VARIATION OF MICROFIBRIL ANGLE OF *Pinus radiata* D. Don IN RELATION TO TREE SPACING IN CHILEAN PLANTATIONS[1]

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ABSTRACT – Four stands of 28-year-old radiata pine (*Pinus radiata* D. Don) grown in the eighth region (Biobio) of Chile were sampled to determine the effect of tree spacing on the microfibril angle. The samples were taken at two different stem levels of the tree, 2.5 m and 7.5 m, with increment strip taken in the Nothern direction. The four experimental stands were characterized by the following spacing 2x2, 2x3, 3x4 and 4x4. The microfibril angle was measured by X-ray diffraction with the SilviScan technology at the FP-Innovation-Paprican Division in Vancouver, Canada. The results showed a significant effect of tree spacing on the microfibril angle in both juvenile wood and mature wood as well as at the two stem levels considered. The minimum (9.42º) was reached in 2x2 stand at 7.5 m in mature wood, while maximum microfibril angle (24.54º) was obtained in 2x3 stand at 2.5 m in juvenile wood. Regarding the effect of tree spacing, 4x4 stand had the lowest microfibril angle,except in mature wood at 7.5 m where 4x4 had the highest microfibril angle (11°) of the four stands.

Keywords: Microfibril angle; *Pinus radiata*; Tree spacing.

VARIAÇÃO DO ÂNGULO DE MICROFIBRILAS DE ***Pinus radiata*** *D. Don EM RELAÇÃO AO ESPAÇAMENTO NAS PLANTAÇÕES CHILENAS*

*RESUMO – Quatro espaçamentos de pinheiro radiata (**Pinus radiata** D. Don) com 28-anos de idade, cultivado na região oitava (Biobio) do Chile, foram amostrados para determinar o efeito da árvore no espaçamento sobre o ângulo de microfibrilas. As amostras foram tomadas em dois níveis diferentes dos ramos da árvore, 2,5 m e 7,5 m, com incremento de* strip*, no sentido Norte. As quatro bancadas experimentais foram caracterizadas por seguir o espaçamento 2x2, 2x3, 3x4 e 4x4. O ângulo de microfibrilas foi medido por difração de raios X com a tecnologia SilviScan, na FP-Inovação-Paprican Divisão, em Vancouver, Canadá. Os resultados indicaram efeito significativo do espaçamento entre árvores no ângulo de microfibrilas em madeira juvenil e madeira madura, bem como nos dois níveis considerados tronco. O mínimo (9,42) foi atingido no espaçamento de 2x2 a 7,5 m na madeira adulta, enquanto o ângulo de microfibrilas máximo (24,54) foi obtido no espaço de 2x3 a 2,5 m na madeira juvenil. Quanto ao efeito de espaçamento, o espaçamento de 4x4 teve o menor ângulo de microfibrilas, salvo na madeira adulta de 7,5 m, em que 4x4 teve o maior ângulo de microfibrilas (11) dos quatro espaçamentos.*

Palavras-chave: Angulo de microfibrilas; ***Pinus radiata****; Espaçamento.*

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1. INTRODUCTION

Radiata pine (*Pinus radiata* D. Don), also called Monterey pine, is one of the most important commercial species in Chile along with Eucalyptus. Originally a native species from California, USA, this species was first introduced in Chile for ornamental purpose and, later, to reduce erosion (TORO; GESSEL, 1999). Radiata pine is grown in plantations and has become over the last decades so important that it could have an impact, in the long term, on other Chilean native forests (BUSTAMANTE; SIMONETTI, 2005). This species covers about 1,7 million hectares and is mainly grown under intensive forest management with treatments like soil preparation, fertilization, pruning, thinning in order to increase the yield per hectare (TORO; GESSEL, 1999).

The growing use of radiata pine in the pulp and paper industry or construction has been a driver of research on its wood characteristics, especially for stiffness. This last property has been shown to be dependent of the initial tree spacing in young trees (LASSERRE et al., 2004) which presented high stiffness in high density stands. Moreover, researches suggested that stand management operations such initial tree spacing and rotation age, had a direct impact on wood volume yield along with wood quality (KENNEDY, 1995; ZOBEL, 1981) and finally on the end use product.

