Radial variation in microfibril angle of *Acacia mangium*.

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Abstract— *Thirteen years old provenance trials of Acacia mangium from five provenances were established at five sites in the state of Sarawak, Malaysia, were sampled for this study. Fifty trees were sampled at random and cut to study radial variation in microfibril angle in the SS2 of secondary wall of the fibre using polarised microscope. Microfibril angle decreased from pith to bark with the greatest decrease occurred within the first two radial sampling near to the pith. It ranged from 5.9° to 28.8° with an overall mean and coefficient of variation of 12.6° and 45.8% respectively. It had a mean value of 21.4° at pith and 6.9° near the bark, which is a decrease of 67.8%. Highly significant different in mircrofibril angle were detected between radials of individual trees at α≥0.001. It was the major contributors to the total variance in which contributed for about 64.8%. Variations between trees were highly significant at α≥0.001 and accounted for 25.5% of the variation in microfibril angle while differences between the two orientations were not significant at α≤0.05.*

Keywords— Acacia mangium, microfibril angle, radial variation, pith to bark, interaction.

I. INTRODUCTION

In future, it is expected that Malaysian and international market will be flooded with wood produced by short rotation fast growing timber species. A major concern with short rotation is the present of higher proportion of juvenile wood known as core wood (Burdon *et al.* 2004). Juvenile wood displays poor characteristics like higher microfibril angle (MFA), lower density, low stiffness, thinner cell walls, and shorter tracheids than mature wood (Cown 1992). Lower densities and reduced fibre dimensions, higher MFA, and low stiffness of juvenile woods are expected to produce a poorer quality product, often causing dimensional instability, for example in loblolly pine (Kretschmann and Bendtsen 1992) and Sitka spruce (Macdonald and Hubert 2002), resulting in poor acceptance in the market (Cown and van Wyk 2004). For these reasons, wood property traits have begun to receive more attention in the tree improvement programs as well as in forest industry (Powell *et al.* 2004).

In addition to specific gravity the other most important wood characteristic, which has direct impact on wood stiffness and strength, is microfibril angle (Butterfield 1998; Bendtsen and Senft 1986; Cave 1969). It also has an influence on shrinkage of wood (Harris and Meylan 1965; Ying *et al.* 1994). Microfibril angle (MFA) is referred to the mean helical angle that the cellulose microfibril in the S_2 layer of the cell wall makes with the longitudinal axis of the cell (Barnett & Bonham 2004). Microfibril angle is a property of the cell wall of wood fibers, which is made up of millions of strands of cellulose called microfibril (Walker & Butterfield 1995 and Butterfield 1998).

Now in Malaysia as *Acacia mangium* is gaining popularity for both timber and for pulping, understanding the wood properties of this species is particularly important to effectively utilize this timber and before any improvement program has been developed to improve its quality. This study was therefore carried out to fulfil this objective. The work described in this paper forms part of a larger study of the genetic and environmental influences, and their interaction on growth, wood properties and mechanical properties in *Acacia mangium*. The main objective of this study is to establish a radial variation in MFA and to study the extent of variations in microfibril angle between trees, orientations and radial subsamples.

II. MATERIALS AND METHODS

2.1 The trial

Thirteen years old provenance trials of *Acacia mangium*, which were established in five sites in the state of Sarawak, Malaysia, were sampled for this study. Five provenances were planted. Details of the trials were reported in Lokmal & Mohd Noor (2010). The trial was conducted using randomised complete block design and was laid in complete factorial of five sites x five blocks x five provenances x 25 trees.

2.2 Tree sampling

Three trees were sampled randomly and cut from each treatment plot making a total of 375 sampling trees. However, due to some heart-rot and termites attack, only 362 trees were managed for final process. For the purpose of this study, 50 trees were sampled at random. The number was a compromise between high variability in MFA within tree and the difficulty of measuring the MFA.

2.3 Wood sampling

A two (2) cm thick disc was cut at 1.3 m height for every tree. A strip of two (2) cm width was cut running through the centre (pith) of the disc along east west orientation of the tree. The strip was cut at the centre into two parts i.e. east and west. Each part was measured and cut into four (4) equal-length samples, hence producing 400 samples (50 trees x 2 orientations x 4 radial positions).

2.4 Measurement of the microfibril angle

The method for measuring microfibril angle was adopted from Leney (1981). It involved several stages and were briefly explained below. All selected samples were separated and placed in a 40-ml beaker and 25 ml distilled water was poured over it. The sample was then boiled in the autoclave at 100°C for a total of 10 hours. Slices were cut from the tangential face of the samples using a sliding microtome with thickness set at 10 micron (about 50% of fibre thickness) in order to produce half-cut fiber required for the measurement of MFA through this method.

