



Sampling sufficiency of the anatomical characteristics of Brazilian hardwood using the resampling method

André Luiz Missio^{1*}, Fábio Mariano Bayer², Darci Alberto Gatto^{1,3} and Pedro Henrique Gonzalez de Cademartori⁴

¹Programa de Pós-graduação em Engenharia Florestal, Laboratório de Produtos Florestais, Universidade Federal de Santa Maria, Av. Roraima, 1000, 97105-900, Santa Maria, Rio Grande do Sul, Brazil. ²Departamento de Estatística e Laboratório de Ciências Espaciais de Santa Maria, Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, Brazil. ³Curso de Engenharia Industrial Madeireira, Centro de Engenharias, Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brazil. ⁴Programa de Pós-graduação em Engenharia Florestal, Centro de Ciências Florestais e da Madeira, Universidade Federal do Paraná, Curitiba, Paraná, Brazil. *Author for correspondence. E-mail: andreluizmissio@gmail.com

ABSTRACT. Sampling sufficiency of the anatomical characteristics of fibres from *Luehea divaricata* wood was investigated through the bootstrap resampling method. Sampling sufficiency of fibre length, fibre diameter, lumen diameter and fibre wall thickness were determined. Three scenarios of sampling sufficiency evaluation were used: general, segregation between juvenile and mature wood, and accumulation throughout the life of the tree. The segregation of juvenile and mature wood showed that sampling sufficiency in juvenile wood was higher than in mature wood, up to three times for the fibre length. In general, results indicated higher values than suggested in specific standards and by other authors. Therefore, resampling bootstrap methodology, even though underused in forestry research, is an alternative for new studies, as it does not have restrictive hypotheses such as data normality.

Keywords: bootstrap, juvenile wood, mature wood, *Luehea divaricata*, sample size.

Suficiência amostral das características anatômicas da madeira usando o método de reamostragem

RESUMO. A suficiência amostral das características anatômicas das fibras da espécie *Luehea divaricata* foi investigada utilizando o método de reamostragem bootstrap. Foram determinadas as suficiências amostrais do comprimento da fibra, do diâmetro da fibra, do diâmetro do lúmen e da espessura da parede da fibra. Três cenários de avaliação da suficiência amostral foram usados: geral, segregação entre lenhos juvenil e adulto, e acumulação durante toda a vida da árvore. Para o cenário de segregação entre lenhos juvenil e adulto, verificou-se que a suficiência amostral no lenho juvenil foi superior à do lenho adulto, chegando a ser três vezes maior para o comprimento da fibra. Os tamanhos amostrais determinados foram, de maneira geral, superiores aos sugeridos por normativas da área e utilizados por outros pesquisadores. Dessa forma, a metodologia de reamostragem bootstrap, até hoje pouco usada na área florestal, surge como alternativa para trabalhos futuros, já que não possui hipóteses restritivas, como a normalidade dos dados.

Palavras-chave: bootstrap, lenho juvenil, lenho adulto, *Luehea divaricata*, tamanho amostral.

Introduction

In most of scientific research, it is not possible or convenient to access all population elements, making the use of a population sample necessary. Sampling techniques are widely used in order to obtain accurate inferences of unknown population parameters. The reach and importance of this technique is evidenced in many areas, such as forest inventory (NEWTON et al., 2012), water preservation in forest ecosystems (BIAO et al., 2010), biodiversity in marine bioevaluation (XU et al., 2012) and agricultural science

(CARGNELUTTI-FILHO et al., 2010; STORCK et al., 2012).

With regards to sampling sufficiency, it is known that larger sampling sizes increase the probability of obtaining the same inferential conclusions in new samples within the same population (PILLAR, 1999). On the other hand, small sampling tends to achieve distorted inferential results. In this context, determining coherent sampling sizes is very important in order to balance the sampling size and the confidence level of inferences.

