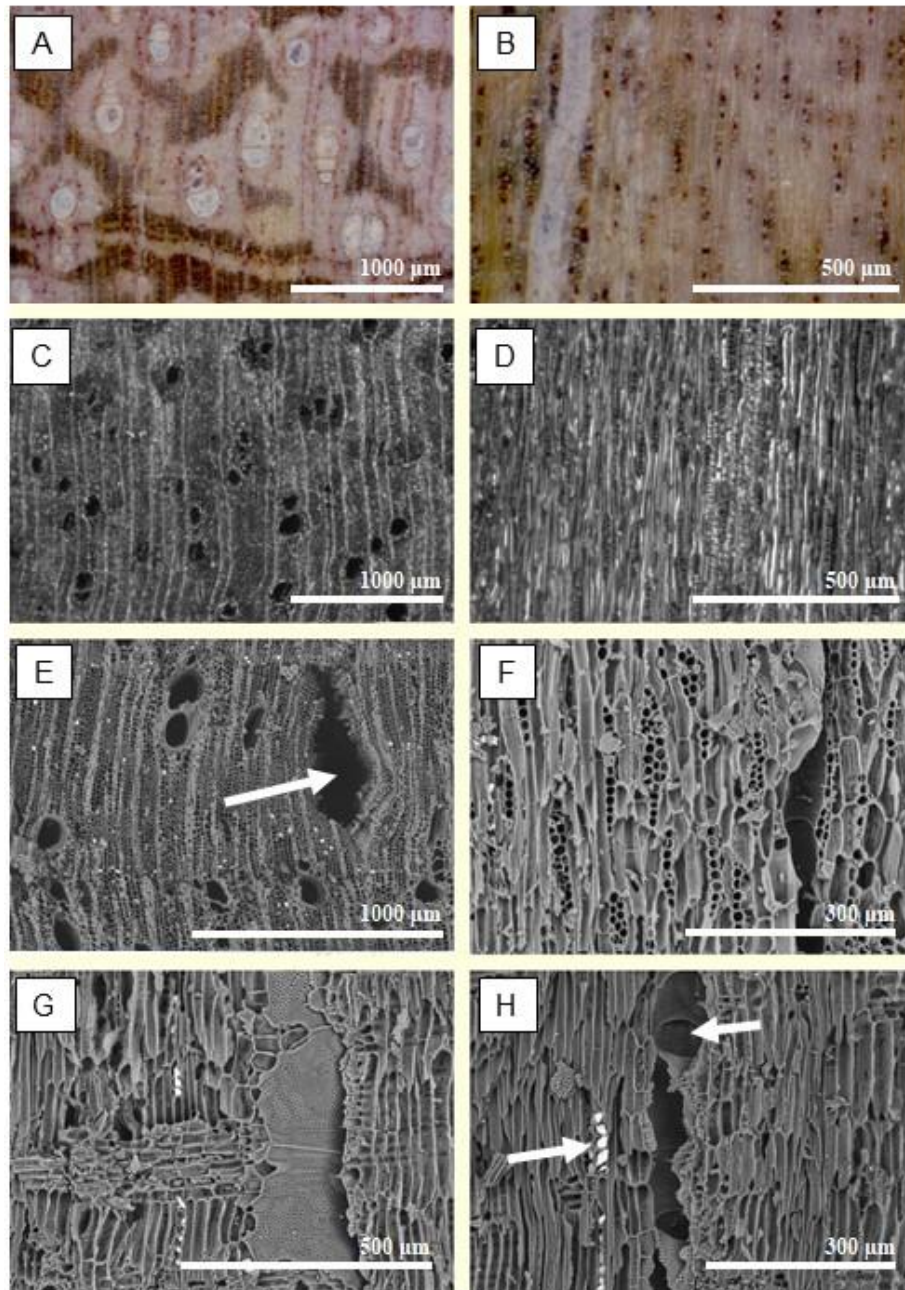


Figure 2. Images of *Inga vera* wood (A, B) and charcoal (C, D). SEM images of charcoal (E-H). (A, C) transversal section, (B, D, F) tangential section, (E) transversal section, arrow indicates splits in rays; (G, H) radial section, arrow indicates crystals and simple perforation plate.