2.5 Maceration process

The slices were placed in 50 ml test tubes with 2 ml of maceration solution (a mixture of 44 parts of glacial acetic acid and 56 parts 30% hydrogen peroxide). The test tubes were heated in a water bath at 90°C - 95°C for 12 hours until the samples were bleached white and easily separated into components cells or fibres when shaken gently. The maceration solution was then poured off. The remaining maceration solution was diluted by adding 20 ml distilled water into the test tube, shaking and removing the mixed distilled water and solution using a 30 ml pipette. This step was repeated three times. A wide-mouth bulb pipette (8 mm inside diameter) was put in the suspension of fibres. The bulb of the pipette was then released quickly to suck a random sample of fibres into the pipette.

2.6 Preparation of the slides

The pipette was held vertically and moved to a position over a slide on a hot plate (80-90°C). The fibre suspension was squeezed out of the pipette on to the centre of the slide.The fibres were allowed to settle on the slide, and part of the water was allowed to evaporate on the hot plate. A cover glass was then put on the slide taking special care not to trap bubbles in between the fibres. Two slides were prepared from each sample to ensure enough microfiber are captured. A microfibril angle was measured once from each of the individual 29 half-cut fibres generating a total of 11,600 MFA for this study. The high sample number of fibres was needed because of the high variability of microfibril angle between fibres within a tree.

2.7 Polarised microscope procedures

With the polariser and analyser of a polarised light microscope set in the cross position (darkest), a first order red wave plate was introduced into the beam below the analyser at an angle of 45° to give a red field. The slide was then introduced into the field of view of the polarised light microscope. It was rotated clockwise to an angle where the colour changed from yellow to red to blue, indicating that it was in the major extinction position (MEP). The red plate was then removed and slight adjustment was made for the best major extinction position in black and white (when the central part of the fibre was the darkest). The angle of the rotary stage was recorded. Then the fibre axis was aligned parallel to the vertical cross hair line in the eyepiece. Again the angle of the rotary stage was recorded. The difference between these two readings was the microfibril angle for the secondary cell wall (S2).

2.8 Data analysis

SAS (SAS 2008) was employed throughout the analysis of this study. Two types of analysis were carried out in this study as described below.

2.9 Analysis of variance

Mean over 29 microfibrils were subjected to analysis of variance using Procedure general linear model (SAS 2008) using the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \delta_k + \eta_{ij} + \gamma_{ik} + \theta_{jk} + \varepsilon_{ijk}$$

Where

Y_{ijk} is the mean microfibril angle in *k*th radial position at *j*th orientation of *i*th tree.

μ is the overall mean.

α_i is the random effect of *i*th tree (*i*=1, 2 .... 50).

β_j is the fix effect of *j*th orientation (*j*=1,2).

δ_k is the fix effect of *k*th radial position (*k*=1,2,3,4)

η_{ij} is the interaction between *i*th tree and *j*th orientation.

γ_{ik} is the interaction between *i*th tree and *k*th radial position.

θ_{jk} is the interaction between *j*th orientation and *k*th radial position.

ε_{ijk} is the random error associated with the *k*th radial position in the *j*th orientation at the *i*th tree.

Student-Newman-Keuls multiple-range test was also performed to identify differences between samples within the radial.

2.10 Variance components

Variance components were estimated using proc varcomp via restricted maximum likelihood (REML) method (SAS 2008) with the same model as in the analysis of variance.

III. RESULTS

3.1 Radial Variation

Microfibril angle decreased from pith to bark. Its mean ranged from 5.9° to 28.8° with an overall mean and coefficient of variation of 12.6° and 45.8% respectively (Table 1). It had a mean value of 21.4° near pith and 6.9° near the bark, which involved a decrease of 67.6%, or a reduction of 14.5°. The most rapid changes occurred between the first two radial sampling i.e. SS1 and SS2 (Figure 1 and Table 1). It involved a reduction of 39.4% (reduction of 8.5°). The decrease in MFA from SS2 to SS3 and SS3 to SS4 were 28.7% and 25.0% involving a reduction of 3.7° and 2.3° respectively.Highly significant different in mircrofibril angle were detected between radials at $\alpha \geq 0.001$. Student-Newman-Keuls multiple range test have shown that all the four radials were differed significantly at $\alpha \geq 0.05$.