Results based on the distribution of sample means are traditionally used to determine

sampling sizes (ISRAEL, 2012) according to procedures indicated by the 'Pan American Standards Commission' (COPANT, 1974). However, though this procedure is straightforward and largely applied, it presents restrictive hypotheses such as data normality. Based on a normality supposition, a pre-determined significance level and an error, the mean sampling distribution is obtained (*t*-Student when variance is unknown) and consequently it is possible to determine the ideal sampling size in order to obtain inference concerning the population mean. These hypotheses could not be suitable in forestry studies or wood sciences because wood is a heterogeneous material that has variations amongst species, within species and within the same tree, resulting in non-normal data and high variability. Moreover, within the same species, heterogeneity can occur in trunk height, between heartwood and sapwood, in the pith-bark direction, between juvenile and mature wood, and between individual cells in macroscopic scale (ADAMOPOULOS et al., 2007; DÜNISCH et al., 2010).

Bootstrap methods (EFRON; TIBSHIRANI, 1994) could be a great alternative to determine sampling sufficiency. Bootstrap is an intensive computational method based on resampling of original data that aims to improve inferences in small sampling (EDWARDS et al., 2011). Instead of supposing a restrictive hypothesis, such as known theoretical distributions, an empiric distribution of the sample is used to perform inferences (GRUNKEMEIER; WU, 2004; EDWARDS et al., 2011). Determining accurate confidence levels and ideal sampling sizes could be performed based on empiric distribution of population parameters of interest, as verified in Pillar (1998, 1999).

Therefore, the versatility and the precision of the Bootstrap method (EDWARDS et al., 2011) enables the evaluation of sampling sufficiency to infer anatomical characteristics of forest species, since these sampling sizes still have not been clearly defined. For example, 35 measurements per sample were used by Malan and Hoom (1992) and Denardi and Marchiori (2005) to characterise the anatomy of fibres or tracheids. The Committee of the International Association of Wood Anatomists (GASSON et al., 1989) reported the need to measure at least 25 fibres. Moreover, according to Zhu et al. (2005), properties of trees gradually vary during its growth. Understanding the difference between juvenile and mature wood is important because it affects forest management and wood

improvement. Most species with a high proportion of juvenile wood present variations in technological properties when related to mature wood. Mechanically, juvenile wood shows lower static bending, lower dynamic strength and lower toughness than mature wood (ADAMOPOULOS et al., 2007). Additionally, physically and chemically such wood presents lower density (BAO et al., 2001; KRETSCHMANN, 2008), lower moisture content (KRETSCHMANN, 2008), lower percentage of latewood, lower cellulose content, higher lignin content, higher longitudinal shrinkage, thinner cell walls (KRETSCHMANN, 1998) and lower natural durability (DÜNISCH et al., 2010).

Taking these facts into account, this study aimed to determine sampling sufficiency of anatomical characteristics of fibres of juvenile and mature wood from *Luehea divaricata* Mart. & Zucc. through the bootstrap method. Determining accurate sampling sizes is essential because an insufficient number of measurements can cause results that do not significantly represent the real anatomical characteristics of a population. On the other hand, if the quantity of measurements is too great, the researcher needs more time, as well as material and financial support. Thus, the results herein reported could be used as reference sampling sizes for subsequent research.

Material and methods

Material collection and preparation of samples

Three açoita-cavalo (*Luehea divaricata*) trees (approximately 55 years old) were randomly selected and harvested according to American Society for Testing and Materials (ASTM, 2000) from a native forest licensed by the State Ministry of Environment, located in Upper Northeast Slopes of Rio Grande do Sul, southern Brazil. All trees presented cylindrical and straight trunks, with the diameter at breast height (DBH, 1.30 m) higher than 30 cm and appropriate phytosanitary conditions.

Disks measuring 0.02 m of thickness and located at 0.1 m of the tree height were cut from the trees. Central samples measuring 0.02 m of width, with growth rings oriented in radial direction and including a pith in the centre were cut from each disk. The samples were immersed in a 70% alcohol solution to maintain their natural characteristics.

Samples of early wood for each growth ring along the diameter of the trees (pith-bark direction) were cut in order to perform the maceration and preparation of slides through the Jeffrey method,

according to Burger and Richter (1991). One hundred and sixty fibres from fifty-one growth rings were measured, with a total of $N = 8160$ measurements as the sampling universe for this study. Fibre length (L), fibre diameter (D_F) and lumen diameter (D_L) of each fibre were measured (Figure 1). The fibre length was measured in a microscope with a 10x enlargement lens, while fibre diameter and lumen diameter were measured in a microscope with a 40x enlargement lens. The thickness of the fibre wall (T_W) was obtained through the half difference between fibre diameter and lumen diameter.

