

## An Evaluation of Fiber Biometry and Nanomechanical Properties of Different *Eucalyptus* Species

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Wood fibers from seven *Eucalyptus* species were collected to investigate the relationships among species, fiber biometry, and nanomechanical properties. The results indicated significant differences in wood density, coarseness, fiber length, fiber width, and cell wall thickness among the different *Eucalyptus* species. The nanomechanical properties of the S<sub>2</sub> cell wall layer also showed significant differences among the *Eucalyptus* species. The elasticity modulus ranged from 16 to 19 GPa, the hardness spanned 0.24 to 0.31 GPa, and the ductility ratio was between 54 and 68. Moreover, significant correlations were observed for hardness versus cell wall thickness ( $r = 0.87$ ), and elasticity modulus versus crystallinity index ( $r = 0.80$ ) and crystallite size ( $r = 0.68$ ). Among the evaluated species, *E. dunnii* showed the highest elasticity modulus, highest hardness average, and the highest crystallinity index. The range of nanomechanical values indicated that *Eucalyptus* wood fibers are suitable for the development of new composite materials or engineering products by selecting the most adequate species for each use according to its properties.

*Keywords:* Fiber length; Fiber width; Coarseness; Cell wall thickness; Hardness; Elastic modulus

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### INTRODUCTION

Natural fibers are attractive materials that are widely used for paper, paperboard, textiles, fiberboards, and a variety of other uses. Natural fibers can also replace man-made fibers as reinforcement and fillers to make environmentally friendly products (Gindl *et al.* 2006b; Cheng *et al.* 2007; Wu *et al.* 2009). To make this application possible, it is necessary to fully understand the characteristics of the raw material for the development of new composite materials or engineering products. Accordingly, it is important to assess the chemical features and fiber biometry of wood, and more specifically, test the mechanical properties of the cell wall S<sub>2</sub> layer (Tze *et al.* 2007; Wu *et al.* 2009). The nanoindentation technique is a helpful tool to better understand the strength properties of the individual fibers at the microscopic level, since it can be used to investigate mechanical behavior of materials at the nanoscale. The test involves the penetration of a sample material using an indenter. The penetration depth and load are recorded, and the elastic modulus and hardness of wood cell walls is calculated (Gindl *et al.* 2004; Wu *et al.* 2009). The test can detect the mechanical properties of the S<sub>2</sub> cell wall layer, which is the major contributor to the mechanical properties of wood cell walls because it constitutes approximately 80% of the total cell wall thickness (Tze *et al.* 2007).

For nanoindentation experiments there is no need for chemical pretreatment to isolate individual wood fibers as required in single-fiber tensile tests (Tze *et al.* 2007), and the measured values are very consistent for elasticity and hardness evaluations (Huang *et al.* 2012). Thus, this technique has been used to investigate the fiber nanomechanical properties of several wood species. Nanomechanical characterizations have been performed on the fiber cell walls of softwoods species (Wimmer and Lucas 1997; Wimmer *et al.* 1997; Gindl *et al.* 2002, 2004; Gindl and Schoberl 2004; Tze *et al.*, 2007; Yu *et al.* 2011; Huang *et al.* 2012; Vincent *et al.* 2014), crops stalks (Wu *et al.* 2010), and several hardwood species (Gacitúa *et al.* 2007; Wu *et al.* 2009; Muñoz *et al.* 2012; Valenzuela *et al.* 2015; Zanuncio *et al.* 2016). In addition, regenerated fibers (Gindl *et al.* 2006a, b), microcrystalline cellulose (Das *et al.* 2010), and cellulose nanofibers (Yildirim and Shaler 2016) have been studied.

*Eucalyptus* trees are widely used in commercial plantation as raw material for pulp, paper, and cellulose derivatives production due to several desirable features: fast growth, straight form, valuable wood properties, wide adaptability to soils and climates, and easy management (Turnbull 1999; Gomes *et al.* 2015; Carrillo *et al.* 2018b). Several reports have addressed the morphological, anatomical, and chemical features of various *Eucalyptus* species as well as the respective pulp and derivatives products (Kibblewhite *et al.* 2000; Ona *et al.* 2001; Ramírez *et al.* 2009a, b; Aguayo *et al.* 2014; Carrillo *et al.* 2015, 2017, 2018a, b). Nanocharacterization research has been also carried out in some *Eucalyptus* species, mainly *Eucalyptus nitens* (Gacitúa *et al.* 2007; Muñoz *et al.* 2012; Valenzuela *et al.* 2015) and *E. grandis* × *E. urophylla* (Zanuncio *et al.* 2016). This work attempts to describe the main anatomical and nanomechanical features of the wood fibers from seven *Eucalyptus* species using microscopic and indentation techniques in order to elucidate their relationship and variation within the *Eucalyptus* species. In a previous study (Carrillo *et al.* 2018a), a comparative evaluation of the cellulose supramolecular structure of the same seven *Eucalyptus* wood species was made. The results from this previous study support this work and are considered for the results discussion. It is expected that this report will provide valuable information about wood property variations in the *Eucalyptus* genre, as well as explore the potential use of these species as raw material for the design and development of new forestry products.

