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CASE STUDY: MICROFIBRIL ANGLE AND ITS RELATIONSHIP WITH BASIC DENSITY IN *PINUS TAEDA L.* WOOD FROM SILVOPASTORAL SYSTEMS

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ABSTRACT

The material resulting from fast-growing plantations is presumed to present a high percentage of juvenile wood, with high microfibril angle (MFA) and low basic density (BD), which would negatively affect the technological properties of wood. The objective of this study was to measure the MFA using the technique of the orientation of bordered and cross-field pit apertures of the tracheids walls, determine BD and verify the correlation between the two variables. The used material was *Pinus taeda* 15 years old from a stand with silvopastoral management. The pit apertures of the earlywood tracheids showed to be more rounded and abundant compared to those of the latewood. The MFA decreased from 56.2 to 42.1 degrees, and wood density increased from 0.31 to 0.42 g/cm^3 from the pith to the bark. The high values of MFA could be explained by the genetic material with low degree of breeding, and also by the wide spacing between trees - which favors the fast growth -, but could also be due to the employed methodology. The correlation between MFA and density in this study is moderate and negative (R= -0.59), indicating that density would not be a parameter sufficient to infer about the structural quality of the wood.

Keywords: Basic density; microfibril angle; *Pinus taeda*; pits; silvopastoral systems

INTRODUCTION

The NE region of Argentina is characterized by the cultivation of fast growing tree species to serve the different timber markets. Silvopastoral systems have thus increased their participation for clear wood, among others items. The raw material from these plantations is presumed to have a high percentage of juvenile wood with a high microfibril angle (MFA) and low basic density, which would negatively affect its technological properties (Brown and McWilliams, 1990).

The MFA has a significant impact on the mechanical wood properties, largely determining its strength (Walker and Butterfield, 1995) and longitudinal shrinkage (Donaldson, 1996), being, thus, a parameter indicating the quality of solid wood products (MacDonald and Hubert, 2002). Both the MFA and BD are the most important indicators of the wood quality for the forest and paper industries (Meylanand Probine, 1969; Donaldson, 1996 and Jordan *et al*., 2006).

The MFA of the cell wall is studied in softwoods mainly because of its effect on strength and longitudinal shrinkage, and in hardwoods because of its relationship with growth and shrinkage stresses (Donaldson, 2008).

The MFA is also used to select trees that produce wood of higher strength (Cave, 1968; Walker and Butterfield, 1995). The inclusion of this variable in breeding programs would replace the selection based on density (Donaldson, 1992; Walker and Butterfield, 1995; Donaldson, 1996).

The secondary wall of tracheids comprises 3 layers: S1, S2 and S3. The cellulose in the cell wall forms microfibrils that provide structural support. The microfibrils are in a helix arrangement and the angle they form with the cell wall is called microfibril angle (Treacy *et al*., 2000). Authors Josza and Middleton (1994) define the microfibrilar angle as the mean helical (spiral) angle that the fibrils of the S2 layer of the cell wall make with the longitudinal axis of the plant.

The orientation of bordered and cross-field pit apertures in the wall of tracheids often indicate the alignment of microfibrils and has been used by several authors to measure the MFA (Bailey and Vestal, 1937; Pillow *et al*., 1953; Cockrell, 1974; Senft and Bendtsen, 1985; Donaldson, 1991; Huang, 1995; Huang *et al*., 1997 and Wang *et al*., 2001).

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Despite its influence on the wood properties, there is limited research on the MFA because difficult to be measured. The objective of this study was to measure the MFA using the technique of the orientation of bordered and cross-field pit apertures in the tracheid wall, as well as to determine the basic density and verify the correlation between both variables.

MATERIALS AND METHODS

The species used in this study was *Pinus taeda*, 15 years old, from a stand of a silvopastoral system located at 26°58.81' and 55°13.44' belonging to the Tabay S.A. company. The soil type is Kandiudults. The climate type of the study area, according to the Köppen classification, is Cfa, macrothermal, constantly humid and subtropical (Rodriguez *et al.*, 2004). The mean rainfall for the period 1981-1990 was 1,947 mm in Posadas, the closest city to the study area. The mean annual temperature was 21.5°C (National Weather Service, 2008) for the same period.