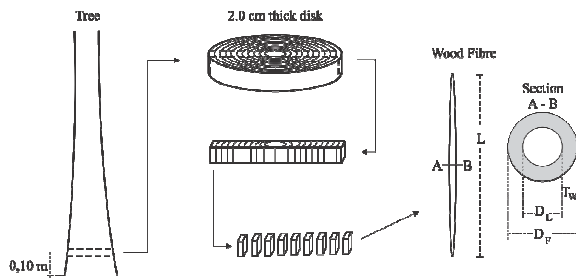


Figure 1. Measurement of fibres of *Luehea divaricata* wood (L = fibre length; D_F = fibre diameter; D_L = lumen diameter; T_W = wall thickness).

Sampling sufficiency by Bootstrap

The sampling sufficiency to infer the parameters of the mean of the anatomical characteristics was performed through bootstrap confidence intervals. This methodology was similar to that used by Pillar (1999, 1998); Cargnelutti-Filho et al. (2010); Cargnelutti-Filho et al. (2012) and Storck et al. (2012) for determining sampling sizes in studies about ecology, limnology and phytotechny.

n sampling sizes that are $n = 3, \dots, 90$ were considered, in which a confidence level of $1 - \alpha$ was determined for the mean μ of anatomical characteristics. The samples with size n were randomly chosen – with replacements – from the universe of available data $N = 8160$. Amplitude (δ) of confidence interval for each n was measured and compared with a measure of error ε equal to 10% of sampling mean (\bar{x}) (CARGNELUTTI-FILHO et al., 2012). Therefore, sampling sufficiency was determined through the lowest sampling size n that obtained δ lower than error of $\varepsilon = 0.1 \cdot \bar{x}$. This procedure could be graphically visualized in Figure 3 and determined by the following algorithm:

1. Set an initial value for n , n_k , and a significance level α ;
2. Determine the interval amplitude (δ_k) at a $(1 - \alpha)$ confidence level for the parameter μ , considering the sample size equals to n_k ;

3. Verify if δ_k is less than $\varepsilon = 0.1 \cdot \bar{x}$;

4. If $\delta_k < \varepsilon$, then n_k is the sufficiency sample size. Else, i.e. if $\delta_k > \varepsilon$, then increment n_k and repeat the steps (2), (3) e (4).

Determining the sampling size using an approach based on sample mean variance \bar{x} would be a traditional approach to step 2 of the aforementioned algorithm. However, this approach supposes restrictive hypotheses, such as data normality. These presuppositions are not always satisfactory or reasonable in studies of anatomical characteristics. In this context, bootstrap confidence intervals can achieve more credible results than results that suppose theoretical distribution of data. Bootstrap confidence intervals are based on empirical distribution of observed samples and need just Monte Carlo simulations for their determination. The confidence interval of mean μ based on bootstrap percentiles (EFRON; TIBSHIRANI, 1994), with significance level α and sample size n is determined through the following algorithm:

1. Sample n observations randomly with replacement from pseudo-population with N elements to obtain a bootstrap data set $\mathbf{x}_1^* = \mathbf{x}_1^*, \dots, \mathbf{x}_n^*$;

2. Based on \mathbf{x}_1^* calculate the bootstrap version of

$$\bar{x}_1^* = \sum_{i=1}^n x_i^* / n$$

the estimative \bar{x}_1^* of the μ , where

3. Repeat the steps (1) and (2) a large number of times (we adopt $B = 10000$), i.e. $b = 1, \dots, B$;

4. Define ordered values of the B bootstrap estimates \bar{x}_b^* ;

5. Determine a percentile interval $1 - \alpha$, where the lower limit (\bar{x}_{lo}^*) of the confidence interval is the $(B \cdot \alpha / 2)$ -th ordered estimative and the upper limit (\bar{x}_{up}^*) is the $[B \cdot (1 - \alpha / 2)]$ -th ordered bootstrap estimative;

6. Make the percentile bootstrap interval equals to $[\bar{x}_{lo}^*, \bar{x}_{up}^*]$.

Thus, the interval $[\bar{x}_{lo}^*, \bar{x}_{up}^*]$ contains the population μ with probability $1 - \alpha$.