The effect of stand density on tree characteristics as well as on wood characteristics has been of great interest. Wood volume (DREW; FLEWELLING, 1979) and internal properties of various coniferous species such ring width, wood density, and stiffness were shown to be affected by initial stand density (ZHANG et al., 2002; KANG et al., 2004). Tree growth rate was also reported to impact the microfiril in Norway spruce (HERMAN et al., 1999) with fast-grown trees having higher MFA. MFA along with other wood features is also shown to vary in radial and vertical direction (BURDON et al., s.d.).

Although it has been shown that stiffness can be predicted by both wood density and microfibril angle (EVANS; ILIC, 2001; YAMASHITA et al., 2000), more and more researches have shown that microfibril angle was the main predicting factor of stiffness (ALTEYRAC et al., 2006a; TSEHAYE et al., 1995; EVANS et al., 1996; BARNETT; BONHAM, 2004; FARBER et al., 2001). The microfibril angle is characterised by the angle of crystalin cellulose spiraling around the tracheids in the secondary cell wall (S2) with the longitudinal axis of the tracheids. It can be determined by several techniques including split extension of the pits apertures (COCKRELL, 1974), soft rot cavity (ANAGNOST et al., 2000; BRÄNDSTRÖM et al., 2002), polarized light microscopy (LENEY, 1981; MANWILLER, 1966; DONALDSON, 1991) X-ray diffractometry (CAVE, 1966; BOYD, 1977; EVANS et al., 1996) and infrared spectrometry (SCHIMLECK; EVANS, 2002). Older techniques generally consist in direct measurement but are also reported to be time-consuming, whereas X-ray diffractometry, developed in the sixties (MEYLAN, 1967), has been improving over the decades and is suitable for fast and simple sample preparation as well as for high speed measurements (EVANS, et al., 2003).

The present study objective was to estimate the impact of initial tree spacing in plantations of radiata pine on the microfibril angle measured with the technology of SilviScan considering the stem level and the type of wood.

2. MATERIAL AND METHODS

The radiata pine sample trees were collected in a trial plantation owned by Forestal Mininco and located in the region of Biobio, Chile, 10 km north of the city of Cabrero (36º58'S; 72º20' O, 100 m above see level). The region is characterized by dried condition in summer and high rain falls in winter, concentrated between May and August (annual rainfall of 1200 mm). The soil of this stand is sandy and consists of alluvial plains with low slope (2%). This is a particularity of this region where soils are moderately deep, rich in sand (above 93%), poor in clay and organic matter and have a high infiltration capacity (CARRASCO, et al., 1993).

The plantation is divided into four stands which are each characterized by their initial tree spacing, from 2x2 (2500 trees/ha), followed by 2x3 (1666 trees/ha), 3x4 (833 trees/ha) to the larger spacing 4x4 (625 trees/ha). All trees of the plantation were 28 years-old at the moment of cutting down and were coming from random genetical families. Beside, this trial plantation was grown without silvicultural intervention.

Nine trees, dominant or codominant, were randomly selected in each stand. Their diameter at breast height (DBH) was measured while standing, and their total height was measured once felt down to the ground.

They were cut down and trimmed with a chain saw into two 5m long logs at 5m and 10m height. A 5 cm thick disc was removed from the middle of each log in which a radial strip of 1.5x1.5 cm^2 section was removed in the northern direction. The samples were treated in alcohol 100% for 48h to prevent damage during the drying and conditioning, and once air dried they were packed and stored at 20 ºC and 65% HR until they were sent to the FP-Innovation Division Paprican laboratory.

Those samples were then processed again in the FP-Innovation laboratory according to the SilviScan protocol. A treatment with acetone was applied to remove all remaining extractives. The samples were then trimmed to 2 mm tangentially so that the X-ray beam penetrates the samples through the 2 mm thick radial/longitudinal plan.

Two samples were eliminated from the 2x3 stand because of presence of defects. The samples were scanned with a resolution of 5 mm. The average MFA was assigned to each ring from pith to bark.