Imágenes de la madera (A, B) y carbón (C, D) de *Inga vera*. MEB imágenes del carbón (E-H). (A, C) sección transversal; (B, D, F) sección tangencial; (E) sección transversal, la flecha indica grietas en los radios; (G, H) sección radial, la flecha indica cristales y placa de perforación simples.

published in *Inside Wood*, along with the study of Ortega *et al.* (1988), who also analyzed *Inga vera*. Furthermore, the results of this study are comparable to those reported by Richter and Dallwitz (2000), with values varying from 60-130-200 μm , for material collected in Mexico, and those reported by Vieira *et al.* (2019), with a mean value of 141 μm . In *Machaerium paraguariense* wood, mean vessel diameter was 107 μm , within the range presented in

Inside Wood for the same genus. The results for both *Machaerium paraguariense* and *Muelleria campestris* wood are proportionate to those of Marchiori *et al.* (2009), Richter and Dallwitz (2000) and Vieira (2017), although these authors did not ascertain differences from pith to bark.

In charcoal, differences in vessel diameter from near pith to near bark regions were not linear in accordance with position and varied among the species. After carbo-

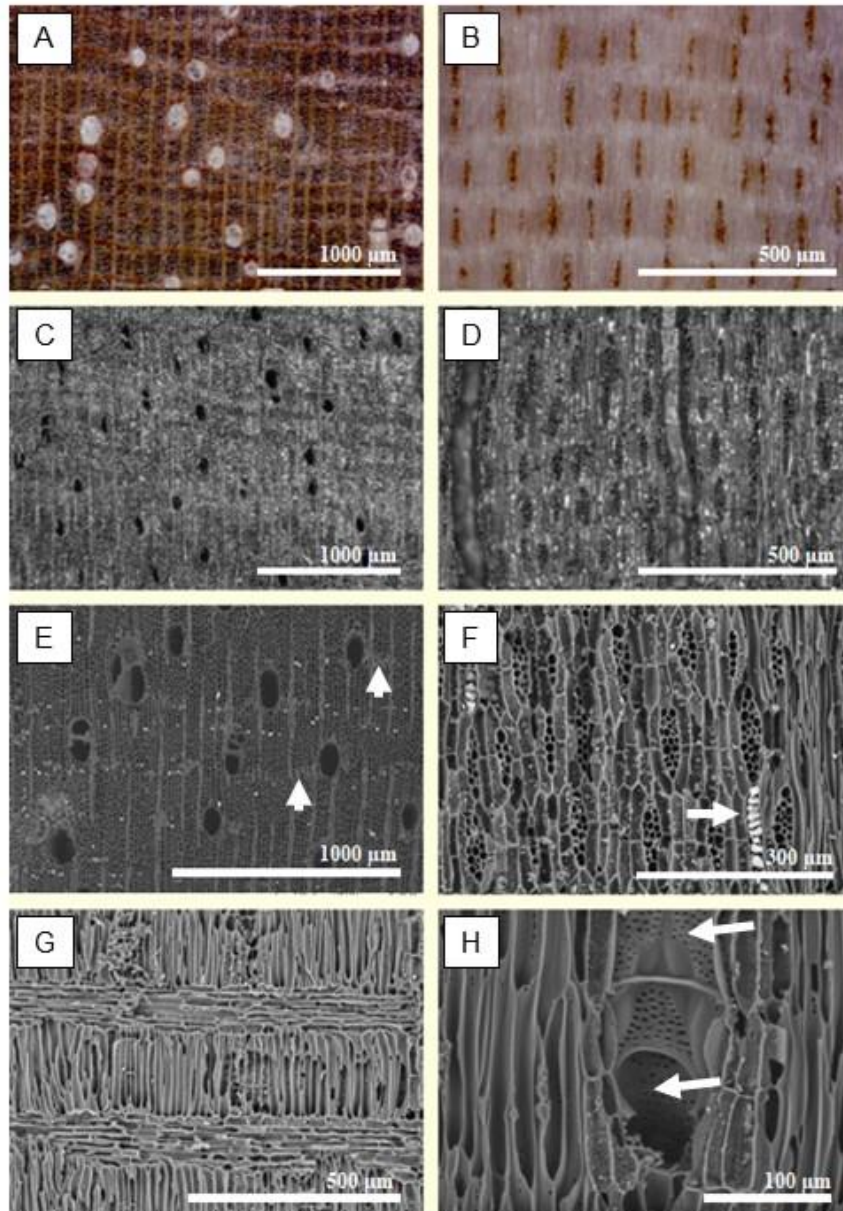


Figure 3. Images of *Machaerium paraguariense* wood (A, B) and charcoal (C, D). SEM images of charcoal (E-H). (A, C) Transversal section, (B, D) tangential section, (E) transversal section, arrow indicates axial parenchyma; (F) tangential section, arrow indicates crystals in parenchyma cells; (G, H) radial section, arrow indicates simple perforate plate and intervessel pits.

Imágenes de la madera (A, B) y carbón (C, D) de *Machaerium paraguariense*. MEB imágenes de lo carbón (E-H). (A, C) Sección transversal; (B, D) sección tangencial; (E) sección transversal, la flecha indica parénquima axial aliforme; (F) sección tangencial, la flecha indica cristales en lo parénquima axial; (G, H) sección radial, la flecha indica la placa de perforación simple y las punteaduras intervesselares.

nization, we observed a reduction in vessel diameter for all positions and species. The contraction of cells was more evident in the intermediate position of *Machaerium paraguariense* (32.99 %) and less pronounced in the intermediate position of *Muelleria campestris* (14.41 %). Regarding reduction of mean vessel diameter by species after carbonization, *Machaerium paraguariense* had the

most important contraction (32.04 %) followed by *Inga vera* (25.41 %) and *Muelleria campestris* (20.36 %). Reduction in vessel diameter after carbonization is related to anatomical features, such as the wall thickness of fibers and disposition of axial parenchyma cells, as well as processing conditions such as heating rate. These changes were also reported in other species, such as four Myrtaceae