Results and discussion

Descriptive analysis

Figure 2 and Table 1 show a descriptive analysis of collected data. According to Figure 2, the juvenile wood shows an increase in all anatomical characteristics until the segregation of wood (21 years, reported by Gatto et al. (2008)). The mature wood (21 years onwards) showed a stabilisation and lower mean values than the juvenile wood for all anatomical

characteristics. The lumen diameter (D_L) presented high variability between growth rings. This could be related to the decrease of growth rings due to increased age of the wood and, consequently, a higher proportion of early wood than latewood. Growth of mature wood is small because the activity of cambium is low, which causes lower values of D_L than in juvenile wood. Weather and spacing of plantation influence the variation in anatomical characteristics of fibres.

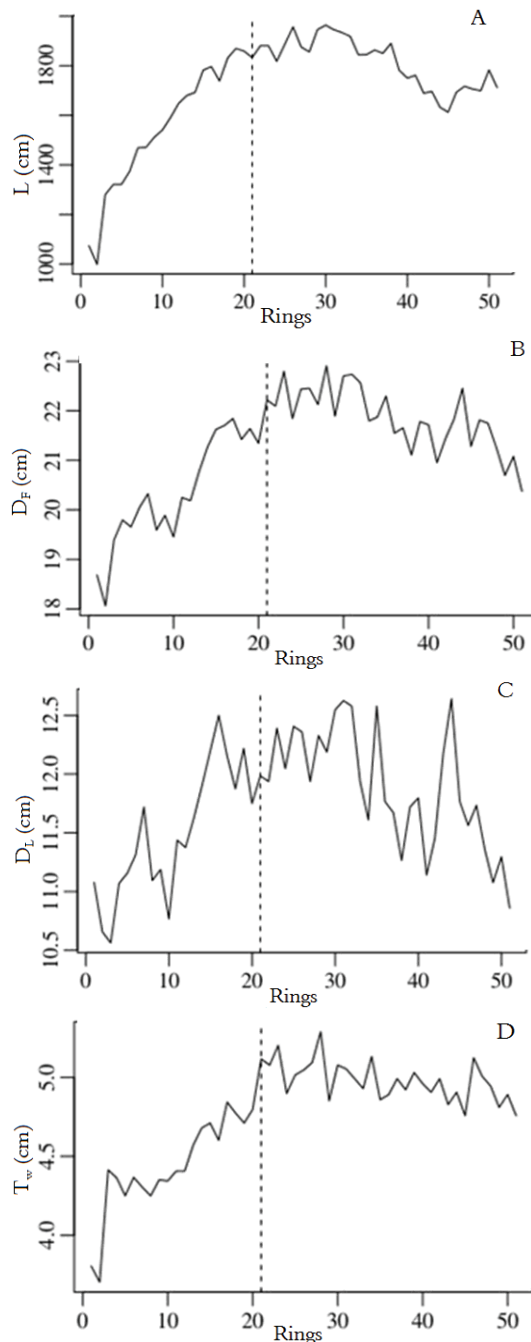


Figure 2. Mean values of the anatomical characteristics of the fibre by growth ring. (a) Fibre length; (b) Fibre diameter; (c) Lumen diameter; (d) Thickness of the fibre wall.

Table 1. Descriptive measures of *Luehea divaricata* wood for three considered scenarios: (i) general scenario without separation (juvenile + mature) and (ii) with separation between juvenile and mature wood.

Scenario	Measures				
	Mean (μm)	SD	CV (%)	Min.	Max.
L					
General	1707	281.06	16.46	600	2650
Juvenile	1556	301.58	19.38	600	2350
Mature	1813	208.63	11.51	1050	2650
D_F					
General	21.26	3.28	15.41	12.50	37.50
Juvenile	20.44	3.12	15.29	12.50	35.00
Mature	21.84	3.26	14.91	12.50	37.50
D_L					
General	11.73	2.42	20.63	5.00	22.50
Juvenile	11.59	2.48	21.53	5.00	22.50
Mature	11.89	2.37	19.91	5.00	22.50
T_w					
General	4.77	1.02	21.34	1.25	8.75
Juvenile	4.47	0.99	22.07	2.50	8.75
Mature	4.98	0.99	19.80	1.25	8.75

SD: standard deviation; CV: coefficient of variation; Min: minimum; Max: maximum; L: fibre length; D_F : fibre diameter; D_L : lumen diameter; T_w : thickness of the fibre wall.