The statistical analysis of data was carried out in SAS (SAS Institute) with a proc MIXED to analyse the effect of tree spacing on the microfibril angle at the two sampling heights and in the two types of wood, juvenile wood (JW) and mature wood (MW). Juvenile wood was defined as rings ranging from 2 to 8 (7 annual rings), and MW was defined as rings ranging from 11 to 17 (7 annual rings) (rings were counted from pith to bark). This selection was adopted to make sure that there was the same number of rings in each type of wood (equal sample size), and also to avoid any occurence of transition wood in the sampling. An estimate of the JW to MW transition occuring at ring number 10 is generaly admitted in Chile for this species (COWN, 1992). Finally the selection of few annual rings in each type of wood for determining the average MFA allowed to reduce the intra-tree variation of MFA due to the cambial age, especially in JW where high radial variation can be observed. Ring number was included in the model to take into account the radial variation of MFA.

The interaction analysis between the factors (stand, stem level and type of wood) were simplified by carrying out four separate analysis, regarding the type of wood and the stem level. This would also simplify the interpretation of the model.

Because the assumption of independance of error could not be verified (samples from the same tree are not independent) and because of the hierarchy in the sampling set, it was prefered to use a mixed model where plantation and annual ring were the fixed effects and tree was set as the random effect. The individual trees were considered the experimental unit for tree spacing factor analysis (Eq. 1), and annual rings were considered the experimental units for stem level factor analysis (Eq. 2). Equation (1) was run four times, respectively in JW/2.5m, JW/7.5m, MW/2.5m and MW/7.5m to test the effect of tree spacing on MFA. Equation (2) was run two times (in JW then in MW) to test the effect of stem level on MFA and the interaction of stem level factor with tree spacing factor.

The mixed models were written as following, in each stem level (2.5 m and 7.5 m) and in each type of wood (JW and MW).

$$Y_{ijk} = \mu + \gamma_k + Stand_i + ring_j\,(tree_k) + \varepsilon_{ijk} \qquad (Eq.\ 1)$$

$$Y_{ikl} = \mu + \gamma_k + SL_l + Stand_i + SL_l \times Stand_i + \varepsilon_{ikl} (Eq.\ 2)$$

Where:

- Y is the average MFA in the i^{th}(from 1 to 4) stand, j^{th} ring in the k^{th} individual tree ;
- $Stand_i$ is the deviation of average MFA in stand i to μ, $\Sigma\sigma_i=0$;
- $Ring_j$ is the deviation of average MFA in j^{th} ring to μ, $\Sigma\sigma_j=0$, nested in tree;
- SL_l is the deviation of average MFA in l^{th} stem level to μ, $\Sigma\sigma_l=0$;
- γ_k is the random effect due to the trees $\gamma_k \sim N(0,\sigma k^2)$; e
- μ represents the overall mean of MFA and $\varepsilon_{ijk} \sim N(0,\sigma_{ikl}{}^2)$ is the error.

Means comparisons were made by using contrasts. A total of 6 contrasts with the "contrast" statement of SAS were used to compare each stand, one by one, with the others. The four stands were categorized after it was verified with the mixed model that the fixed effect of tree spacing was significant. Each category was assigned a letter to point at stands which were statistically different from others.

3. RESULTS

As a preliminary result, the four stands were tested for their external characteristics which are given in

Figure 1. There was significant difference between the four stands for both DBH and tree height.

It was noticed that tree height and DBH had two different trends regarding the effect of tree spacing. While DBH increased linearly with increasing spacing, tree height presented an irregular trend marked by a low 3x4 average tree height. The DBH variable showed high variations between plantations ranging from 23.9 cm for 2x2 to 38.3 cm for 4x4 stand. Tree height varied from 25.7 m in 3x4 to 34.4 m in 4x4. It was observed for DBH variable on the stand density plot (Figure 1) a sudden change of slope from 833 t/ha to 625 t/ha which was higher than the slope between 2500 t/ha to 833 t/ha. The 4x4 stand appeared to have a higher growth rate than a normal trend would have suggested.

A summary of MFA variations among plantations, at the two stem levels and in the two types of wood is given in Figure 2. The statistical analysis showed a significant effect of tree spacing (fixed effect) on MFA at 1% level of significance in all four cases.

