RELATIONSHIPS BETWEEN ANATOMICAL FEATURES AND INTRA-RING WOOD DENSITY PROFILES IN *Gmelina arborea* APPLYING X-RAY DENSITOMETRY

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ABSTRACT: Four annual tree-rings (2 of juvenile wood and 2 of mature wood) were sampled from fast-growth plantations of *Gmelina arborea* in two climatic conditions (dry and wet tropical) in Costa Rica. Each annual tree-ring was divided in equal parts in a radial direction. For each part, X-ray density as well as vessel percentage, length and width fiber, cell wall thickness and lumen diameter were measured. Wood density and profile patterns of cell dimension demonstrated inconsistency between juvenile and mature wood and climatic conditions. The Pearson correlation matrix showed that intra-ring wood density was positively correlated with the cell wall thickness and negatively correlated with vessel percentage, fiber length, lumen diameter and width. The forward stepwise regressions determined that: (i) intra-ring wood density variation could be predicted from 76 to 96% for anatomical variation; (ii) cell wall thickness was the most important anatomical feature to produce intra-ring wood density variation and (iii) the vessel percentage, fiber length, lumen diameter and width were the second most statically significant characteristics to intra-ring wood density, however, with low participation of the determination coefficient of stepwise regressions.

Key words: X-ray densitometry, Gmelina arborea, tree-ring, wood anatomy, wood density.

RELAÇÃO ENTRE AS CARACTERÍSTICAS ANATÔMICAS E A DENSIDADE INTRA-ANÉIS DE CRESCIMENTO POR DENSITOMETRIA DE RAIOS X EM ÁRVORES DE Gmelina arborea

RESUMO: Anéis de crescimento foram extraídos do lenho de árvores de plantações de rápido crescimento de Gmelina arborea em dois tipos de clima (tropical seco e úmido). Cada um dos anéis de crescimento foi cortado em seções iguais no sentido radial e, em uma delas, foi determinada a densidade pela técnica de densitometria de raios X. A porcentagem de área do lenho ocupada pelos vasos e as dimensões das fibras (comprimento, largura, diâmetro do lume, espessura da parede) foram avaliados na segunda seção. A matrix de correlação de Pearson mostrou que a variação da densidade intra-anel de crescimento foi positivamente correlacionada com a espessura da parede celular e negativamente correlacionada com a porcentagem de vasos, comprimento, largura e diâmetro do lume das fibras. A análise de regressão "forward stepwise" determinou que: (i) a variação intra-anel de crescimento é altamente influenciada (de 79 a 96%) pelas variações dos elementos anatômicos do lenho; (ii) a espessura da parede celular foi a característica anatômica do lenho com maior influência na variação da sua densidade; (iii) a porcentagem dos vasos, comprimento, diâmetro do lume e largura das fibras exerceram a segunda maior influência na densidade intra-anel de crescimento, embora com baixos valores de coeficientes de correlação.

Palavras-chave: Densitometria de raios X, Gmelina arborea, anel de crescimento, anatomia do lenho, densidade do lenho.

1 INTRODUCTION

Wood density is related to anatomical, physical and chemical properties of the wood. Its effects on wood products as pulping, drying and machine processing are well documented (KOUBAA et al., 2002). Wood density is related to the amount of cell wall substance presented in a given piece of wood, as well as, the spatial arrangement of the wall substance. Also important, is the relative proportions of different cell types presented and its dimensions (DECOUX et al., 2004). Wood density variation is presented in the tree, in the radial direction cross, the longitudinal direction stem and within the annual tree-rings (NICAULT et al., 2001).

In hardwood species, the wood density variation depends on the amount or proportion of different cell types and spatial arrangements. Four different cell types are present in hardwood, each more or less specialized in structure and restricted in function; rays; vessels; fibers; axial parenchyma (PANSHIN & ZEEUW, 1970). It is important to know the magnitude and intra-ring wood variation pattern in order to determine the wood uniformity degree (ECHOLS, 1973). For example, the lack of wood uniformity and chemical properties are one of the greatest problems that the wood industry faces (OLSON & ARGANBRIGHT, 1977). Echols (1973) attributed the degree of uniformity of the wood density as a limiting factor of the end use of the wood considering the effect in the sawing,

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drying, machining, gluing, finishing and other wood quality parameters.