Table 1 shows that the coefficient of variation (CV) for all anatomical characteristics of juvenile wood was higher than in mature wood. These results are similar to those reported by Ferreira et al. (2011), in which a significant difference between CV of mature (5.01%) and juvenile wood (12.64%) in L of *Hevea brasiliensis* (50 years) was observed. The same authors observed that mean values of L were low in juvenile wood, corroborating with results verified by Bao et al. (2001) and in this study.

Juvenile wood showed lower values than mature wood because L, D_F , D_L and T_w are in formation during the natural process of fast initial growth of the tree. Particularly, the segregation of wood is less noticeable in D_L (Figure 2c) due to a high variability between growth rings. Nevertheless, a tendency of D_L decrease based on segregation year was observed.

Therefore, the variability and the mean values of the anatomical characteristics are directly related to the proportion of juvenile wood in the tree. This fact indicates that sampling sufficiency in research about juvenile and mature wood could be differentiated since the variability of collected data directly influences in determining ideal sample sizes.

Determination of the sample sizes

The results discussed in this section were obtained through the methodology previously described. The determination of sampling sufficiency was performed considering $B = 10000$ bootstrap resampling, significance levels α equals to 0.05 and 0.10, measurement error ε at 10% of mean estimative and three scenarios. The scenarios were

(i) general case without any separation or classification of $N = 8160$ observations; (ii) segregation between juvenile and mature wood results considering the descriptive analysis; (iii) accumulated sampling sufficiency considering the accumulation of growth rings until the ring (year) of interest, i.e., sampling sufficiency to accumulated m first growth rings with $m = 1, 2, \dots, 51$ was determined. The computational implementation was developed in R language (R DEVELOPMENT CORE TEAM, 2012).

The sampling sufficiency of anatomical characteristics of *Luehea divaricata* fibres for the general scenario is shown in Figure 3 and Table 2. The sample size for L was 30 at $\alpha = 0.10$ and corroborated with the methodology applied by Malan and Hoon (1992) and Denardi and Marchiori (2005). On the other hand, the sampling sufficiency suggested by the Committee of the International Association of Wood Anatomists (GASSON et al., 1989) indicates a minimum of 25 measurements, which is statistically adequate. For $\alpha = 0.05$, all aforementioned references, except Gasson et al. (1989), the suggested sample sizes are not sufficient for L of *Luehea divaricata*, which could causes a distortions in the results.

Table 2. Sampling sufficiency of the anatomical characteristics of the of *Luehea divaricata* fibre considering the general scenario (i) and the scenario of separation in juvenile and mature wood (ii).

Scenario	Characteristics			
	L	D _F	D _L	T _w
$\alpha = 0.10$				
General	30	26	49	50
Juvenile	43	26	50	55
Mature	15	16	46	44
$\alpha = 0.05$				
General	43	38	67	69
Juvenile	59	37	71	76
Mature	21	35	61	61

L: fibre length; D_F: fibre diameter; D_L: lumen diameter; T_w: thickness of the fibre wall.

According to Figure 3b, results found for D_F were similar to those suggested by Gasson et al. (1989). The methodologies used by Malan and Hoon (1992) and Denardi and Marchiori (2005) suggest 35 measurements, which is close to the 38 measurements found in this study for the general case and considering $\alpha = 0.05$ level of significance (see Table 2). The sampling sufficiency for D_L and T_w (Figure 3c and d, respectively) at $\alpha = 0.10$ and $\alpha = 0.05$ was higher than the aforementioned studies. The mean values of D_L and T_w were according to those suggested by Gasson et al. (1989), i.e. 25 measurements. Nevertheless, these results of sampling sufficiency were two or three times higher than suggested by the standard.