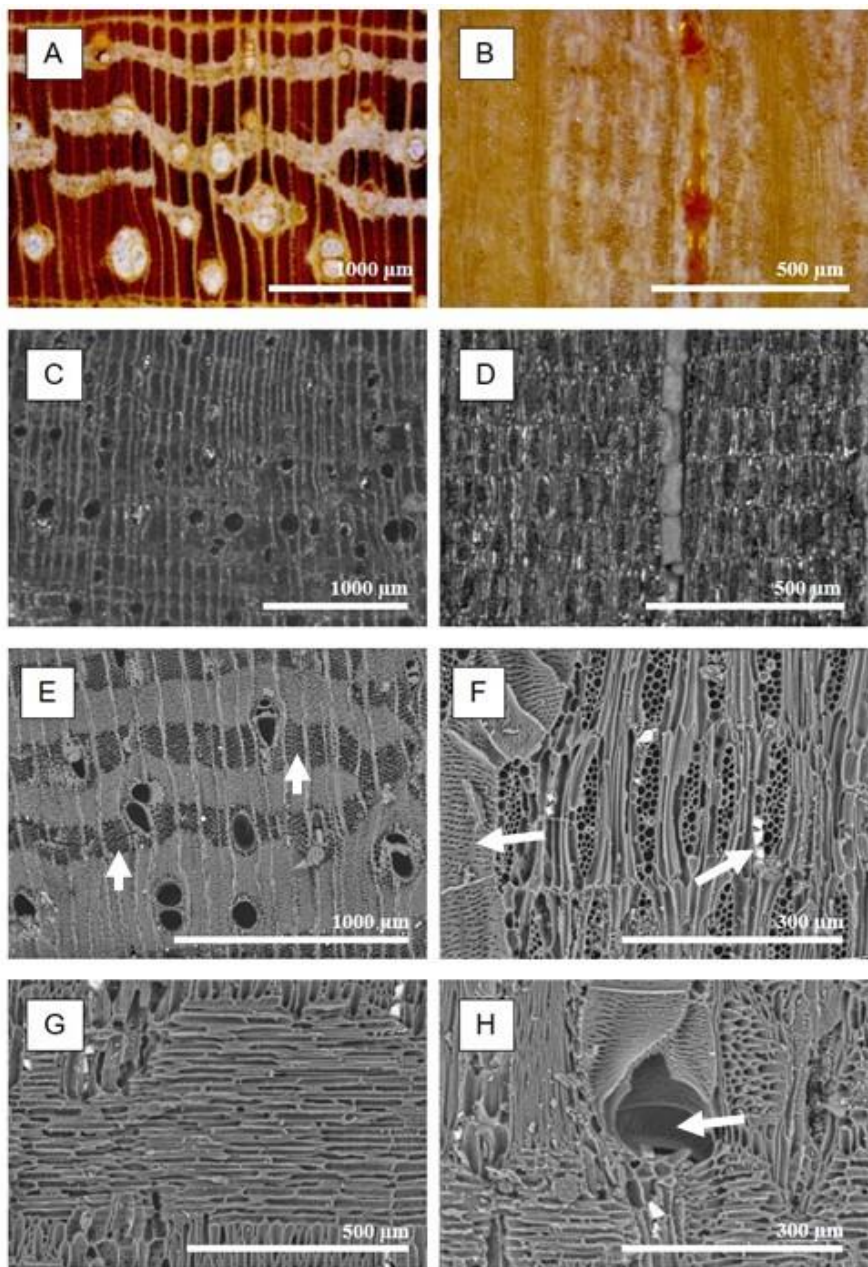


Figure 4. Images of *Muelleria campestris* wood (A, B) and charcoal (C, D). SEM images of charcoal (E-H). (A, C) Transversal section, (B, D) tangential section, (E) transversal section, arrow indicates the axial parenchyma; (F) tangential section, arrow indicates intervessel pits and crystals in parenchyma cells; (G, H) radial section, arrow indicates simple perforate plate.

Imágenes de la madera (A, B) y carbón (C, D) de *Muelleria campestris*. MEB imágenes de lo carbón (E-H). (A, C) Sección transversal; (B, D) sección tangencial; (E) sección transversal, la flecha indica el parénquima axial; (F) sección tangencial, la flecha indica punteaduras intervasculares y cristales en las células del parénquima axial; (G, H) sección radial, la flecha indica placa de perforación simples.

Table 2. Mean values and standard deviation of tangential diameter and vessel density of studied species.

Valores medios y desviación estándar del diámetro tangencial y densidad de los vasos de las especies estudiadas.

Species	Material	Near Bark	Intermediate	Near Pith
Vessel diameter (µm)				
<i>Inga vera</i> *	Wood	139.88 Aa (21.91)	136.38 Aa (25.26)	109.17 Ab (31.92)
	Charcoal	106.24 Ba (25.49)	96.44 Bb (28.30)	84.18 Bc (28.96)
<i>Machaerium paraguariense</i>	Wood	119.82 Aa (23.18)	103.04 Aa (18.34)	97.85 Ab (20.69)
