FIBRE LENGTH IN RELATION TO THE DISTANCE FROM VESSELS AND CONTACT WITH RAYS IN ACACIA MANGIUM

Ridwan Yahya¹,²,*, Katsuhiko Koze² and Junji Sugiyama²

SUMMARY

Fibre length, as a function of radial or tangential distance from a vessel was estimated from serial cross sections. This new method is easier and faster than earlier methods which rely on photographic prints of transverse images for the analysis. When necessary virtual tangential and radial sections were produced from 3D data to enable fibre length estimation. Fibre length up to the 5th and 2nd fibres away from a vessel in radial and tangential directions, respectively, were significantly shorter than fibres at greater distance from the vessels. Fibre length strongly correlated with radial vessel distance \( r = 0.83 \) up to the 5th fibre and then leveled off. Vessel-adjacent fibres on the radial or tangential side of a vessel did not significantly differ in length. However, the rate of length increase differed significantly in the radial and tangential directions. Percentage of contact with rays varied independently of fibre length.

**Key words:** Alignment, serial images, fibres, rays, vessels.

INTRODUCTION

The demands for forest products as new biomass resources have intensified recently, and thus supply is unable to meet demand. Improved recycling and planting of fast-growing species are potential solutions to this problem (Kojima et al. 2009a). The total area of tree plantations in South Asia is now approaching 2 million ha, and the largest of these plantations (about 1.2 million ha) are located in Indonesia, where the major plantation species is fast-growing *Acacia mangium* Willd. (Yamashita et al. 2008). It is utilized primarily for pulp and secondarily for medium density fibreboard (MDF) or particleboard (Barry et al. 2004). Important attributes of this species include rapid early growth and tolerance of a range of soil types and pH (National Research Council 1983).

Vessel proportion of *A. mangium* is 12.1% (Yahya et al. 2010). The vessel proportion and the number of vessels per mm² tend to be negatively correlated with pulp yield and paper strength properties (Amidon 1980). Vessel picking is another problem in the processing of hardwood; it results from wide vessel elements being picked from the surface of the paper during the printing process and deposited on the printing surface. This causes ink-free spots on the printed page (Hudson et al. 1998).

1) Research Institute for Sustainable Humanosphere, Kyoto University, Gokasho, Uji, Kyoto 611-0011, Japan.
2) Faculty of Agriculture, University of Bengkulu, Kota Bengkulu 38371 A, Indonesia.
* Corresponding author [E-mail: ridwany@rish.kyoto-u.ac.jp].
A positive correlation exists between fibre length and burst strength (Casey 1952; Miyake 1968; El-Hosseiny & Anderson 1999; Ona et al. 2001), tensile strength (Casey 1952; Miyake 1968); tear strength (Casey 1952; Haygreen & Bowyer 1996) and folding endurance (Dinwoodie 1965; Ona et al. 2001). Holocellulose, α-cellulose and lignin contents of acacias can all be reliably predicted by fibre length (Yahya et al. 2010).

There is a possibility to decrease vessel size through tree improvement programmes. Vessel size has a high heritability ($H^2$); for instance, Bertolucci et al. (1992) found that vessel size had a $H^2 = 0.72$ in *Eucalyptus grandis*. This means that it should be possible to make special clones with desired small vessels.

The vessels influence fibre length by their transverse enlargement during differentiation, after derivation from fusiform initial cells (Larson 1994). As for the differences of the fibre length in the radial direction (RD), Honjo et al. (2006) found that wood
fibres up to three or more cells away are shorter, but that greater distances from a vessel hardly effected fibre length any further.

However, so far there is no information on the differences of fibre length as a function of distance from a vessel in the tangential direction (TD). In this study, therefore, we analyzed fibre length variation and vessel distance both in radial and tangential directions. According to Larson (1994) and Honjo et al. (2006), fibres adjacent to vessels should be shorter than those at greater distance.

In another approach, Zhang et al. (2003) studied contact and non-contact proportions of axial elements and rays of 3 hardwood species and found that a higher elongation rate of the wood fibre leads to a higher potential to develop new contact with the rays during elongation. Rays have an important role in the radial transport of photosynthates; they are the main routes for supplying the cambium cells and their derivatives with nutrients (Sauter & Kloth 1986; Van Bel 1990; Rennenberg et al. 1997). This in turn leads to the assumption that a higher percentage of contact with rays will lead to greater intrusive growth and longer fibres.

The question is then whether wood fibre length is more strongly affected by the distance from a vessel than by the percentage of contact with rays. If the distance from a vessel is most important, a breeding programme to decrease vessel proportion has greater perspectives, as it is not only increasing pulp yield but also would increase fibre length. This is important in A. mangium, because it has been reported that the high growth rate of A. mangium leads to shorter fibres (Kojima et al. 2009b).

The conventional maceration method cannot be used to investigate the relationship between fibre length and fibre position because the positional information is lost (Honjo et al. 2006). It is also difficult to investigate the relationships by conventional light microscopy because single sections include only parts of the cells. The relationships can only be obtained from a three-dimensional (3D) representation of the microstructure. The most economical method to obtain 3D information at the microscopic level is serial sectioning (Honjo et al. 2006).