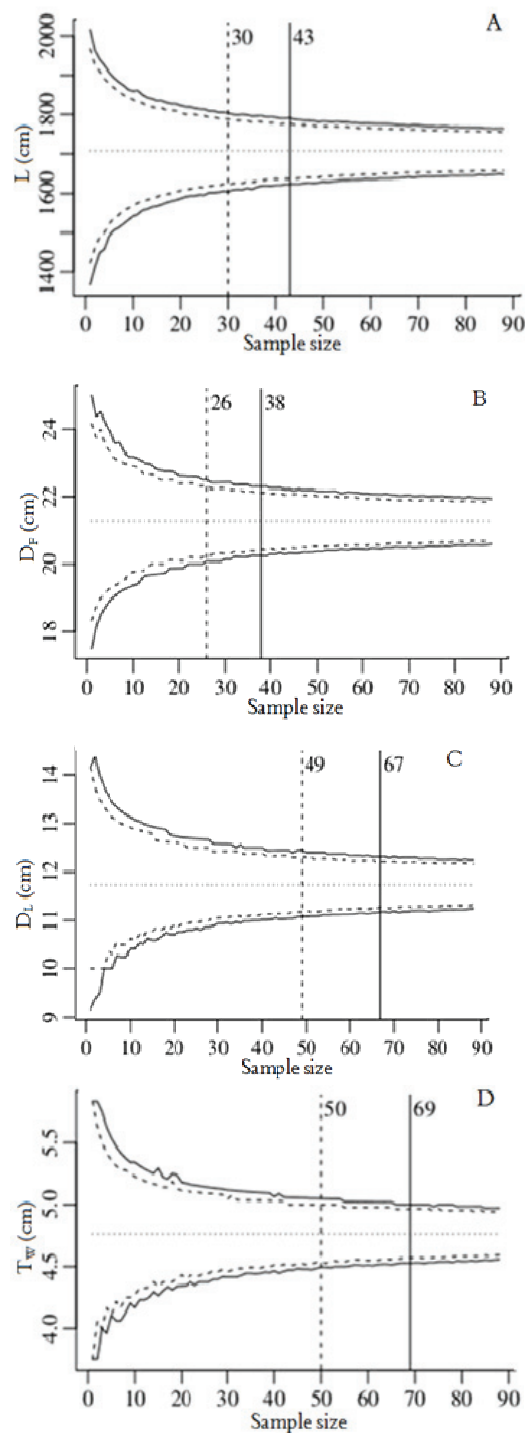


Figure 3. Bootstrap confidence intervals (IC) of 90% (dashed line) and of 95% (solid line) for means of the anatomical characteristics of *Luehea divaricata* wood considered for several sample size in the general scenario. The results are set based on $B = 10000$ resampling iterations at each sample size. The vertical lines indicate the sample sufficiency, considering error of 10% of the mean estimate. (a) Fibre length; (b) Fibre diameter; (c) Lumen diameter; (d) Thickness of the fibre wall.

The sampling sufficiency considering the segregation of juvenile and mature wood is shown in

Table 2. The sample sizes of juvenile wood were higher than those of mature wood. The highest difference was in L, in which the sampling sufficiency in juvenile wood was approximately three times higher than in mature wood. Moreover, only sampling sufficiency in L (mature wood) and D_F (both juvenile and mature wood) is according to that suggested by Malan and Hoon (1992) and Denardi and Marchiori (2005). Taking into account the 25 measurements suggested by the Gasson et al. (1989), only L (mature wood) at $\alpha = 0.05$ and $\alpha = 0.10$, and D_F at $\alpha = 0.10$ could provide the necessary sample sizes to perform inferences in parameters with adequate confidence level. The sampling of anatomical characteristics for species with a high proportion of juvenile wood could be carefully performed in order to avoid sampling error. Moreover, timber industries are decreasing the cutting age of trees due to market demands. Consequently, wood quality is low because the proportion of juvenile wood is high, during which wood properties are in formation.

The anatomical characteristics of fibres are not good to differentiate species, but are very important in wood technology due to their influence in paper quality. The individual characteristics of fibres have no correlation with the strength of paper. On the other hand, characteristics related to the facility of linkage between fibres and fibre stiffness, such as felting rate ($p = L/D_F$), flexibility coefficient (D_L/D_F), cell wall fraction ($R = 2 \cdot T_W/D_F$) and Runkel index ($R = 2 \cdot T_W/D_L$) significantly influence pulp quality and mechanical properties of paper (NISGOSKI et al., 2012).