	Charcoal	81.52 Ba (22.91)	69.05 Bb (17.72)	67.34 Bb (17.55)
<i>Muelleria campestris</i> *	Wood	118.27 Aa (33.21)	105.33 Ab (23.26)	94.73 Ac (15.87)
	Charcoal	83.90 Bb (25.53)	90.15 Ba (23.28)	78.05 Bb (20.95)
Vessel density (n/mm ²)				
<i>Inga vera</i>	Wood	6.00 Ab (3.42)	5.77 Ab (3.01)	7.58 Aa (4.35)
	Charcoal	5.76 Aa (2.62)	5.46 Aa (2.32)	6.49 Aa (4.49)
<i>Machaerium paraguariense</i> *	Wood	4.18 Bb (1.69)	5.53 Ba (2.29)	5.76 Ba (1.85)
	Charcoal	7.64 Aa (2.70)	7.89 Aa (2.13)	8.18 Aa (2.38)
<i>Muelleria campestris</i> *	Wood	8.42 Ab (3.51)	7.61 Ab (2.71)	10.38 Ba (3.55)
	Charcoal	7.49 Ab (2.85)	7.84 Ab (2.59)	12.22 Aa (5.08)

*Species where interaction between material and position was not significant at 95 % probability. For each species, equal letters in means do not present statistical differences by the Scott-Knott test at 95 % probability. Capital letters in column refer to changes after carbonization and small letters are related to radial position in disk (near bark, intermediate, near pith).

species (Stange *et al.* 2018), in angelim species (Muñiz *et al.* 2016) and other species from the Fabaceae family (Nisgoski *et al.* 2012). Another factor that can influence vessel diameter is the possible change in circular shape of cells after carbonization (Gasson *et al.* 2017).