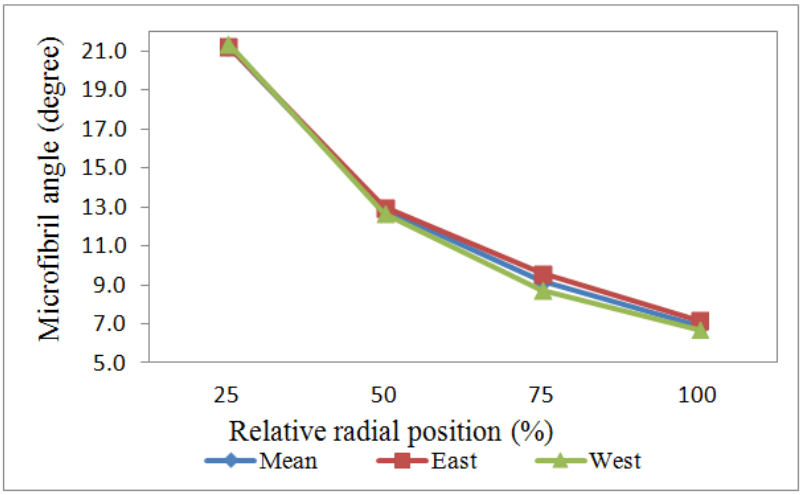

FIGURE 1: VARIATION IN MICROFIBRIL ANGLE FROM PITH TO BARK IN *ACACIA MANGIUM*.

TABLE 1
VARIATION IN MICROFIBRIL ANGLE FROM PITH TO BARK IN *ACACIA MANGIUM*.

Radial	Mean	Min	Max	CV (%)	Std error
SS1	21.4°[a]	16.6°	28.8°	15.1	0.32
SS2	12.9°[b]	11.5°	15.5°	7.2	0.09
SS3	9.2°[c]	7.6°	11.6°	10.4	0.10
SS4	6.9°[d]	5.9°	8.8°	9.10	0.06
Overall	12.6°	5.9°	28.8°	45.8	0.29

Notes: SS1 sample nearest to pith; SS4 sample closest to bark; Mean with the same letter are not significantly different at α≤0.05 via Student Newman Keuls.

3.2 Variation between trees

Mean of individual tree varied from 11.0° to 15.3° with an average and coefficient of variation of 12.6° and 9.7% respectively. Differences between trees were also highly significant at α≥0.001 (Table 2). Student-Newman-Keuls multiple-range test was performed and separated all trees in to 22 significantly distinct groups reinforced the high variation between trees. Tree accounted for 25.5% of the total variance in MFA.

3.3 Variation between orientations

Mean of MFA for east and west orientation 12.8° and 12.4° respectively. The differences between the two orientations were not significant at α≤0.05 (Table 2 and Figure 3).

TABLE 2
ANALYSIS OF VARIANCE AND VARIANCE COMPONENT FOR MICROFIBRIL ANGLE

Source	df	Mean square	Variance	Variance (%)
Tree (T)	49	12.1^{***}	11.0	25.5
Orientation (O)	1	16.8^{ns}	0.1	0.1
Radial (R)	3	4015.1^{***}	28.1	64.8
TXO	49	0.4^{ns}	0.1	0.1
TXR	147	3.6^{***}	2.6	6.0
OXR	3	4.2^{***}	1.1	2.5
error	147	0.4	0.4	0.9
Total	399		43.3	100.0

Notes: *, **, *** significant at p≥0.05, p≥0.01 and p≥0.001. ^{ns} not significant at p≤0.05

3.4 Interaction between Trees and Orientations

Interaction between trees and orientations was not significant (Table 2). Although there was highly significant different between trees, the different between both orientations for individual tree was very small (Figure 3).

3.5 Interaction between Trees and Radials

Interaction between trees and radials was highly significant (Table 2). Further examinations have shown that the interaction was due to different rate of reduction in microfibril angle within each radial of individual tree (Figure 2). This was very obvious especially within the first quarter from pith where tremendous change in MFA occurred.

3.6 Interaction between orientations and radials

Interaction between orientations and radials were highly significant (Table 2). Further examinations have shown that the interaction was due to different rate of reduction in microfibril angle within each radial of different orientations especially in the region of 50%-75% from pith.