Reducing the budget and the time to determine anatomical characteristics are directly related to proportions of juvenile wood. Increased ratio between juvenile and mature wood could be achieved through forest management (PEÑA; PERIS, 2006) and greater forest cycles that are alternatives to decrease the proportion of juvenile wood, as the first presents higher industrial quality (ADAMOPOULOS et al., 2007; KRETSCHMANN, 2008; DÜNISCH et al., 2010) and lower sampling sufficiency.

The difference between juvenile and mature wood alone could not satisfy the necessity of timber industries in order to characterise anatomical characteristics of fibres. Figure 4 shows accumulated sampling sufficiency as a function of the age of trees (for each growth ring). This property was determined considering the accumulation of growth rings until the ring (year) of interest.

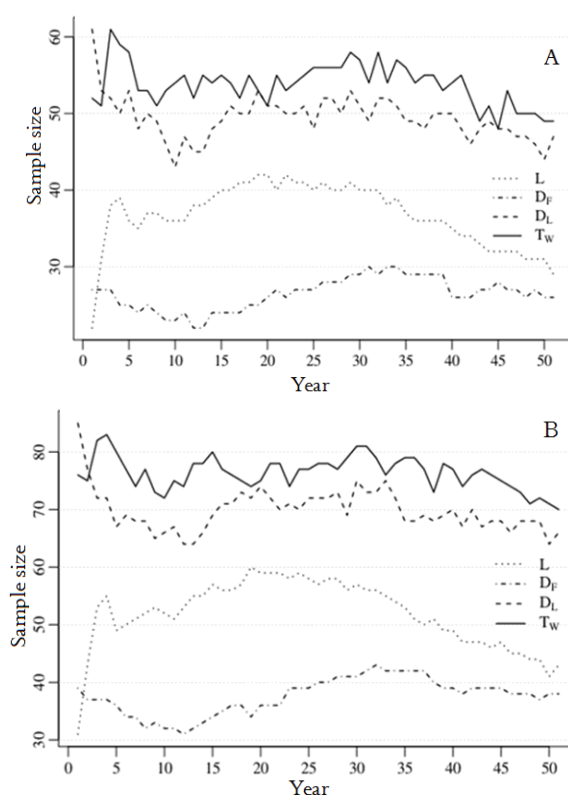


Figure 4. Accumulated sampling sufficiency of the fibre length (L), fibre diameter (D_F), lumen diameter (D_L) and thickness of the fibre wall (T_W) of the *Luehea divaricata* as a function of the age of tree for two levels of significance. (a) $\alpha = 0.10$; (b) $\alpha = 0.05$.

Determining the sampling sufficiency of juvenile and mature wood from a 5-year-old tree is not interesting, mainly because mature wood is present only after segregation. Therefore, accumulated sampling sufficiency in the fifth year includes only the growth rings until this step, i.e., from first to fifth ring. According Figure 4a, sampling sufficiency at $\alpha = 0.10$ for L in the fifth year was 35 samples, while for the juvenile wood it was 43 and for the mature wood it was 15 (Table 2), proving the importance of this method. If the aim is to use species with short forest cycles such as from *Eucalyptus* and *Pinus* genus, the accumulated sampling sufficiency could be important for each forest management at 7, 14 and 21 years considering the data of desirable age.

According to the results shown in this study, it is clear that further research about sampling sufficiency of anatomical characteristics of fibres should be performed, mainly with fast-growth species that have high proportions of juvenile wood. The results found in this study through the bootstrap computational method are different to the recommendations of the Committee of the International Association of Wood Anatomists (GASSON et al., 1989), mainly for T_W and D_L and except for L and D_L , the last at $\alpha = 0.10$.

Therefore, if sample sizes lower than those found in this study are used, the inferences on parameters of anatomical characteristics of wood do not have a desirable confidence level. In this context, sampling sufficiency obtained in this study is proposed to species with anatomical and technological characteristics similar to *Luehea divaricata*.

Conclusion

The sampling sufficiency of anatomical characteristics of fibres from *Luehea divaricata* was investigated through the bootstrap method considering several scenarios and different confidence levels. In the scenario of separated juvenile and mature wood, sampling sufficiency in the juvenile wood was higher than in the mature wood, specifically three times to L. The results found at 95% of confidence level, which is the standard in forestry research, were higher than those suggested by Gasson et al. (1989) and used by other researchers. This confirms the necessity of studies with each species and the differentiation between juvenile and mature wood.

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