Vessel density. Decrease in vessel density was observed in wood from the regions near the pith towards the bark, with statistical significance from the intermediate to near pith region, except in *Machaerium paraguariense*, which had differences between the regions near the bark towards intermediate (33.3 %). In *Inga vera* wood, mean vessel density was 6, similar to the numbers reported by Richter and Dallwitz (2000) and Vieira *et al.* (2019). In wood from *Machaerium paraguariense*, the mean value observed was comparable to that reported by Vieira *et al.* (2019), while for *Muelleria campestris* it was similar to that reported by Marchiori *et al.* (2009), Richter and Dallwitz (2000) and Vieira (2017).

In charcoal, changes were only observed in *Muelleria campestris*, with decrease in radial direction (33.3 % from near pith towards intermediate and 41.7 % from near pith towards near bark). In other species, there were no differences based on radial position. In a study with Myrtaceae species, Stange *et al.* (2018) also verified that changes in vessel density were not linear regarding position and oc-

curred in accordance with species. Carbonization had little influence on vessel density. Samples from all positions of *Machaerium paraguariense* and the near pith region of *Muelleria campestris* had significant increase. The highest change was near the bark for *Machaerium paraguariense* (82.78 %), although considerable values were noted in the other locations (42.68 % in intermediate and 42.01 % near the pith region). This parameter seems to be influenced by species intrinsic characteristics, since Stange *et al.* (2018) observed increases in vessel density in all positions after carbonization of four Myrtaceae species.

Regarding mean vessel density, large divergence was observed among the species. A reduction of 7.92 % occurred in *Inga vera* after carbonization, and increase of 10.60 % in *Muelleria campestris* and 32.76 % in *Machaerium paraguariense* were detected. Stange *et al.* (2018) observed values much higher than for the species studied here with samples of *Eugenia pyriformis* Cambess (92.3 %), *Campomanesia xanthocarpa* (Mart.) O. Berg (60.3 %) and *Myrcia retorta* Cambess (50.7 %). Likewise, the influence of species characteristics on changes in vessel density after carbonization has also been reported in literature, such as for Anacardiaceae (Gonçalves and Scheel-Ybert 2016) and Fabaceae species (Muñiz *et al.* 2016). The release of volatile matter and formation of some cracks and voids can also interfere in vessel density (Assis *et al.* 2016).

Table 3. Mean values and standard deviation of ray dimensions and frequency of studied species.

Valores medios y desviación estándar de las dimensiones y frecuencia de los radios.

Species	Material	Near bark	Intermediate	Near pith
Ray height (μm)				
<i>Inga vera</i>	Wood	189.14 Aa (67.94)	190.39 Aa (77.81)	163.39 Ab (68.56)
	Charcoal	165.59 Ba (60.18)	168.22 Ba (55.47)	155.26 Aa (78.27)
<i>Machaerium paraguariense</i>	Wood	107.41 Aa (21.22)	109.63 Aa (28.49)	108.86 Aa (21.42)
	Charcoal	112.05 Aa (18.31)	111.65 Aa (16.99)	110.71 Aa (28.75)
<i>Muelleria campestris</i>	Wood	145.84 Aa (33.82)	137.60 Aa (37.88)	139.51 Aa (29.42)
	Charcoal	127.58 Ba (27.96)	116.28 Bb (22.66)	118.15 Ba (18.19)
Ray width (μm)				
<i>Inga vera</i> *	Wood	20.08 Aa (6.81)	20.32 Aa (8.46)	15.54 Bb (5.14)
	Charcoal	22.81 Aa (9.05)	18.95 Ab (6.32)	23.02 Aa (16.93)
<i>Machaerium paraguariense</i>	Wood	16.33 Ba (4.73)	14.13 Bb (5.18)	15.77 Ba (5.73)
	Charcoal	25.45 Aa (9.16)	24.73 Aa (6.04)	25.28 Aa (7.98)
<i>Muelleria campestris</i>	Wood	30.17 Ba (11.31)	26.69 Ab (8.07)	24.21 Ab (9.69)
	Charcoal	33.23 Aa (6.27)	28.74 Ab (6.32)	25.90 Ac (7.07)
Ray frequency (n/mm)				
<i>Inga vera</i>	Wood	8.40 Ba (2.07)	8.46 Ba (1.93)	8.35 Ba (2.13)
	Charcoal	10.42 Aa (1.91)	10.62 Aa (2.07)	10.31 Aa (1.76)
<i>Machaerium paraguariense</i> *	Wood	7.54 Ba (1.24)	7.33 Ba (1.18)	6.90 Bb (1.16)
	Charcoal	9.20 Aa (1.27)	9.36 Aa (1.45)	9.46 Aa (1.37)
<i>Muelleria campestris</i> *	Wood	8.88 Ba (1.87)	8.64 Ba (2.01)	8.84 Ba (1.61)
	Charcoal	10.68 Ab (1.88)	10.82 Ab (1.70)	12.54 Aa (1.93)