The X-ray densitometry profile has been used to determine intra-ring wood density (SCHINKER et al., 2003) and was used to increase information on wood formation, physiological processes and the amount or proportion of different cell types and spatial arrangements (KOGA & ZHANG, 2004; NICAULT et al., 2001). Also, a variety of techniques and equipment is available to measure the cell dimensions, like image analysis softwares, improving the speed and the measurement procedures (MOELL & DONALDSON, 2001).

Recent investigation has determined the effects of anatomy characteristics on intra-ring wood density variation based on X-ray densitometry technique using the digital image analysis software (DECOUX et al., 2004; KOUBAA et al., 2002; WANG et al., 2002). However, the scientific information on the relationships between wood anatomy and density profiles based on X-ray densitometry are scarce in hardwoods, reported to *Alnus rubra* (RAO et al., 1997), *Quercus petraea* (GUILLEY & NEPVEU, 2003) and some species from Canada (COWN & PARKER, 1978).

Density fluctuations in annual tree-rings were determined with anatomical cross sections in several species from Amazon inundation forests and were possible to distinguish the annual tree-ring boundaries due to latewood distinctness (WORBES et al., 1995). A significant intra tree-ring wood density variation was found in *Terminalia ivorensis* trees, from Africa, as a result of

different radial porosity patterns (NEPVUE, 1976). However, the wood density profile intra tree-rings of the hardwood species obtained applying the X-ray densitometry were not related to the wood anatomical features, like fiber and vessel dimensions. The objectives of this study are: (i) to determine the correlation between width and length fiber, lumen diameter and vessel proportions with intra-ring wood density profile and (ii) to study these correlations in juvenile and mature wood from fast-growing *Gmelina arborea* trees in Costa Rica.

2 MATERIAL AND METHODS

Plantations characteristics and tree selection

Two *Gmelina arborea* fast-growth tree plantations located in the north part of Costa Rica were selected. The Northwest region has an annual average precipitation between 1500 to 2 000 mm, average temperatures of 25-28 °C and a severe drought (December - March) with almost 0 mm. This area is commonly known as dry tropical climate. The Northern region, classified as wet tropical climate, has an annual average precipitation of 3000-5 000 mm, average temperature of 20-25°C and during the months of January and March it presents a average precipitation of 70 mm/ month. Nine to 10 years old plantations were chosen with the same management practice. The initial spacing was 3x3 mm and one thinning was applied at 4 years old. The growth rate was 2.43 cm years⁻¹ for a sampled plantation from wet tropical conditions and 2.00 cm years⁻¹ from dry tropical conditions (Table 1).

Table 1 – Dasometric condition of the sampled plantation and tree sampled.

Tabela 1 – Condições dasométricas das plantações e árvores amostradas.

	Characteristic	Wet tropical	Dry tropical
Sampled Plantation	Tree age (years)	10	9
	Mean annual rainfall (mm)*	4903	1780
	Density of plantation (n ha ⁻¹)	800	764
	Diameter at breast height (cm)	24.35	20.10
	Average growth rate (cm/ano)	2.43	2.00
	Total height (m)	19.56	20.30
Sampled trees	Diameter at breast height (cm)	23.80	18.8
	Average growth rate (cm/ano)	2.38	1.88
	Total height (m)	20.00	19

^{*}Mean annual rainfall from 1992 to 2002

Source: Instituto Costarricense de Electricidad (ICE).

One plot (400 m² each) was established in each sampled plantation. The diameter at breast height (DBH) and total and crown height were measured. One tree representing average DBH, straight trunk, normal branching, and no disease or pest symptoms were selected in each trial (Table 1). Each sample tree was marked facing north and a stem section (3 cm thickness) was cut at DBH. The radii of the north positions were selected for density and anatomical analysis.