## EXPERIMENTAL

### *Eucalyptus* Wood Samples

Six-year-old *Eucalyptus* trees were provided by a Chilean forestry company located in the Biobío Region of southern Chile. The species provided were *Eucalyptus badjensis*, *E. benthamii*, *E. dunnii*, *E. globulus*, *E. nitens*, *E. smithii*, and two hybrids *E. nitens* × *E. globulus*, coded *En* × *Eg* (1) and *En* × *Eg* (2). The seven *Eucalyptus* species grew under the same field and planting conditions. Wood chips were used for wood density determination, according to the TAPPI Standard T258 om-94 (1996), for nanomechanical evaluation and fiber biometry characterization. Experimental analyses were carried out in duplicate. As shown in Table 1, the chemical composition of the wood chips was determined in a previous study with the same sampled species (Carrillo *et al.* 2018a).

**Table 1.** Compositional Analysis of the *Eucalyptus* Wood Samples (Carrillo *et al.* 2018a)

Sample	Holocellulose (%)	Alpha-cellulose (%)	Glucans (%)	Xylans (%)	Lignin (%)	Extractives (%)
<i>E. badjensis</i>	69 ± 1	33.5 ± 0.3	51.7 ± 0.3	11.1 ± 0.6	25.2 ± 0.4	2.4 ± 0.3
<i>E. benthamii</i>	63.6 ± 0.7	31.9 ± 0.1	51.2 ± 0.1	8.3 ± 0.5	27.7 ± 0.7	6.7 ± 0.1
<i>E. dunnii</i>	64.0 ± 0.5	35.5 ± 0.8	54.3 ± 0.7	9.3 ± 0.9	24.9 ± 0.3	4.6 ± 0.3
<i>E. globulus</i>	73.5 ± 0.5	38.9 ± 0.1	56.9 ± 0.9	10.2 ± 0.4	23.2 ± 0.1	1.5 ± 0.1
<i>E. nitens</i>	68 ± 1	34.6 ± 0.1	51.9 ± 0.7	10.5 ± 0.5	25.2 ± 0.2	1.9 ± 0.1
<i>E. smithii</i>	70.5 ± 0.1	37.7 ± 0.3	51.2 ± 0.1	8.8 ± 0.1	25.7 ± 0.9	2.32 ± 0.02
<i>En</i> × <i>Eg</i> (1)	68.4 ± 0.9	34.1 ± 0.2	55.4 ± 0.7	10.8 ± 0.3	25.7 ± 0.4	1.97 ± 0.03
<i>En</i> × <i>Eg</i> (2)	70.3 ± 0.4	35.9 ± 0.2	55.2 ± 0.2	10.7 ± 0.5	25.6 ± 0.7	2.91 ± 0.03

### Fiber Biometry

Wood chips were treated according to the protocol reported by Mansfield and Weineisen (2007). A chisel was used to obtain matchsticks (0.1 × 0.1 × 0.5 cm) from wood chips. The obtained matchsticks were macerated and treated using Franklin solution (30% H<sub>2</sub>O<sub>2</sub> and CH<sub>3</sub>COOH, 1:1 v/v) for 8 h at 70 °C. The solution was decanted, and the remaining fibrous material was washed with water until a neutral pH was achieved. Average fiber length and coarseness were determined in a Lorentzen & Wettre Fiber Tester (Kista, Stockholm, Sweden) using 200 mg of sample that was previously disintegrated in 200 mL of distiller water for 10 min. During the suspension analysis, the equipment was set to measure 35,000 fibers of each sample. Fines were characterized as 0 to 0.2 mm in length to ensure that broken fibers and fines were not included in the final averages of fiber measurements (Carrillo *et al.* 2015, 2017).