MFA ranged from a minimum of 9.42º (7.5 m, in MW and in 2x2 stand) to a maximum of 24.54º (2.5 m, in JW in 2x3 stand). It was observed a higher range of variation due to the type of wood than due to the stem level. Amplitude of variation of MFA from JW to MW was about 8º/11º while it was about 1º/4º between 2.5 m and 7.5 m.

*Means with the same letter are not significantly different at 5%.

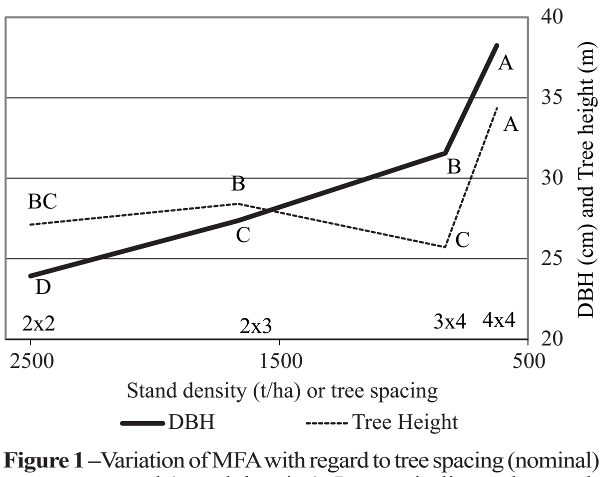

Figure 1 – Variation of MFA with regard to tree spacing (nominal) and (stand density). Letters indicate the result of Duncan test*.

Figura 1 – *Variação do MFA, que diz respeito ao espaçamento (nominal) e (densidade de suporte). As letras indicam o resultado do teste de Duncan.*

The 2x2 and 4x4 stands presented in JW a low MFA which usually characterizes a low growth rate and high stiffness.

3.1. Stem level effect

In JW, it was found that the average MFA at 2.5 m (22.38º) was significantly higher than the one at 7.5 m (18.46), whatever the stand since no significant interaction was observed between tree spacing and stem level factors.

In MW, it was found that the average MFA at 2.5 m (11.23º) was significantly higher than the one at 7.5 m (10.61º), but in that case an interaction was found between stand and stem level factors. Indeed, in contrast to the other cases, 4x4 stand presented higher MFA at 7.5 m than at 2.5 m.

3.2. Type of wood effect

Of all four cases, only MW/7.5m presented a regular trend of variation with regard to tree spacing. In other cases, stands 2x3 and 3x4 presented higher MFA while the two extremes (2x2 and 4x4) presented lower MFA. In two cases (JW/7.5m and MW/2.5m), MFA of 2x2 and 4x4 were not statistically different at 5% significance whereas in JW/2.5m MFA of 4x4 was statistically lower than those of 2x2. In MW/7.5m, the reverse was observed; MFA of 2x2 was statistically lower than those of 4x4.

4. DISCUSSION

The 4x4 low average MFA (Figure 2), in comparison to other stands, associated with higher growth rate (Figure 1), were found incoherent regarding some litterature (KENNEDY, 1995; LASSERRE, et al., 2004). Results were compared with other experiments (MOE, not published) carried out on the same sampling set which showed high stiffness for specimen removed from trees in the 4x4 stand. Some research has reported that the biological adaptation of trees to higher wind, especially when they are young, in widely spaced stands, was characterized by a lower stiffness (and so a large MFA) to bend easily (BARNETT; BONHAM, 2004).