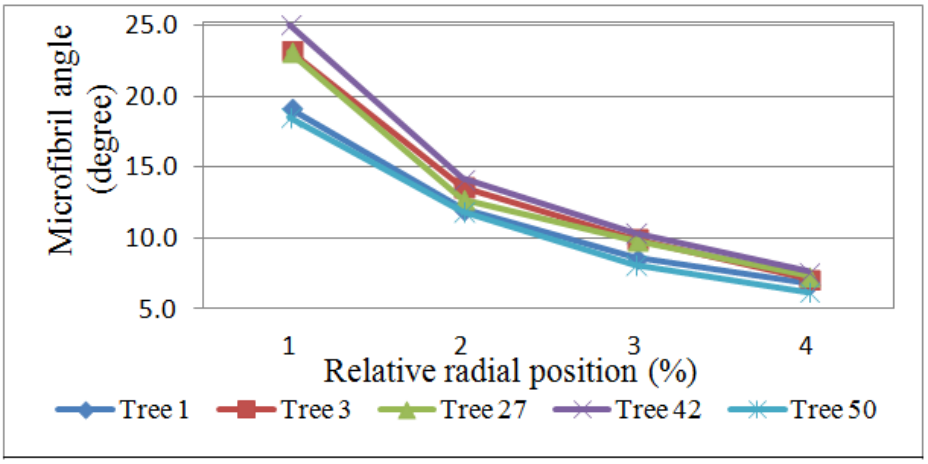

FIGURE 2: VARIATION IN MICROFIBRIL ANGLE IN FIVE SELECTED TREES FROM PITH TO BARK IN *ACACIA MANGIUM*.

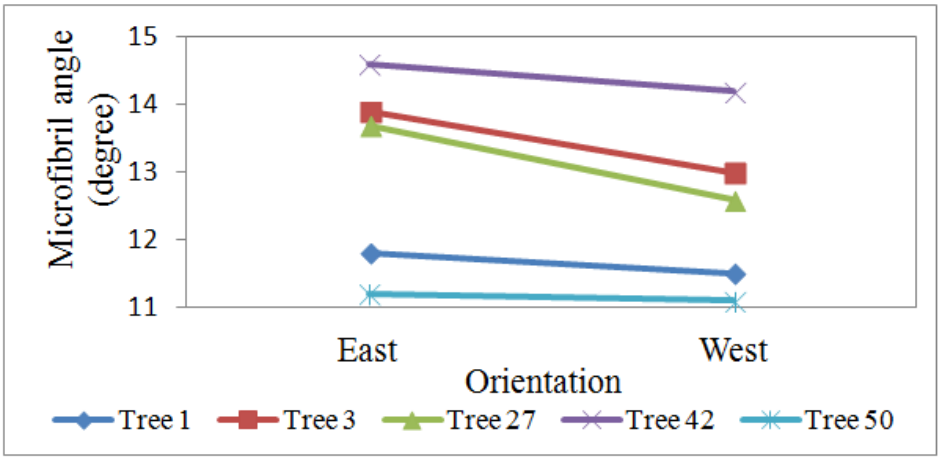

FIGURE 3: VARIATION IN MICROFIBRIL ANGLE IN FIVE SELECTED TREES IN EAST AND WEST ORIENTATIONS OF *ACACIA MANGIUM*.

IV. DISCUSSIONS

4.1 Radial Variation

A decrease in microfibrils angle from pith to bark in *Acacia mangium* is consistent with work on *Pinus radiata* by Baltunis *et al.* (2007), Donaldson & Burdon (1995), Donaldson (1997). This was also reported in other species such as loblolly pine (Megraw *et al.* 1998, and Myszewski *et al*. 2004) and in *Picea abies* (Lundgren 2004). A significantly higher value of MFA near pith was probably due to the present of juvenile wood in the region of core wood, which eventually led to the inconsistent and poor strength at the centre (Cown 1992). Bendtsen (1978), further revealed that juvenile wood displayed shorter tracheids, with thinner cell walls and consequently lower wood density, while in mature wood in which the MFA is smaller, the trachieds are longer with thicker walls and consequently higher wood density.

The most drastic reductions which occurred between SS1 and SS2 may suggest the presence of juvenile wood within this region; however, almost the same magnitude of reductions which extended between SS2 and SS3 may further suggest that the juvenile regions may presence up to SS3 or within 75% radially from pith. Microfibril angle in the S2 layer of the tracheid cell wall is the only known physical characteristic of wood that is capable of affecting large changes in the stiffness of wood (Meylan and Probine 1969). Independent studies have shown that decreasing in MFA have increased MOE and MOR from pith to bark in eastern cottonwood (Bendtsen and Senft 1986) and in quaking aspen (Roos *et al.* 1990).