### Nanomechanical Characterization

Cubes 3 mm × 3 mm in size were cut from wood chips obtained close to the bark section. The wood cubes were impregnated with Spurr epoxy resin (Spurr 1969) to provide mechanical support for cutting in a Leica RM2265 rotary microtome (Leica, Wetzlar, Germany), and to prevent damage during indentation of the fibers cell wall. The transverse surfaces of the samples were leveled with a glass knife and smoothed with a diamond knife. The indentation area obtained was about 1 mm<sup>2</sup> with a low and uniform roughness to increase the accuracy of the indenter measurements. The samples were conditioned for at least 24 h at 21 °C and 60% relative humidity in the room that housed the nanoindenter. Nanoindentations were performed using a Hystron TriboIndenter TI-900 (Hystron Inc., Minneapolis, MN, USA), using a cube corner diamond tip.

The elastic modulus of the secondary cell wall layer was obtained through a load-hold-unload cycle in areas of the S<sub>2</sub> cell wall layer. The loading cycle was worked to obtain an accelerated mapping of properties (XPM). For the load cycle, a 5 × 5 array with a separation of 1 μm between each indentation was used (Fig. 1), with a maximum load of 100 μN and a total time of 0.3 s. An area of 5 μm<sup>2</sup> was analyzed, and at least 25 measurements were taken for the S<sub>2</sub> cell wall layer of each *Eucalyptus* species. The reduced elastic modulus was obtained through Eq. 1,

$$E_r = \frac{\sqrt{\pi}}{2} \frac{S}{\sqrt{A}} \quad (1)$$

where  $E_r$  corresponds to the reduced elastic modulus resulting from the elastic deformation of the diamond tip ( $i$ ) and sample ( $s$ ),  $S$  is the slope of the discharge curve  $dP/dh$  when the discharge starts, and  $A$  is the contact area between the material and the maximum load of the indenter. The elastic modulus of the sample ( $E_s$ ) was determined using Eq. 2 (Gindl *et al.* 2004),

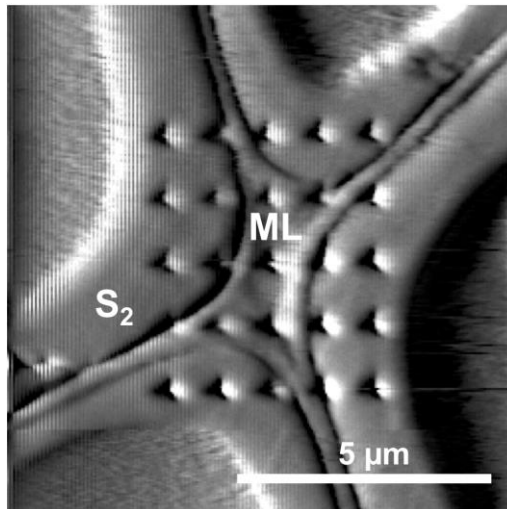
$$E_r = \left[ \frac{1-v_s^2}{E_s} + \frac{1-v_i^2}{E_i} \right]^{-1} \quad (2)$$

where Poisson's ratio  $v_s$  and  $v_i$  represent the sample and diamond tip, respectively, and  $E_i$  is the elastic modulus of the diamond (1140 GPa).

Hardness ( $H$ ) is the maximum load divided by the contact area, projected from indentation. Hardness was calculated through the Eq. 3 (Wu *et al.* 2009),

$$H = \frac{P_{max}}{A} \quad (3)$$

where  $P_{max}$  is the maximum load and  $A$  is the contact area.



**Fig. 1.** A 2D image showing the indentations (triangular shapes) on the  $S_2$  cell wall layer of *Eucalyptus* fibers. The indentations located in the middle lamella (ML) and in the  $S_2$  border were discarded.

### Transversal Anatomical Characterization

The transversal characterization was performed on the same cube sample used for nanoindentation tests. The wood cubes were mounted on stubs to apply a conductive coating using a metallizer (SPI-MODULE sputter-coated). The coating was performed with gold for 60 s. Images were obtained using a JEOL JSM-6380LV (Tokyo, Japan) scanning electron microscope (SEM) connected to a personal computer for image capture. Forty fibers were randomly selected, and their cell wall thickness, fiber width, and lumen width were measured at 1000 times total magnification. All these parameters were measured using the JEOL SEM software.