The stand was established in 1999, with an initial planting density of 1666 stems/hectare. In 2000, the "giant jesuitic carpet grass" (*Axonopus catharinensis* (sp.nov.inéd.) was introduced.

The silvopastoral system consisted of a regime of intensive pruning and thinning, resulting, at age of 7, in an average diameter of the stand at the breast height (DBH) of 25.9 cm, a pruning height of 8 to 8.5 m, and 373 stems/ha after thinning to waste and commercial purposes. A permanent inventory system was established since 2007 to evaluate the evolution of the timber resource and its response to silvicultural management. **Figure 1a** shows the variation in number of stems per hectare and **Figure 1b** shows the DBH evolution. With the management system, at 15 years old the stand had a mean DBH of 43.9 cm and a final population of 150 stems/ha.

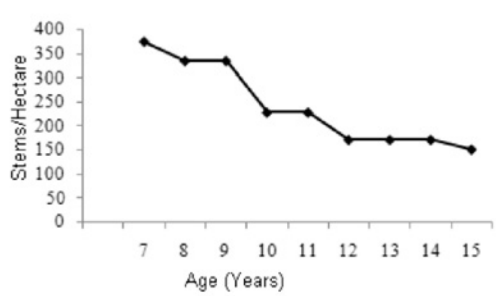

Figure 1a. Variation in number of stems/ha in the stand at age 7 to 15 years

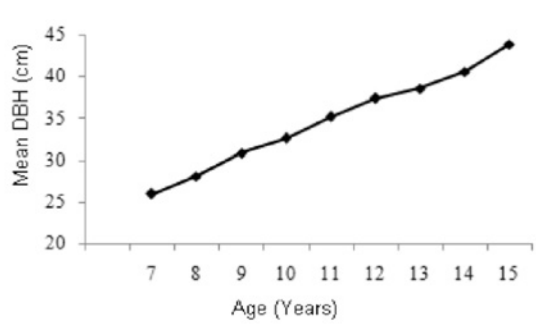

Figure 1b. Evolution of the mean tree diameter at breast height (DBH) of the stand at age 7 to 15 years

Table1. Data of the variables of felled trees

Tree #	DBH (cm)	tcbh (m)	TH (m)	Stratum
3	36.6	11.2	24.7	S
22	39.0	12.2	24.3	S
25	45.0	9.1	24.3	CD
20	47.0	8.8	23.5	CD
26	50.0	8.6	24.3	D
7	56.3	8.4	24.1	D

Where: DBH = tree diameter at breast height;
tcbh = tree crown base height; TH = total height;
S = suppressed; CD = codominant; D = dominant

Trees were classified in strata based on their phytosociological position as suppressed, codominant and dominant (Köhl *et al.*, 2006). A total of 6 trees were felled, 2 from each stratum. The trunk was cut in commercial length sizes. **Table 1** provides a description of the 6 trees used for this study.

Discs 2.5 cm thick were taken from the base of each tree (10 cm from the ground), and properly identified. A central strip was taken from each disc, the length of which was equal to its diameter in order to evaluate the microfibril angle and basic density.

Microfibril angle

Blocks of 2x2x2 cm were taken from the central strip of the three radial sections, from the pith to the bark, and were identified as: inner (A: block immediately adjacent to the pith); middle (B: approximately 50% of the radius) and outer (C: block adjacent to the bark). The blocks were boiled for 24 hours (until the material was softened), and then longitudinal tangential sections were cut, 5 mm thick, "stick" type. This material was treated in test tubes with Franklin maceration solution (1:1 glacial acetic acid and hydrogen peroxide 130 vol.), covered with an aluminum foil and taken to an oven at 60°C for 30 hours (the time was changed) to separate the cellular elements. After delignification it was washed with distilled water to remove all the solution. The material was placed on slides and stained with 1% safranin to promote the coloration of the tracheids, to easy the microscope observation. A small fraction of the colored tracheids was transferred with aid of a histological needle to another slide, half a drop of glycerin was added and it was covered with a cover slip. Five temporary sheets were produced for each of the radial positions, so as to ensure that at least 5 measurements of the MFA would be obtained per sheet, with a minimum of 25 measurements per sample.