The results on tree attributes showed an usual trend of DBH which increase matched the increase of tree spacing. In contrast the tree height variation presented an irregular trend especialy due to the 3x4 stand average tree height which was lower than expected. The 3x4 stand was expected to show an average tree height ranged between 28.4 m and 34.4 m to match the tree spacing ranging but was only 25.7 m. The 4x4 stand was expected, according to the slope, a lower value for both DBH and tree height. It was thought later that the 4x4 stand could have grown under specific conditions of growth that interfered with the main tree spacing factor studied. Indeed 4x4 stand is located the nearest to a river close to the plantation which could have help trees to get more water in summer when the climate is dry. Further investigations are necessary to explain such low MFA associated to high growth rate in the 4x4 stand. In JW it was observed that 4x4 presented the lowest MFA, whatever the stem level (no significant interaction found from Eq. 2 between tree spacing and stem level). It was also obtained from SilviScan data set the transition age from JW to MW, and the JW proportion based on the radial profile of MFA. It appeared a trend showing that 4x4 had an early transition age than others but was not statistically significant, and it had a lower JW proportion (Table 1). It was also observed an area of JW and MW at 2.5m that were respectively low and suddenly high. In the early stage of the plantation, the 4x4 trees may have experienced such conditions that slowed their growth and, later on, changing conditions may have stimulated them to grow faster. That would explain the low MFA observed in this stand.

* Means with the same letter are not significantly different at 5%.