*Species where interaction between material and position was not significant at 95 % probability. For each species, equal letters in means do not present statistical differences by the Scott-Knott test at 95 % probability. Capital letters in columns refer to changes after carbonization and small letters are related to radial position in disk (near bark, intermediate, near pith).

Ray height. In wood, no linear pattern was observed in all three species and the variation among positions was from 0.65 % to 5.99 %. The highest discrepancy (14.18 %) was from intermediate towards the near pith of *Inga vera*. The same species had mean ray height of 181 μm , lower than the 500 μm observed by Richter and Dallwitz (2000). Moreover, in *Machaerium paraguariense*, mean ray height was 109 μm , lower than the values observed by Vieira *et al.* (2019). For *Muelleria campestris* wood, the mean value was 141 μm , similar to those obtained by Marchiori *et al.* (2009) in wood from Rio Grande do Sul, and smaller than those observed by Richter and Dallwitz (2000).

In charcoal, reduction in ray height occurred from the near pith towards the intermediate region in *Muelleria campestris*. In other species, there was no influence of position on ray height. Carbonization did not statistically influence ray height in *Machaerium paraguariense*. For *Muelleria campestris*, all positions presented reduction in ray height

from wood to charcoal (12.52 % near bark, 15.49 % intermediate and 15.31 % near pith), probably as a result of the higher quantity of parenchyma cells in comparison to the other two species. In *Inga vera*, only in the near pith region were there no substantial changes in dimension, while for other positions, reductions of 12.45 % in the near bark position and 11.64 % for intermediate region were observed. According to Muñiz *et al.* (2012), rays are laterally connected to fibers, which limit rays movement, since the contraction of the fibers in the axial direction is small during the carbonization process. On the other hand, Stange *et al.* (2018) described reduction of ray height in *Campomanesia xanthocarpa* and *Eugenia pyriformis* and increase in *Myrcia retorta* species in all radial positions.

With regard to mean ray height values by species, a contrast was observed after carbonization, with reduction of 9.69 % in *Inga vera* and 14.44 % in *Muelleria campestris*, and with increase of 2.62 % in *Machaerium pa-*

raguariense. Different behavior has also been reported in literature, associated with ruptures of cells due to cell wall expansion, which can vary in accordance with fiber or parenchyma expansion (Gonçalves *et al.* 2012, Muñiz *et al.* 2012, Nisgoski *et al.* 2019). Ruptured rays were verified in some eucalyptus species (Gonçalves *et al.* 2014) and reductions in ray height after carbonization have been described, for example, in *Dipteryx odorata* (Nisgoski *et al.* 2012), *Diploptropis purpurea* (Rich.) Amshoff (Muñiz *et al.* 2016), *Eugenia pyriformis*, *Campomanesia xanthocarpa* and *Plinia peruviana* (Poir.) Govaerts (Stange *et al.* 2018). Increase in ray height has been found in *Cedrelinga catenaeformis* (Muñiz *et al.* 2012a), *Ocotea porosa* (Nees *et Mart.*) Barroso (Nisgoski *et al.* 2014) and *Myrcia retorta* (Stange *et al.* 2018).

Ray width. For ray width, a difference was observed in wood from near pith towards near bark, nevertheless there was no pattern in all species. Significant increase was observed from near pith towards the bark region in *Inga vera* (23.52 %). The same pattern was also noted from near pith to intermediate (9.29 %) and to near bark (11.54 %) region in *Muelleria campestris*. In *Machaerium paraguayariense*, the intermediate region had thinner rays than those of the near pith (10.40 %) and near bark regions (13.47 %). Stange *et al.* (2018) also observed divergence in this characteristic in the radial position of Myrtaceae species, with predominance of thinner rays in the near pith region.