## Data Analysis

Statistical analysis of the anatomical and nanomechanical characteristics were performed using the SAS software system version 9.2 (Cary, USA). To determine significant differences between the *Eucalyptus* samples, analysis of variance (ANOVA) followed by Tukey's statistical test were performed at  $p < 0.5$ . Correlation analysis between the wood features were performed using Pearson's correlation coefficient.

## RESULTS AND DISCUSSION

### Wood Density and Fiber Biometry

Wood density and fiber biometry of the different *Eucalyptus* trees are shown in Table 2. Wood density values ranged from 420 to 484 kg/m<sup>3</sup>, with *E. globulus* and *E. smithii* as the higher wood density trees and *E. badjensis* as the lower one. These values agreed with whole-tree average densities reported by McKinley *et al.* (2002) for 8-year-old *E. globulus* and *E. nitens* (476 kg/m<sup>3</sup> and 440 kg/m<sup>3</sup>, respectively).

Regarding fiber biometry, fiber length ranged from 0.59 to 0.70 mm, with *E. badjensis* and *E. smithii* exhibiting the lowest and highest values, respectively. Fiber width and lumen width ranged 11 to 15  $\mu$ m and 4 to 9  $\mu$ m, respectively. The lowest fiber and lumen widths were found in *E. benthamii*, while the highest widths were found in *E. dunnii*. Fiber data results agree with studies of fiber biometry in *Eucalyptus* species (Muneri and Raymond 2001; Ona *et al.* 2001; Ohshima *et al.* 2004; Ramírez *et al.* 2009b; Carrillo *et al.* 2015, 2017). Muneri and Raymond (2001) evaluated the fiber length of 5- to 9-year-old *E. globulus* and *E. nitens* trees from different sites, reporting values of 0.66 to 0.75 mm and 0.56 to 0.72 mm, respectively. Cell wall thickness values spanned between 1.9 and 2.3  $\mu$ m, which is a lower range than the values reported by Ramírez *et al.* (2009b) for 7-year-old *E. globulus* trees, and by Carrillo *et al.* (2015) for 15-year-old *E. globulus* trees. Coarseness is defined as fiber mass per fiber length. Coarseness is a good index for predicting pulp properties and is closely related to the biometric properties of fibers and basic density of wood (Via *et al.* 2004; Mansfield and Weineisen 2007; Carrillo *et al.* 2015). The higher coarseness values were observed in *E. smithii* (8.9 mg/100 m) and *E. globulus* (8.5 mg/100 m), while the lower values were seen in *E. badjensis*, *E. benthamii*, and *En*  $\times$  *Eg* (1) species, with approximate values of 7.0 mg/100 m.

**Table 2.** Wood Density and Fiber Biometry of the Different *Eucalyptus* Species

Sample	Wood Density (kg/m <sup>3</sup> )	Fiber Length (mm)	Fiber Width ( $\mu$ m)	Lumen Width ( $\mu$ m)	Cell Wall Thickness ( $\mu$ m)	Coarseness (mg/100 m)
<i>E. badjensis</i>	420 <sup>b</sup> $\pm$ 21	0.59 <sup>d</sup> $\pm$ 0.04	13 <sup>b</sup> $\pm$ 2	7 <sup>bc</sup> $\pm$ 2	2.3 <sup>a</sup> $\pm$ 0.4	7.03 <sup>b</sup> $\pm$ 0.02
<i>E. benthamii</i>	444 <sup>ab</sup> $\pm$ 14	0.67 <sup>b</sup> $\pm$ 0.01	11 <sup>d</sup> $\pm$ 2	4 <sup>e</sup> $\pm$ 2	2.2 <sup>ab</sup> $\pm$ 0.5	7.1 <sup>b</sup> $\pm$ 0.1
<i>E. dunnii</i>	438 <sup>ab</sup> $\pm$ 20	0.68 <sup>b</sup> $\pm$ 0.01	15 <sup>a</sup> $\pm$ 3	9 <sup>a</sup> $\pm$ 3	2.3 <sup>a</sup> $\pm$ 0.6	7.7 <sup>ab</sup> $\pm$ 0.2
<i>E. globulus</i>	481 <sup>a</sup> $\pm$ 13	0.69 <sup>a</sup> $\pm$ 0.01	13 <sup>bc</sup> $\pm$ 3	7 <sup>bc</sup> $\pm$ 3	2.1 <sup>bc</sup> $\pm$ 0.4	8.5 <sup>a</sup> $\pm$ 0.1
<i>E. nitens</i>	433 <sup>ab</sup> $\pm$ 9	0.62 <sup>c</sup> $\pm$ 0.01	11 <sup>d</sup> $\pm$ 2	6 <sup>d</sup> $\pm$ 2	2.0 <sup>bc</sup> $\pm$ 0.4	8.0 <sup>ab</sup> $\pm$ 0.6
<i>E. smithii</i>	484 <sup>a</sup> $\pm$ 17	0.70 <sup>a</sup> $\pm$ 0.01	11 <sup>cd</sup> $\pm$ 2	6 <sup>cd</sup> $\pm$ 2	1.9 <sup>c</sup> $\pm$ 0.3	8.9 <sup>a</sup> $\pm$ 0.6
<i>En</i> $\times$ <i>Eg</i> (1)	469 <sup>ab</sup> $\pm$ 5	0.69 <sup>ab</sup> $\pm$ 0.02	13 <sup>ab</sup> $\pm$ 3	9 <sup>ab</sup> $\pm$ 3	2.0 <sup>c</sup> $\pm$ 0.4	6.9 <sup>b</sup> $\pm$ 0.3
<i>En</i> $\times$ <i>Eg</i> (2)	447 <sup>ab</sup> $\pm$ 16	0.69 <sup>a</sup> $\pm$ 0.01	11 <sup>d</sup> $\pm$ 2	6 <sup>d</sup> $\pm$ 2	2.2 <sup>ab</sup> $\pm$ 0.5	8.2 <sup>ab</sup> $\pm$ 0.4