Photographs of 30 tracheids with 400X magnification where taken with a trinocular microscope attached to a digital camera. They were later used to measure the MFA through the pits using the software UTHSCSA "Image Tool" for Windows, version 3.0 (Wilcox *et al.*, 2002), which had been previously adjusted. In each photograph of the tracheids, measurements were made of all pits, obtaining for each sample more than 90 MFA measurements, for a total of 1,410 measurements.

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Basic density

At the central strip, 2x2x2 cm test specimens (IRAM 9544, 1973) were marked from the pith to the bark (right and left), for a total of 131 samples. The sample volume was determined by means of the volumetric displacement method applying the Archimedes principle. The specimens were then taken to an oven at 103±2°C temperature until constant weight to estimate the basic density (BD), applying equation 1.

$$BD = \frac{P_A}{V_S} \qquad (1)$$

Where: BD = basic density (g/cm^3), P_A = anhydrous weight (g), V_S = saturated volume (cm^3).

For the purpose of statistical analysis, the specimens were grouped into three sections relative to the radial length. The inner section (A) was 0% to 33%, the middle section (B) was 33.1% to 67%, and the outer section (C) was 67.1% to 100% of the radius.

RESULTS AND DISCUSSION

The microphotographs of the tracheids (**Figures 2a** and **2b**) show that the pits in the tracheids of the inner section are more abundant and have a rounded shape, whereas the pits of the outer section are fewer and have an elongated shape.

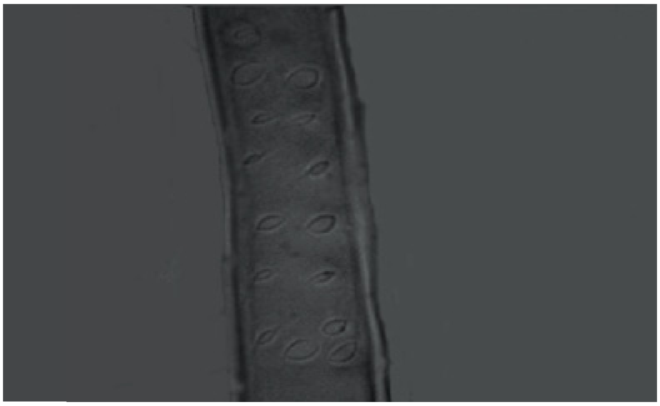

Figure 2a. Tree 22, section A - 400X magnification

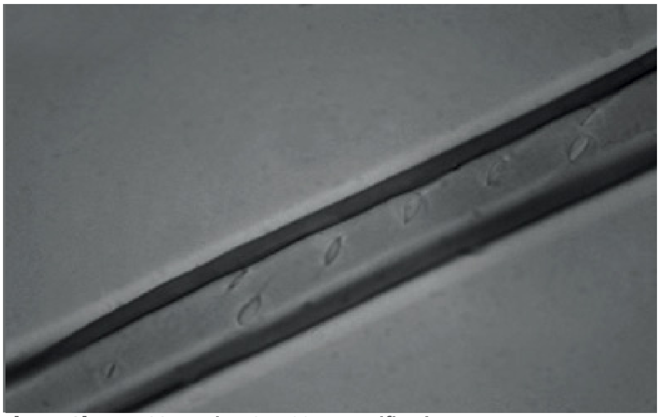

Figure 2b. Tree 22, section C - 400X magnification

Figure 3a. Tree 25, section A - 400X magnification

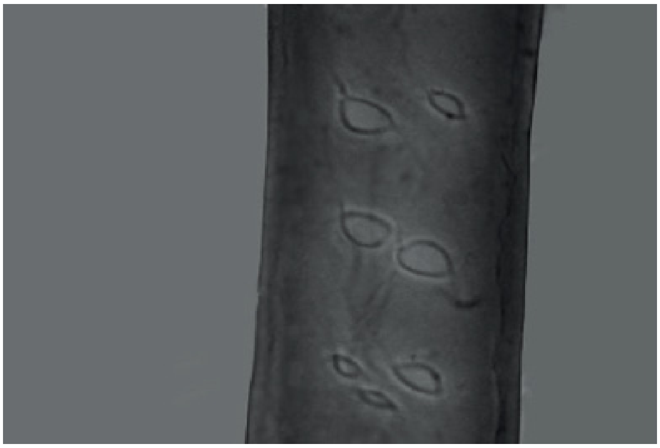

Figure 3b. Tree 25, section C - 400X magnification

A different behavior was observed in specific case of tree #25; the pits of the tracheids in the outer section were larger than the pits of the tracheids in the inner section. The fact that the tracheid samples were taken randomly - with no separation within the growth ring in earlywood and latewood - could be the reason of this behavior. **Figure 3a** shows a latewood tracheid in the inner section, and **Figure 3b** shows an earlywood tracheid in the outer section. However, this tree also had a higher MFA (**Figure 4a**) and