Densitometric measurements and annual tree-ring selection

After the thin laths $(1\pm0,045;$ mean $\pm SD)$ were cut using a twin-bladed saw (Figure 1) and X-rayed on film using X-ray equipment (Hewlett Pakard Faxitron 43805 N, time: 5 minutes; energy: 16 Kv; intensity: 3 mA). The films (Kodak, Diagnostic Film X-Omat XK1, 24 x 18 cm) were developed using the normal procedures (AMARAL & TOMAZELLO, 1998). The radiographs of *Gmelina arborea* wood samples were scanned on a 256 gray scale with 1000 dpi resolution. X-ray microdensity measurements were taken on this digital image by CERD software (MOTHE et al., 1998).

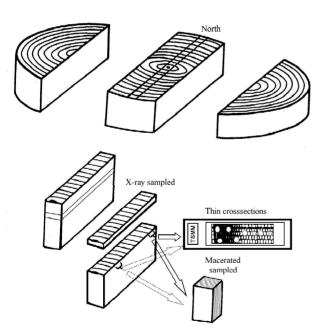


Figure 1 – X-ray, macerated and thin cross-sections sampled from a stem section of *Gmelina arborea* trees.

Figura 1 – Raios X, maceração e seções transversais das amostras do lenho de árvores de Gmelina arborea.

The wood density data was recorded from pith to bark and the density profile obtained. Four annual treerings were selected, two for dry tropical conditions, juvenile wood (DJ) and mature wood (DM), and two from wet tropical conductions also juvenile wood (WJ) and mature wood (WM). Various reasons were given for selecting these annual tree-rings: (i) juvenile and mature wood should be present in trees from wet and dry tropical conditions; (ii) the highest density was presented in trees from dry tropical conditions; (iii) false annual rings were present in trees from dry tropical conditions; (iv) the lowest density was present in trees from wet tropical conditions; (v) the highest and lowest intra-ring wood density variation was presented in trees from dry and wet tropical conditions, respectively, and (vi) porous diffuse and semi-ring porous were present. Afterwards, wood density was again recorded in the annual tree-rings selected, but the density data was divided in equal parts by CERD program: 20 measures for WM and DJ, 25 for WJ and 15 for DM (Table 2).

Wood anatomical measurements

Fiber dimension and vessel proportion were determined in each annual tree-ring selected in the wood samples. They were measured in equal parts divided by each annual ring in the radial direction where wood density was measured with CERD software. The percent of vessels was measured on thin cross-sections from each annual ring selected from the above part of the north radii (Figure 1). Thin wood sections about 10-15 mm thick were cut using a sliding microtone (Leica SM2000R) and these sections were stained with safranin and permanently mounted on microscope slides. An optical microscope with a LCD digital camera (Evolution LC color PL-A662) was used to capture digital images from microscope slides with a magnification of 25X. Sequences of images were captured for each annual tree-ring selected. Photoshop 5.0 software was used to rebuild, divide into an equal radial direction and draw 20 equal rectangles for WM and DJ; 25 parts for WJ and 15 parts for DM annual tree-ring samples. The height of the rectangle was equal to microcut width (Table 2). Vessel area was measured in each rectangle and its percentage calculated (total vessel area x 100/rectangular area). The digital images of the annual tree-rings were processed with the Image Analysis System for Wood (SAIM) developed for the Laboratory of Wood Anatomy and Tree-Ring, Department of Forest Sciences of the University of São Paulo.

Table 2 – Characteristics and dimensions of the annual rings san	pled	l.
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Tabela 2 – Características e	e dimen	sões dos	anéis de	crescimento	amostrados.
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Climatic conditions	Wood	Sample	Annual ring age (age)	Annual ring width (μm)	Porous type	Radial segment	Radial segment width (µm)
Wat tuonical	Juvenile	WJ	3	20 000	Semi-ring	25	800
Wet tropical	Mature	WM	8	8 060	Diffuse	20	400
D T	Juvenile	DJ	3	6 080	Semi-ring	20	300
Dry Tropical	Mature	DM	9	4 500	Semi-ring	15	300

The fiber dimensions (fiber length and width, lumen diameter and cell wall thickness) were measured in the maceration wood. Slices from the same equal parts divided by each annual tree-ring in the radial direction were cut and macerated using Franklin's method (RUZIN, 1999). Twenty-five fibers were established for measurement. The maceration of each division was replicated three times on a glass slide and 8 fibers were measured by projecting them to a magnification of X250 for fiber length and X1000 for lumen diameter and fiber width. Digital images of the fiber were captured and processed with SAIM. The fiber wall thickness was calculated by the difference of fiber width and lumen diameter divided in half.