\*Different letter means significant differences within a column at  $p < 0.05$ .

## Nanomechanical Properties

Nanomechanical properties obtained for the *Eucalyptus* wood samples are shown in Fig. 2. The Spurr epoxy resin has an influence on mechanical properties of embedded wood cell walls, increasing their hardness (around 20%) (Gindl *et al.* 2004; Meng *et al.* 2013). This has prompted the implementation of alternative preparation methods to avoid the influence of embedding mediums on wood cell wall (Meng *et al.* 2013). In this work, with a comparative purpose, all the samples were subjected to the same impregnation treatment. Thus, the influence of the epoxy resin on nano-mechanical properties is expected to be the same in all the evaluated specimens, which makes it possible to make an adequate comparison among the different *Eucalyptus* species.

Elastic modulus ( $E$ ) averages were significantly different among the *Eucalyptus* species ( $p$ -value = 0.0361), ranging from 16 to 19 GPa. The highest average was observed in *E. dunnii*, while *E. badjensis* and *E. smithii* showed the lowest average values (Fig. 2a). The  $E$  variation coefficient ranged between 15 and 24%, where *E. benthamii* showed the highest heterogeneity (Fig. 2a).

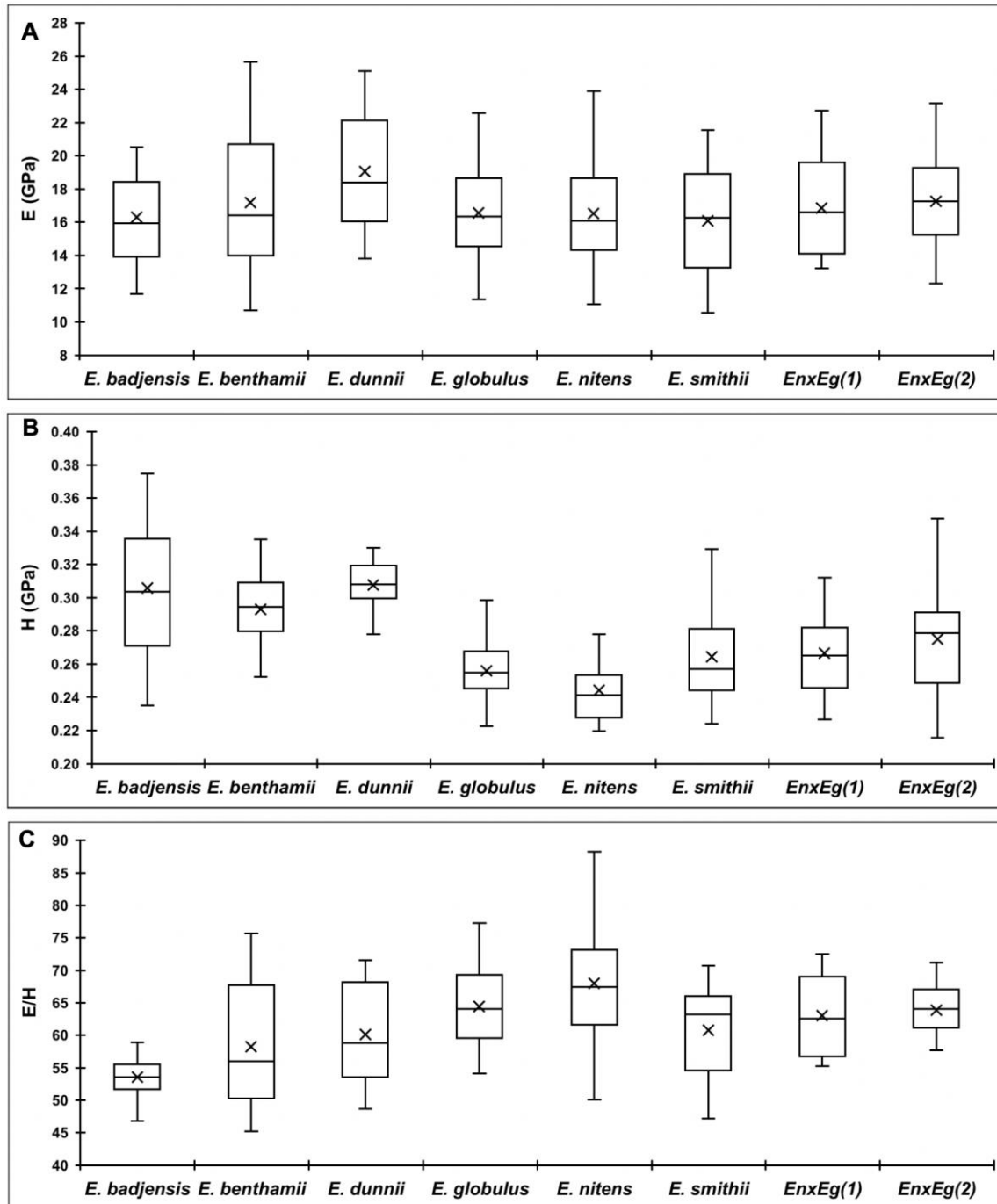
Hardness ( $H$ ) average values ranged from 0.24 to 0.31 GPa, with *E. badjensis* and *E. dunnii* being the highest, and *E. nitens* the lowest (Fig. 2b), displaying significant differences between the different *Eucalyptus* species ( $p$ -value < 0.0001). The  $H$  variation coefficient ranged from 4 to 13%, with *E. badjensis* and *En* × *Eg* (2) exhibiting more heterogenous data, while *E. dunnii* exhibiting the most homogeneous.