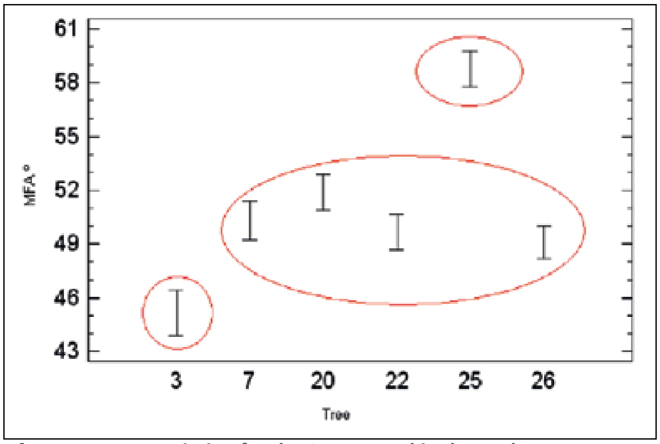

Figure 4a. MFA variation for the 6 trees used in the study

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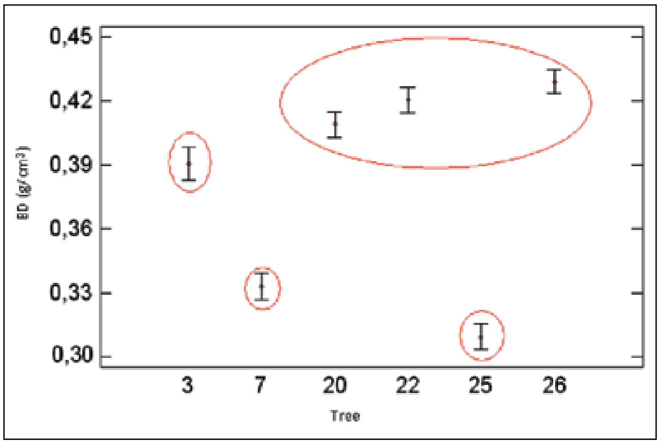

Figure 4b. BD variation for the 6 trees used in the study

a lower average BD (**Figure 4b**) with respect to the other trees.

The MFA decreases from the pith to the bark, with average values of 56.24°, 54.62° and 42.06° for the inner (A), middle (B) and outer (C) sections, respectively (**Figure 5a**), being the three sections statistically different at a confidence level of 95%. The radial decrease of the MFA value was also noted by other authors (Megraw, 1985; Herman *et al.*, 1999, Larson *et al.*, 2001; Jones *et al.*, 2005), and similar values for *Pinus taeda* were found by Huang *et al.*, 1997.

The results might indicate that the wood is only juvenile. Bendtsen (1978), Senft *et al.* (1985) and Ballarín (2003) when comparing juvenile to mature wood said that the microfibril angles of the S2 layer - as well as other features such as lumen diameter, lignin content, reaction wood and longitudinal shrinkage - are higher in juvenile wood. However, the tracheid length, cell wall thickness, cellulose content, density, strength and stiffness are higher in mature wood.

On the other hand, Jankowski (1979) considered that the higher the microfibril angle the greater the longitudinal shrinkage, which decreases from the pith to the bark, being this change more pronounced in the section of juvenile wood and gradual in mature wood. Vidaurre *et al.* (2011) argued that the longitudinal shrinkage