Statistical analysis

Analysis of variance (ANOVA) was applied to analyze the relationships of the wood density with the two climatic conditions and wood types evaluated. Afterwards, the anatomical characteristics were plotted to demonstrate the correlations between wood density and anatomical features in the different climatic (dry or wet tropical) and wood types (juvenile or mature). The forward stepwise regressions were applied in each annual tree-ring to establish the importance of anatomical features on wood density variation, considering the values of the coefficient of correlation.

3 RESULTS AND DISCUSSION

The Pearson matrix correlation showed a high degree of statistically significant relation between wood density and anatomical features in all annual tree-rings (Table 3) and each anatomical characteristic was plotted with density (Figure 2) which showed the correlation type (positive or negative). Intra-ring wood density from the juvenile wood (WJ and DJ) was highly correlated to all anatomical features in both dry and wet tropical conditions, whereas fiber width in WM and DM and lumen diameter in WM were not correlated with the intra-ring wood density (Table 3).

Annual tree-rings from the juvenile period presented the majority of the correlations between anatomical features and mature wood in the two different climatic conditions. The vessel percentage was not correlated with other anatomical features in WM, however, it was correlated with cell wall thickness and fiber within DM. The fiber length presented minor correlations with other fiber dimensions (wall thickness, lumen diameter and fiber width) in WM and DM (Table 3). Intra-ring wood density was positively correlated with cell wall thickness (Table 3, Figure 2a) and negatively correlated with vessel percentage (Table 3 Figure 2b), lumen diameter (Table 3, Figure 2c), fiber length (Table 3, Figure 2d) and fiber width (Table 3).

The correlation between wood density in softwood tree species has demonstrated positive with the cell wall thickness and negative with cell diameter (DECOUX et al., 2004; WANG et al., 2002). Little research had reported the correlation between anatomical features and intra-ring wood density in hardwood species, in Quercus robur it was found that in mature wood, the latewood density calculated by CATscanning was negatively correlated with vessel percentage, but did not vary significantly with fiber diameter, fiber wall thickness or fiber wall area per unit fiber (RAO et al., 1997). In Ouercus petraea there was a positive correlation between wood density with fiber percentage in late and early wood, and a negative correlation for wood density with ray parenchyma proportion in late and early wood, axial parenchyma in a latewood and a percentage and diameter of vessel (GUILLEY & NEPVEU, 2003).

The variation of cell elements within the tree-ring is well known for many wood species and its distribution is attributed to aspects as tree age, seasonal environmental conditions, genetic or management conditions (ZOBEL & BUIJTENEN, 1998) and this variation influences wood density on annual tree-rings (WANG et al., 2002). The correlations found between intra-ring wood density and anatomical features in *Gmelina arborea* (Figure 2) showed clearly that wood density is an expression of the vessel proportion and fiber dimension distribution (anatomical structure) in annual tree-rings.

Table 3 – Pearson correlation between anatomical features and wood density in annual tree-rings of *Gmelina arborea* trees.

Tabela 3 – Matriz de correlação de Pearson entre as características anatômicas e a densidade da madeira em anéis de crescimento de árvores de Gmelina arborea.

Type of wood	Anatomical features	Wood density	Vessel percentage	Fiber length	Cell wall thickness	Lumen diam.	Fiber width
WJ (N=25)	Wood density	1					
	Vessel percentage	-0.94**	1				
	Fiber length	-0.81**	0.78**	1			
	Cell wall thickness	0.79**	-0.71**	-0.70**	1		
	Lumen diameter	-0.75**	0.67**	0.82**	-0.60**	1	
	Fiber width	-0.70**	0.65**	0.80**	-0.49*	0.98**	1
WM (N=20)	Wood density	1					
	Vessel percentage	-0.57*	1				
	Fiber length	-0.56*	0.17	1			
	Cell wall thickness	0.63**	-0.10	-0.38	1		
	Lumen diameter	-0.43	0.15	0.38	-0.74**	1	
	Fiber width	-0.23	0.07	0.28	-0.56*	0.84**	1
DJ (N=20)	Wood density	1					
	Vessel percentage	-0.74**	1				
	Fiber length	-0.77**	0.65**	1			
	Cell wall thickness	0.91**	-0.73**	-0.63**	1		
	Lumen diameter	-0.82**	0.48*	0.71**	-0.61**	1	
	Fiber width	-0.57**	0.28	0.51*	-0.33	0.86**	1
DM (N=15)	Wood density	1					
	Vessel percentage	-0.73**	1				
	Fiber length	-0.69**	0.50	1			
	Cell wall thickness	0.85**	-0.83**	-0.55*	1		
	Lumen diameter	-0.60*	0.13	0.21	-0.32	1	
	Fiber width	0.23	-0.62*	-0.33	0.62*	0.47	1