The ductility ratio ( $E/H$ ) spanned from 54 to 68, and significant differences were observed between the different *Eucalyptus* species ( $p$ -value < 0.0001). The highest average value was observed in *E. nitens* fibers, while the lowest was observed in *E. badjensis* (Fig. 2c). The  $E/H$  variation coefficient spanned from 5 to 17%.

The data obtained were similar to nanomechanical values published elsewhere for hardwoods species (Wu *et al.* 2009) and for *Eucalyptus* samples (Muñoz *et al.* 2012; Valenzuela *et al.* 2012). Muñoz *et al.* (2012) evaluated 12-year-old *E. nitens* from different sites. They reported  $H$ ,  $E$ , and  $E/H$  values for the  $S_2$  cell wall layer ranging from 0.23 to 0.43 GPa, 8.95 to 16.99 GPa, and 26.40 to 62.94, respectively. Valenzuela *et al.* (2012) also evaluated 12-year-old *E. nitens* from different sites, reporting  $S_2$  cell wall layer  $E$  and  $H$  average values of approximately 10 GPa and 0.29 GPa, respectively. The  $E/H$  values for the same study were approximately 40. In addition, the authors suggested a correlation between cracking levels and the  $E/H$  ratio. Therefore, *Eucalyptus* samples with the lowest  $E/H$  values subjected to small deformations should be more fragile and more easily weakened by micro-fracture effects (Muñoz *et al.* 2012). The  $E/H$  ratio has been used to describe the stiffness of materials; brittle materials have low  $E/H$  values, such as glass ( $E/H=12$ ), and ductile materials have high  $E/H$  values, such as aluminum ( $E/H=117$ ) (Bolshakov and Pharr 1998). Other studies have reported a correlation between  $E$  values and micro-fibrillar angle (MFA), where  $E$  decreased with increasing MFA in hardwoods species (Wu *et al.* 2009) and softwoods (Tze *et al.* 2007).