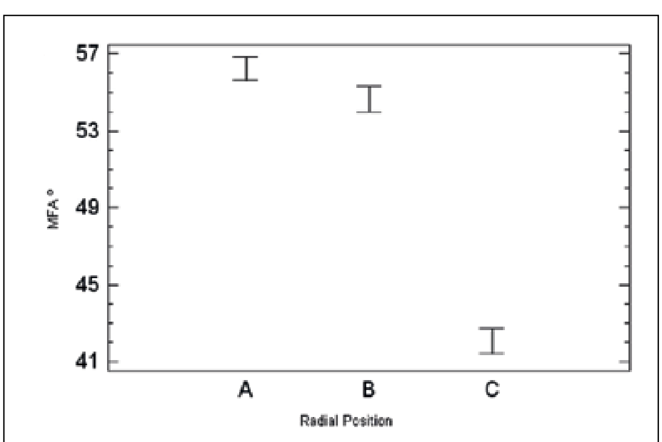

Figure 5a. Radial variation of mean microfibril angle (MFA) according to radial positions A, B and C

in juvenile wood may be 9% higher (or more) than in mature wood, which can affect the dimensional stability of products made with this type of wood, in addition to promoting the emergence of torsions, cracks and splits during and after sawing.

The orientation of microfibrils in the S2 layer of the cell wall of tracheids of juvenile wood varies widely within and between the different trees (Megraw, 1985). Although all 6 trees used in this study received the same silvicultural treatment, tree #3 has a lower average MFA (Figure 4a) and a relatively high mean density (Figure 4b) with respect to the other trees, whereas tree #25 has a higher average MFA (Figure 4a) and a lower mean density (Figure 4b). Figure 4a shows that the mean MFA values form 3 groups: tree #3 with the lowest MFA, tree #25 with the highest MFA and the 4 remaining trees falling between these values. We may thus suggest that tree #3 is the best specimen because of its lower MFA and good density, offering the strongest wood, greater structural stability, less longitudinal shrinkage, increased pulping performance and better quality fibers. In this sense, this tree would be the best candidate for genetic breeding. Tree #25 would provide wood of lower quality with respect to the others.

Although MFA values in all sections are very high, indicating that it is juvenile wood in all cases, the radial decrease of the MFA between the inner and the outer sections is remarkable (Figure 5a).

The stabilization of the MFA indicates the completion of the juvenile period and a significant improvement of wood properties from a technological point of view (Gorisek and Torelli, 1999). Bendtsen and Senft (1986) reported that the microfibril angles of *Pinus taeda* had not reached stable values yet at the age of 30 years.

Jones *et al.* (2005), for the purpose of characterizing the wood of *Pinus taeda* aged between 21 and 26 years from different sites and physiographic regions, determined the MFA by NIR (near infrared) spectroscopy, obtaining values of 10.98° minimum, 45.21° maximum and 26.64° mean. Later, Schimleck *et al.* (2007) increased the sample size and obtained for the same species MFA values of 9.6°, 51.0° and 26.3° minimum, maximum and mean, respectively. Furthermore, Donaldson (2008) mentioned that several authors agree that the average MFA of the S2 layer of mature softwood is between 5° and 20° to the fiber axis; however, he noted that juvenile wood has an average MFA between 25° and 35°. Values of 50° in rings closer to the pith were reported by Megraw (1985), Bendtsen and Senft (1986) particularly at the tree base, contributing to the low strength of base logs. Similar values were found by Jordan *et al.* (2006) and by Isik *et al.* (2008).

According to MacDonald and Hubert (2002), trees with large spacing among them tend to exhibit - among other characteristics - lower mean density and greater fibril angle, which results in lumber of inferior mechanical properties and less dimensional stability. The high MFA angles found in this study could be explained in part by the genetic material used, which had a low

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Table 2. **Correlation coefficients between microfibril angle (MFA) values determined with different techniques**

	Pits of the S2 layer of the tracheids' 2nd wall
Polarized light	0.83
Sludge technique (microtome slicing)	0.71
Sludge technique (maceration)	0.78
Ultrasound	0.79
X-ray diffraction	0.56

Source: Huang *et al.* (1997)

degree of improvement, and by the large spacing among trees, which promotes their rapid growth. On this basis, silvicultural practices that minimize the MFA value - together with an appropriate selection of genetic material - might be the best strategy to avoid the undesirable effects on wood caused by high MFA values, thus contributing to improved wood properties.

The high MFA values obtained in this study could also be due to the methodology used. Huang *et al*. (1997) mentioned that the use of the tracheid pits to estimate the MFA tends to overestimate the MFA of the tracheids of earlywood. Senft and Bendtsen (1985) considered that this methodology has the disadvantage that the pits cannot always be found in sufficient quantities or at specific sites to meet the researcher's needs. In some areas there may be a relatively high number of readily observable pits and in others they are found sporadically or may be absent. In some species, pits are simply not visible. In this study with *Pinus taeda*, there were abundant pits in some tracheids and fewer in others, allowing to infer that the former belong to earlywood and the latter to latewood.