^{*}Significant at 5% level.; **Significant at 1% level. WJ: wet and juvenile wood; WM: wet and mature wood; DJ: dry and juvenile wood; DM: dry and mature wood.

Stepwise regression

The stepwise regression (P < 0,05) showed that intra-ring wood density variation can be predicted from 75.6 to 95.7% considering the wood anatomical variation, and 24.4 to 4.3 can be explained for other anatomical characteristics not present in this research (Table 4), like ray and parenchyma variation (GUILLEY & NEPVEU, 2003; RAO et al., 1997). Some hardwood species (*Liquidambar styraciflua*, *Liriodendron tulipifera*, *Fagus grandifolia* and *Subgenus erythbalanus*) showed the effect of axial

and radial parenchyma on wood density variation within tree-rings (EZELL, 1979).

Cell wall thickness predicted higher proportions of intra-ring wood density than other dimensions of the fiber (Table 4) in *Gmelina arborea*, however, there were differences among annual tree-ring samples. An 83.7 and 73.0% of wood density variation could be predicated by fiber wall thickness in DJ and DM, respectively but this prediction decreased to 2.8 and 46.5% in WJ and WM, respectively (Table 4). Vessel

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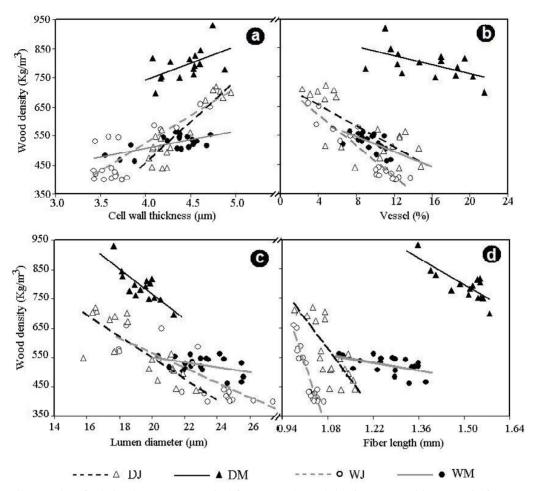


Figure 2 – Scatter plot of relation between anatomical features and wood density measured by X-ray in four-annual ring of *Gmelina arborea* trees: (a) cell wall thickness, (b) vessel percentage, (c) lumen diameter and (d) fiber length. WJ: wet and juvenile wood; WM: wet and mature wood; DJ: dry and juvenile wood; DM: dry and mature wood.

Figura 2 — Gráficos de dispersão entre as características anatômicas e densidade da madeira por raios X em 4 anéis de crescimento anuais de árvores de Gmelina arborea: (a) espessura da parede celular (b) porcentagem de vasos, (c) diâmetro do lume e (d) comprimento da fibra. WJ: úmido e madeira juvenil; WM: úmido e madeira adulta; DJ: seco e madeira juvenil; DM: seco e madeira adulta.

percentage and fiber length were statically significant too. The first parameters predicted wood density from wet tropical conditions, at 88.70 and 25.10% in WJ and WM respectively, but this percentage was not statistically correlated in wood from dry tropical conditions (Table 4). The lumen diameter was statically significantly in DJ, the 10.0% variation in wood density could be an explanation for the variation in this fiber dimension. The fiber width and length were important in the DM, 14.40 and 6.70% wood density variation could be explained for these dimensions.