In charcoal, there was no difference in ray width concerning sample position in *Machaerium paraguayariense*. Increase was observed from near pith towards near bark in *Muelleria campestris* (22.06 %). In *Inga vera* charcoal, ray width was lower in the intermediate position when compared to the other regions (16.92 % near bark and 17.68 % near pith). After carbonization, there was significant increase in ray width in all samples of *Machaerium paraguayariense*, with variation of 35.83 % for near bark, 42.86 % in intermediate wood and 37.62 % for near pith. Correspondingly, the same behavior was observed for near pith (32.49 %) and near bark (11.97 %) in *Inga vera* and near bark in *Muelleria campestris* (10.14 %). According to Stange *et al.* (2018), these results may be related to ruptures that occur due to the expansion of cell walls.

Regarding mean ray width values, increase was observed in all species, varying from 8.27 % in *Muelleria campestris* to 38.77 % in *Machaerium paraguayariense*, and intermediate in *Inga vera*, with 17.07 % increase. These differences can be the result of multiseriate and uniseriate ray percentage. Literature reports divergent behavior of species related to chemical changes in substances stored in parenchyma cells: decrease in ray width in the Fabaceae family was reported in *Enterolobium schomburgkii* (Muñiz *et al.* 2012a), *Dipteryx odorata* (Nisgoski *et al.* 2012) and *Parkia pendula* (Muñiz *et al.* 2016); while increase in ray width was observed in *Cedrelinga catenaeformis* (Muñiz *et al.* 2012a), *Hymenolobium petraeum* and *Va-*

tairea paraensis (Muñiz *et al.* 2016). Moreover, increase in ray width was observed in Cerrado (savanna) species (Gonçalves *et al.* 2012) and Myrtaceae species (Stange *et al.* 2018).

Ray frequency. Ray frequency in wood, in general, was not influenced by radial position in the trunk. Only the near pith region of *Machaerium paraguayariense* had a value lower (12.5 %) than that presented by the other regions. In *Inga vera* wood, mean ray frequency was 8 / mm, in the range reported by Richter and Dallwitz (2000) and Vieira *et al.* (2019). In *Machaerium paraguayariense*, mean ray frequency was 7 / mm, higher than what was observed by Vieira *et al.* (2019). For *Muelleria campestris*, 9 rays / mm were observed, lower than what was reported by Marchiori *et al.* (2009), who found mean ray frequency of 12 (10-15) in wood from Rio Grande do Sul, and similar to the value obtained by Vieira (2017).

In charcoal, ray frequency showed reduction of 15.38 % from near pith towards the intermediate/near bark region in *Muelleria campestris*, and 9.09 % from intermediate to near pith and near bark of *Inga vera*. In all species and positions, carbonization affected ray frequency. Regarding mean values, ray frequency increased by similar values: 24.35 % in *Inga vera*, 29.12 % in *Muelleria campestris* and 28.94 % in *Machaerium paraguayariense*. In other species of the Fabaceae family, Muñiz *et al.* (2016) verified significant increase in ray frequency in *Diploptropis purpurea*, *Hymenolobium petraeum* and *Vatairea guianensis*. Different behavior was observed by Ávila *et al.* (2017) regarding mass loss and cell contraction after carbonization, attributed to the influence of intra-specific and ecological characteristics.

CONCLUSIONS

The changes in cell dimensions caused by the carbonization process were confirmed. In all species, the vessel diameter decreased; vessel density decreased in *Inga vera*, though it increased in the other species; ray height decreased in *Inga vera* and *Muelleria campestris*, however increased in *Machaerium paraguayariense*; and ray width and frequency increased in all species.

Despite the alterations resulting from carbonization, cell arrangement and type were not influenced, therefore the inclusion of these species in a database would be effective to support efforts to control deforestation in the south of Brazil. Charcoal anatomy can be applied for species characterization, and in comparison, with reference data, can support illegal logging control.

ACKNOWLEDGMENTS

The authors thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001, and Programa Uniedu Pós-Graduação – Santa Catarina – Brazil.

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