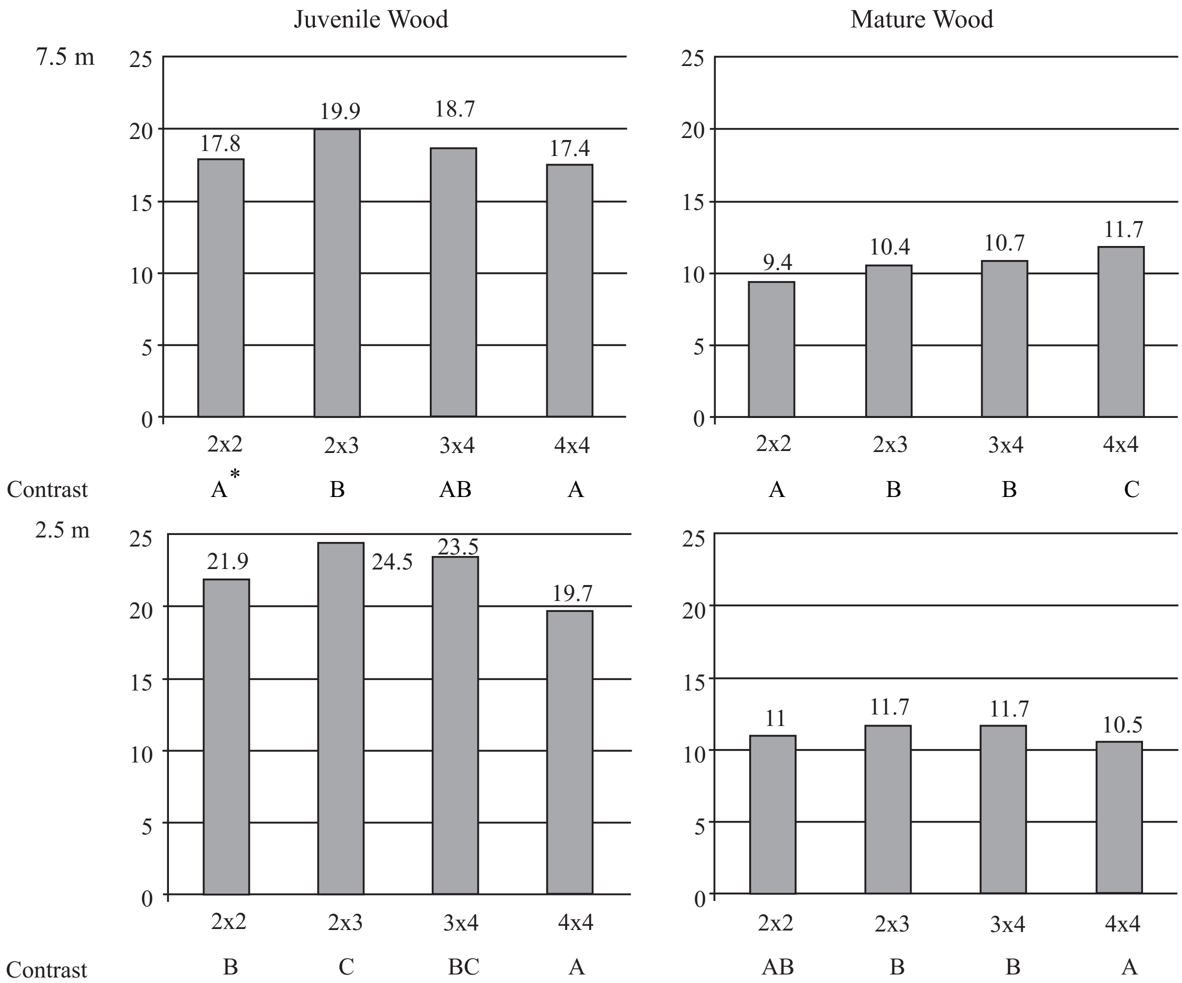

Figure 2– Effect of tree spacing on MFA in four cases. Comparison of four stands by contrasts.
Figura 2 *– Efeito de espaçamento da árvore sobre a AMF em quatro casos. Comparação de quatro carrinhos por* contrastes.

Table 1 – JW/MW area, proportion and transition age (Duncan test in brakets*), at 2.5 m.
Tabela 1 *– JW/MW área, proporção e idade de transição (teste de Duncan em brakets), com 2,5 m.*

Stand	JW age	JW area (cm²)	MW area (cm²)	JW proportion (%)
2x2	8.8 (AB)	162	159	50 (AB)
2x3	12 (A)	285	132	65 (A)
3x4	9.9 (AB)	225	260	44 (B)
4x4	7.6 (B)	285	500	34 (B)

* Means with the same letter are not significantly different at 5%.

In any case, the explaination is not clear yet, and results suggest a good yield for trees of 4x4 stand.

The MFA radial pattern presented similar variation from pith to bark than usually observed in other softwood species (ERICKSON; ARIMA, 1974; SAREN et al., 2004) with a trend characterized by a decrease of MFA from pith to bark with a dramatic slope in JW and a plateau in MW. Whatever the stem level or the stand, MFA in JW were always higher than in MW, logically due to this strong radial variation (BURDON et al., 2004; SAREN et al., 2004). So, MFA radial variation from pith to bark was consistent with usual trend observed in radiata pine (SCHIMLECK; EVANS, 2002) and in other softwood species such Norway spruce (SAREN, et al. 2004) or black spruce (ALTEYRAC, et al., 2006 (b)). The radial variation of MFA is used to determine juvenile wood mature wood transition (ALTEYRAC, et al., 2006 (b)) and is also an indicator of growth rate. In juvenile wood a high growth rate is characterised with high MFA while in mature wood a lower growth rate induces low MFA. MFA is in turn an important factor of determination of stiffness (EVANS; ILIC, 2001; DONALDSON, 1992) and shrinkage, and is reported by Burdon et al. (2004) to vary in both radial and vertical directions. As mentionned in the results, variation of MFA with height was characterized by a diminution of the angle from 2.5 m to 7.5 m which was consistent with already observed in radiata pine and other softwood species (BURDON, et al., 2004) showing that, upward the tree, wood properties are more of mature nature at any given annual ring from pith (ALTEYRAC, et al., 2005).

Consequently, not only trees of the 4x4 spacing were bigger than expected but they had a lower MFA which could confer to this stand a particular interest of high yield wood volume and high wood quality.

Trees of 2x3 and 3x4 stand presented statistically similar MFA, whatever the type of wood and the stem level. Their MFA was logically higher than those of 2x2 stand. The "normal" trend observed for the three first stands (2x2, 2x3 and 3x4) confirmed the inconsistency of the 4x4.

5. CONCLUSIONS

According to the results of this study, there was a significant effect of initial tree spacing on the microfibril angle of radiata pine grown in Chilean plantation. The spacings 2x2, 2x3, 3x4 and 4x4 presented statistically different average value of microfibril angle whatever the type of wood or the stem level. MFA at 7.5 m was lower than those at 2.5 m and MFA in JW was lower than those of MW. Trees in the 4x4 spacing were found relatively tall (tree height) and big (DBH) characterizing fast grown conditions, however it was shown that they had a relatively low MFA. In such growing conditions, it was expected a higher average MFA. This specificity was not explained and needed further investigation. However, untill further explaination, trees coming from the 4x4 stand were considered as a good silvicultural option allowing to produce more wood volume with lower MFA.

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