## Relationship between *Eucalyptus* Wood Features

Table 3 shows the correlation index between the different *Eucalyptus* wood properties evaluated in this work. As mentioned previously, coarseness has a close relationship with wood density (Via *et al.* 2004; Mansfield and Weineisen 2007; Carrillo *et al.* 2015). However, a significant correlation between both properties was not observed in this study.



**Fig. 2.** Nanomechanical properties of *Eucalyptus* wood in the S<sub>2</sub> cell wall layer. **A:** Elastic modulus ( $E$ ); **B:** hardness ( $H$ ); and **C:** ductility ratio ( $E/H$ ). Box and whisker plots show the average ( $x$ ), the median (horizontal line), the 50% interquartile range (box), and the maximum and minimum value (whiskers).

Cell wall thickness versus wood density showed a significant but negative correlation ( $r = -0.69$ ), which was unexpected. Wood density is determined primarily by anatomical structures such as vessel features, fiber width, cell wall thickness, and parenchyma proportion. Chemical composition, especially bulking by extraneous materials, can also play an important role in determining wood density (Carrillo *et al.* 2015,

2017). Several authors have reported different correlation coefficients between anatomical properties of *Eucalyptus* (Kube *et al.* 2001; Wimmer *et al.* 2002; Ohshima *et al.* 2004; Carrillo *et al.* 2015, 2017), which reflect the wide variability in anatomical features presented within *Eucalyptus* species.

On the other hand, relationships between nanomechanical properties, such as hardness, and fiber morphological features have been suggested (Muñoz *et al.* 2012; Savva *et al.* 2010; Vincent *et al.* 2014), while Wu *et al.* (2009) suggested an influence of the cell wall thickness during the nanoindentation test. In agreement with these reports, a positive and significant correlation between cell wall thickness and *H* in *Eucalyptus* wood samples was observed. These results contradict Huang *et al.* (2012), who found that in mature conifer wood, nanohardness was not affected by cell wall thickness. Other factors such as the complex cell wall structure and its chemical composition can influence the nanomechanical properties (Wu *et al.* 2009; Vincent *et al.* 2014). Wimmer and Lucas (1997) attributed a distinctly reduced elastic modulus in the middle lamella to the absence of cellulose in this region. However, results from this study showed no significant correlation between chemical composition and nanomechanical properties (Table 3). In this sense, Gindl *et al.* (2002) suggested that the elasticity and stiffness of the wood cell wall is affected by the arrangement, organization, and quantity of the wood components that shape the cell wall architecture.

**Table 3.** Pearson Correlation Index between Evaluated Variables of the *Eucalyptus* Wood Species (n=8)

	Wood Density	Fiber Length	Fiber Width	Lumen Width	CWT	C	E	H	E/H	CrI	L
Wood Density	1										
Fiber Length	0.79*	1									
Fiber Width	-0.12	-0.01	1								
Lumen Width	0.08	0.11	0.83*	1							
CWT	-0.69*	-0.35	0.48	0.09	1						
C	0.55	0.43	-0.29	-0.19	-0.46	1					
E	-0.18	0.27	0.62	0.46	0.41	-0.11	1				
H	-0.51	-0.20	0.56	0.22	0.87*	-0.53	0.31	1			
E/H	0.19	0.07	-0.28	0.08	-0.53	0.42	0.05	-0.82*	1		
CrI	0.65	0.65	0.67*	0.61	0.19	-0.08	0.80*	0.26	-0.08	1	
L	0.44	0.62	0.65	0.72*	-1.00	-0.07	0.68*	0.02	0.09	0.94*	1
Holocell	0.54	0.14	-0.18	0.05	-0.41	0.58	-0.54	-0.50	0.44	-0.25	-0.14
Alpha-cell	0.68*	0.50	0.10	0.22	-0.37	0.86*	-0.01	-0.41	0.39	0.20	0.25
Glucans	0.39	0.49	0.45	0.55	0.08	0.12	0.39	-0.08	0.37	0.66	0.64
Xylans	-0.26	-0.43	0.17	0.43	0.09	-0.21	-0.19	-0.08	0.43	-0.21	-0.11
Lignin	-0.25	-0.01	-0.48	-0.53	0.03	-0.45	-0.14	0.21	-0.43	-0.20	-0.27
Extractives	-0.33	0.10	-0.01	-0.39	0.49	-0.38	0.39	0.54	-0.61	0.24	0.01