This methodology, despite its limitations, is easy to apply, does not require a major investment and allows the evaluation of the microfibrils orientation of the S2 layer of the secondary wall of *Pinus taeda* wood. It is estimated that this technique could not be advisable to measure the MFA in hardwood species.

Huang *et al*. (1997) determined the MFA of *Pinus taeda* with different measurement techniques. **Table 2** shows the correlation coefficients between the MFA values obtained by different techniques.

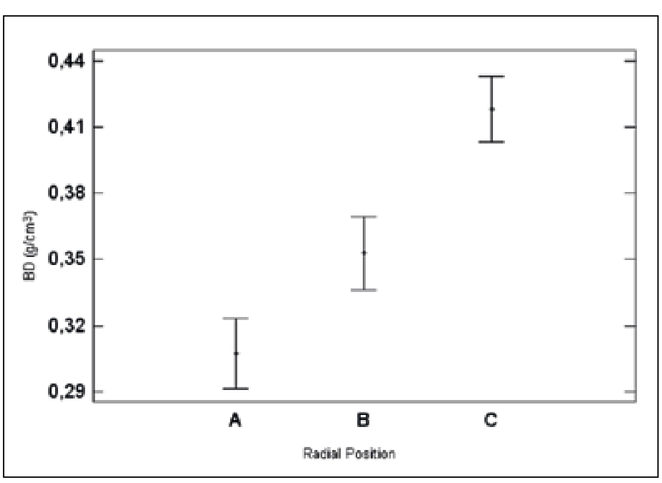

Figure 5b. Variation of mean basic density (BD) according to radial positions A, B, C

The basic density of wood varies depending on the tree (Figure 4b) and increases in radial direction (Figure 5b) with average values of 0.31, 0.35 and 0.42 g/cm^3 in sections A, B and C, respectively, with statistically significant differences between these values, for a confidence level of 95%. This pattern is comparable with those obtained in other studies (Megraw, 1985; Muñiz-Bolzon, 1993; Pereyra and Gelid, 2002; Weber, 2005; Von Wallis *et al*., 2007).

The BD can also be affected by the chemical composition of the cells, the cell wall thickness, the cell diameter and the proportions of earlywood and latewood. Moreover, the BD is directly related to the percentage of latewood and increases radially to the bark. This behavior occurs both in slow and fast-growing trees.

The correlation between the MFA and BD in this study is moderate and negative

($R = -0.59$, ρ-value = 0.0095), indicating that basic density would not be sufficient as a parameter to infer the structural quality of the wood, since 41% of the MFA variation is not explained by this variable.

Isik *et al*. (2008) argued that basic density acts as a variable predictor and explains 36% of the MFA variation. For high values of MFA and low values of BD in *Pinus taeda*, the relationship between both variables is weak, whereas it improves for BD values above 500 kg/m^3 (Schimleck *et. al*, 2007).

CONCLUSIONS

In the studied *Pinus taeda* trees, the microfibril angle (MFA) decreases from the pith to the bark and the basic density (BD) increases in the same direction.

The obtained MFA results can be worrisome because trees with high values of microfibril angles have high values of longitudinal shrinkage. This latter property promotes the emergence of defects, such as torsions, curvatures, splits and cracks before, during and after the mechanical processing of the wood, and it also hinders the drying process.

A moderate and negative relationship between the MFA and BD was found, so these properties - together with other features of wood (wall thickness, fiber length, proportions of earlywood and latewood, juvenile and mature wood) -, may be excellent indicators of the wood quality.

Since these studies are new for materials in our area, more tests should be conducted with *Pinus taeda* from other management systems, of improved genetic material and older ages.

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ACKNOWLEDGMENTS

We thank Eduardo De Coulon for providing his property for the installation of permanent plots; engineer Nahuel Pachas, who installed the plots that supplied to us the raw material; the students of the master and doctoral programs in Wood Technology at the Superior School of Agriculture Luiz de Queiroz: Mariana, Vinicios and Tasio for their help in laboratory tasks and specimen slides. ■

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