With these results it is possible to establish that the cell wall thickness can be a good indicator of the intraring wood density variation of *Gmelina arborea* in fast-growing plantations, mainly in dry tropical conditions. The determined coefficient was greater than 73.00% and the correlation coefficient from the Pearson matrix was again greater than 0.85 (Table 4) in both cases. However, the cell wall thickness was less important in wood from wet tropical conditions, its presence decreased to 2.80% in WJ and 46.50% in WM. On the other hand, vessel percentage was a good indicator of wood density in WJ (Table 4).

Table 4 – Multiple regression analysis of anatomical features and wood density measured X-ray densitometric in annual tree-rings of *Gmelina arborea* trees.

Tabela 4 – Análise de regressão múltipla das características anatômicas e da densidade da madeira de anéis de crescimento anuais de árvores de Gmelina arborea.

Type of wood	Anatomical features	Number step	R ² Multiple	Modified R ²	F-valor	P-valor	
WJ	Vessel percentage	1	0.887	0.887	180.80	<0.0001**	
	Cell wall thickness	2	0.915	0.028	7.09	0.0142*	
	Lumen diameter	3	0.928	0.013	3.91	0.061	
	Fiber length	4	Not significant				
	Fiber width	5		Not sig	gnificant		
WM	Cell wall thickness	1	0.465	0.465	14.77	0.0013**	
	Vessel percentage	2	0.716	0.251	14.15	0.0017*	
	Fiber length	3	0.756	0.040	2.48	0.136	
	Lumen diameter	4	Not significant				
	Fiber width	5	Not significant				
DJ	Cell wall thickness	1	0.837	0.837	92.40	<0.0001**	
	Lumen diameter	2	0.947	0.109	34.81	0.0071**	
	Fiber length	3	0.955	0.009	3.23	0.091*	
	Vessel percentage		Not significant				
	Fiber width	5	Not significant				
DM	Cell wall thickness	1	0.730	0.730	31.12	<0.0001**	
	Fiber width	2	0.874	0.144	13.72	0.003**	
	Fiber length	3	0.941	0.067	12.42	0.005**	
	Vessel percentage	4	0.957	0.016	4.16	0.078	
	Lumen diameter	5		Not sig	gnificant		

^{**}Significant at 1% level.; *Significant at 5% level; WJ: wet and juvenile wood; WM: wet and mature wood; DJ: dry and juvenile wood; DM: dry and mature wood.

Anatomical distribution in annual tree-rings in *Gmelina arborea* (hardwood species) differed from the softwood species. It is well documented that cell wall thickness and fiber dimension play a major role in the intraring wood density variation (WIMMER, 1995) and establishes that cell wall thickness variation has a greater effect on wood density than cell tangential, radial diameter or lumen area (DECOUX et al., 2004). However, the results show that besides the fiber dimensions, the vessel proportions also present effects on intra-ring wood density variation (Table 4).

Differences in the prediction of intra-ring wood density from the anatomical characteristics between juvenile and mature wood were presented. In a recent investigation of the anatomical element variation in Gmelina arborea, it was established that the vessel proportion and diameter increased slightly from the pith to bark, but the frequency decreased abruptly in radial direction; cell wall thickness increased nearly 45% from juvenile to mature wood (MOYA, 2004). Probably, the high vessel frequency in juvenile wood from the dry and wet tropical (WJ and WM) produced more effects on wood density than the low frequency in mature wood. However, the mature wood that increase in fiber dimensions and cell wall thickness produced greater effects in the variation of intra-ring wood density.

4 CONCLUSIONS

The distribution and abundance of anatomical elements are different in annual tree-rings between or within

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trees. The wood density variation throughout the annual tree-rings is correlated with cell dimension, mainly cell wall thickness, vessel proportion, fiber and width, length and lumen diameter; showing clearly that wood density is an expression of these anatomical features on annual tree-rings of *Gmelina arborea*.

Cell wall thickness was the greatest influence on intra-ring wood density variation under dry tropical conditions, and was less significant under wet tropical. The vessel percentage gained importance under these conditions, mainly in wood of the juvenile period.

The main anatomical wood elements were analyzed to determine their influence on wood density throughout annual tree-rings, however, other wood cells could also affected the intra-ring wood density, not explained by vessel proportion or fiber dimension.

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