\* Significant at  $p < 0.05$ .  
 CWT: Cell wall thickness. C: Coarseness. E: Elasticity modulus. H: hardness.  
 E/H: ductility ratio. CrI: crystallinity index. L: lateral crystallite size. Holocell: holocellulose.  
 Alpha-cell: alfa-cellulose

Wu *et al.* (2013) suggested that in some cellulosic materials, the mechanical properties are dependent on the direction relative to the cellulose crystalline structure and chain arrangement within the crystal structure. In a previous work, the crystalline



properties of cellulose from the seven *Eucalyptus* wood species studied in this work were evaluated (Carrillo *et al.* 2018a). Table 4 shows the results obtained in the work for the crystallinity degree (CrI) and crystallite size (*L*) of the same *Eucalyptus* species evaluated in this study. A significant correlation was observed between the crystallite size and crystallinity degree of cellulose in wood with *E* values (Table 3), which could be related to the arrangement of the cellulose microfibrils that influence the elastic modulus of the wood cell wall (Wimmer and Lucas 1997; Gindl *et al.* 2004; Muñoz *et al.* 2012). According to Das *et al.* (2010), a high hardness could be expected in cellulosic samples due to high crystallinity and large crystallite size. However, those correlations were weak and not significant (Table 3).

**Table 4.** Crystallinity Index (CrI) and Lateral Crystallite Size (*L*) of the Different *Eucalyptus* Wood Species (Carrillo *et al.* 2018a)

Sample	Crystallinity Index (%)	Lateral Crystallite Size (nm)
<i>E. badjensis</i>	51.6 ± 0.5	1.82 ± 0.01
<i>E. benthamii</i>	54 ± 1	1.9 ± 0.1
<i>E. dunnii</i>	57.6 ± 0.8	2.06 ± 0.07
<i>E. globulus</i>	55.3 ± 0.6	1.99 ± 0.04
<i>E. nitens</i>	52 ± 1	1.88 ± 0.04
<i>E. smithii</i>	53 ± 2	1.91 ± 0.09
<i>En</i> × <i>Eg</i> (1)	56 ± 2	2.06 ± 0.06
<i>En</i> × <i>Eg</i> (2)	54 ± 1	1.89 ± 0.09

Wu *et al.* (2010) suggested that the polymerization degree, in addition to crystallization and holocellulose content, contributed to a higher hardness in cotton stalk cell walls, which could be an interesting parameter to include in future work. Additionally, it has been observed in regenerated fibers (Lyocell and viscose fibers) that the degree of orientation of both crystalline and amorphous cellulose, which is an indication of the lateral bonding degree, could influence the hardness of fibers (Gindl *et al.* 2006a).

*Eucalyptus* species that grew in the same field and same plantation conditions differ in their chemical composition and fiber biometry. As expected, *E. globulus* frequently shows the best chemical and fiber features for pulping procedures, such as a low lignin and extractives content, high holocellulose content, high wood density, high coarseness, and high fiber length. However, other *Eucalyptus* species may also be suitable as raw material for forest products, working as reinforcement and/or filler in the development of new composite or engineering materials (Muñoz *et al.* 2012), since their native wood fibers provide material with adequate nanomechanical and supra-structural properties. Foresters can discriminate among species and genotypes by applying several non-destructive techniques to predict wood chemical composition (Jones *et al.* 2006), wood density (Isik and Li 2003; Carrillo *et al.* 2017), and wood mechanical properties (Kelley *et al.* 2004). In this work, no significant correlation between these features and nanomechanical properties were established. According to Pearson results, XRD analysis might be an alternative for nondestructive estimation of nanomechanical properties in *Eucalyptus* trees. However, despite that the evaluated samples corresponded to unrelated *Eucalyptus* trees growing in the same field and plantation conditions, additional analysis of a higher number of trees is required in order to increase the statistical significance of the results.

## CONCLUSIONS

1. *Eucalyptus* species growing in the same field and same plantation conditions developed wood with different chemical composition and different fiber biometry, while the nanomechanical properties of the S<sub>2</sub> cell wall layer of native fibers also displayed significant differences. The highest elasticity modulus and hardness averages were observed in *E. dunnii*, while *E. nitens* exhibited the highest ductility ratio.
2. Significant and positive correlations were established between hardness versus cell wall thickness and the elasticity modulus versus crystallinity index and crystallite size.
3. No significant correlation was observed among nanomechanical properties and the chemical composition of *Eucalyptus* wood.

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