Fibers with high MFA at the centre of the tree, which were produced when the tree was in the sapling stage, endow the wood with a low Young's modulus. This enables the sapling to bend during strong wind without breaking. As the tree grows, the stem has to become stiffer to support the increasing weight of the stem and crown. The lower MFA at the outer wood means

the tree has higher Young's modulus which enables them to fulfill the role. The other possibility is probably due to its colonising habit (Wiemann & Williamson, 1988), which combine rapid early growth in stature with the production of a weak stem due to high microfibril angle. As the tree grows, reducing microfibril angle in inevitable as to increase stem stiffness to maintain structural stability. These changes are probably associated with the ecological habit of pioneer species of wet tropical lowland forest. Differences in mircrofibril angle between radial was highly significant at $P \leq 0.001$. This is in contrast with the work by Lima *et al.* (2004) on 11 clones of hybrid between *Eucalyptus grandis* and *E. urophylla* who found no significant different in microfibril angle between radials.

The decreasing radial variation alone contributed for 64.1% of the total variation in MFA, implied that the wood strength in the region of core wood would be low and caused further problem during wood processing. This was consistence with the finding by Butterfield (1998) who concluded that MFA was the dominant wood characteristics underlying the poor wood quality in many fast-grown and short-rotation plantation softwoods. However this situation creates an opportunity in tree improvement by reducing the MFA in the central regions. This will result in improved wood quality at the central hence improve wood strength. Evans & Ilic (2001) found that MFA accounted for 96% of the variation in modulus of elasticity of *Eucalyptus delegatensis*. The scenario was unwelcome as in future most timbers reaching the markets were most likely will be coming from fast grown short rotation with high proportion of juvenile wood.

4.2 Variation between trees

Effect of trees was significant and contributed for 25.5% toward the total variation in MFA. Though the amount was only a third of radial variance, it was substantial to ensure a successful gain in tree selection. Very high MFA variation between radials within trees implicated that attempts to improve MFA must take this into account and must make sure that considerable variation between trees exists. Similar observation was reported in *Pinus radiata* (Donaldson & Burdon 1995). Differences among trees, a large part of observed variation was probably attributed to the differences in genetic makeup of the individual tree provenance which was not taking care during sampling and microclimate surrounding the individual tree. Evans *et al*. (2000) also found very large variation in microfibril angle between hardwood trees of red alder (*Alnus rubra*) and proposed for tree selection for steeper MFA in the central region. The same strategy was suggested by Donaldson & Burdon (1995) following their work in *Pinus radiata*.

4.3 Variation between orientations

There was no effect of orientation on MFA in this study. Although there was some variation in MFA between east and west orientation, it was very small and was not significant. The small variation observed in this study was probably an inherent. It may also due to the influence of strong wind blow which came from the east direction annually during heavy-rain monsoon season which also triggered formation of new fiber. To our knowledge, there was no work been done comparing the MFA between two opposite direction to compare with this study.

4.4 Interaction between trees and orientations

Interaction between trees and orientations was not significant and only accounted for 1% of the total variance in MFA. Since there was no significant different between orientations, the interaction between trees and orientations is probably and solely due to highly significant differences in microfibril angle between trees (Table 2). To our knowledge, there was no work been done comparing the MFA between trees and orientations to compare with this study.

4.5 Interaction between trees and radials

Interaction between trees and radials was highly significant (Table 2). Further examination of the data proved that the interaction was due to differences in rate of reductions between radials of different trees (Figure 2). To our knowledge, there was no work been done comparing the MFA between trees and radials to compare with this study.

4.6 Interaction between orientations and radials

Interaction between orientations and radials was significant (Table 2). Further examination of the data proved that the interaction was due to different rate of reductions in MFA between radials of both orientations (Figure 1). To our knowledge, there was no work been done comparing the MFA between orientations and radials to compare with this study?

V. CONCLUSIONS

All trees exhibited the same decreasing pattern of microfibril angle radially from pith to bark. The largest decrease occurred

in the first two sub samples from pith followed by a gradual decrease in the last two sub samples towards bark. Radial variation in microfibril angel is the most important factor affecting variation in MFA. It accounted for 61% of the total variation. Variation between trees is substantial and contributed for 22.3% of the total variation. Although this variation is only one third of the radial variation, it provides a basis for an improvement in MFA hence the wood strength in *Acacia mangium*.

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