When each section is cut, mounted and imaged separately, section images must be montaged and realigned to accurately analyze and visualize the 3D structure. Several methods have been developed to reconstruct serial cross sections and study the 3D architecture of larger structures such as vessels (Zimmermann & Tomlinson 1967; Kitin et al. 2004; Hugget & Tomplinson 2010), tracheids (Cichan & Taylor 1984), and fibres (Zhang et al. 2003; Honjo et al. 2006). Commonly the methods for 3D analysis of fibres are complex, tedious and time consuming. In the present study we introduce a faster technique to obtain 3D reconstructed data from serial optical micrographs as a way to investigate the above mentioned relationships.

**MATERIALS AND METHODS**

**3D reconstruction from serial cross section**

For this study a seven-years-old tree of *Acacia mangium* Willd. was randomly selected from a trial area of Musi Hutan Persada, a private forest plantation, South Sumatra, Indonesia. Wood blocks of 10 × 5 × 20 mm (R × T × L) were prepared from two portions near pith and bark, respectively.
The wood blocks were first softened by heating in a small autoclave with alcohol 50% : glycerol 50% at 160 °C for 15 min. Subsequently, 200 serial cross sections of 25 µm thickness were cut and mounted on glass slides. Each section was observed using a confocal laser scanning microscope (LSM 5 Pascal Ver. 2.5; Carl Zeiss, Heidelberg, Germany) and one optical slice in the middle of the section thickness was used for further analysis in order to avoid the distortion of structure caused by sectioning (Fig. 1). The image size was 2048 × 2048 pixels and the resolution was set to 0.450 µm/pixel.

The series of images were converted to black-and-white images and aligned using the free software “Reconstruct” (Reconstruct, March 2, 2010. http://synapses.clm.utexas.edu/tools/reconstruct/formats.stm). The aligned sets of images were scrutinized by image analysis software (Image J ver.1.42q Wayne Rasband, National Institute of Health, USA), using the function to handle sequential images and 3D viewers (Fig. 1).

**Fibre length, distance from vessels, and contact with rays**

Fibre length adjacent to and distant from vessels and their percentage contact with rays were observed using the serial cross sections. Observed fibre lengths were grouped in two directions from the vessel: the radial direction and the tangential direction. Since *Acacia mangium* has various types of paratracheal parenchyma we took great care in differentiating fibres from axial parenchyma strands: parenchyma strands have thinner walls and larger lumina than the fibres (Sahri et al. 1993). We always selected radial and tangential fibre series in contact with vessel-adjacent fibres and not separated from the vessel by paratracheal parenchyma.

The total fibre length was obtained by multiplying the section thickness (25 µm) by the number of cross sections and by image J software, respectively in which the focused wood fibres appeared. When necessary virtual tangential and radial sections were produced from 3D data to confirm the measurement (Fig. 1).

Contact of fibre and rays was calculated according to the following formula:

\[
\text{Contact of fibre and rays (%)} = \frac{\text{Number of sections that show contact between a fibre with rays}}{\text{the wood fibre length}} \times 100
\]

The relationships between fibre length and their distance to a vessel were calculated using simple regression analysis. A T-test was used to compare fibre length between vessel-adjacent and vessel-remote fibres in the radial and tangential direction. Analysis of variance was used to determine if significant differences were present in the fibre length among distance from vessels, percentage-contact with rays, and their interaction.

**RESULTS AND DISCUSSION**

**Alignment images and digital data sets**

We aligned serial images of the cross sections to expand the 2D image data to 3D. This is because 3D data have great potential for easy analysis of 3D wood structure. In one day, we successfully reconstructed 200 serial images using the free software “reconstruct”. This data set was used to estimate wood fibre length.
We found that estimation of the wood fibre length from the aligned images by imageJ software is easy and fast. It takes not more than 2 minutes, because finding tips of both ends of a fibre is done by only scrolling images on the computer (Fig. 2). Aligned images are useful and convenient to observe abundant fibres; even non-straight fibres could precisely be followed.

Previous studies on serial cross sections by Zhang et al. (2003), Kitin et al. (2004) and Honjo et al. (2006) did not attempt to align images from their serial sections. In the serial cross-sections method at most 10 cross sections per day can be prepared and photographed (Alkemper & Vorhees 2001). Since 50–150 cross sections are necessary to obtain representative information (DeHo et al. 1972) the preparation of one single sample can take weeks.

**Fibre length and distance from vessels in the radial and tangential direction**

Fibre length varied with the distance from a vessel. Figure 3 shows that in the radial direction, average fibre length increased from 610 µm in the vessel-adjacent fibre to 920 µm in the 6th fibre from the vessel and then was relatively constant up to the 10th fibre away from a vessel (1075 µm). The length of fibres up to the 5th fibre from a vessel in the RD was significantly shorter than that of more distant fibres (Table 1). The average length of the first five fibres near a vessel was 642 µm, and 40.3% shorter than that of the more distant fibres.

Fibre length strongly correlated with vessel distance up to the 5th fibre from a vessel ($r = 0.83$) (Fig. 3). The distance from fibres to vessels in the RD contributed 70%

| Table 1. Comparison of fibre length based on distance and direction to a vessel. |
|---------------------------------------|---------------------------------------|
| Variables                             | Fibre length (µm)                     |
| 1. Fibre distance in RD               |                                      |
| 5 fibres to vessel                    | 642.0                                 |
| 6–10 fibres from vessel               | 1075.0**                              |
| 2. Fibre distance in TD               |                                      |
| 2 fibres to vessel                    | 656.0**                               |
| 3–10 fibres from vessel               | 1004.0                                |
| 3. Vessel-adjacent fibres             |                                      |
| RD                                    | 642.0                                 |
| TD                                    | 656.0                                 |
| 4. Direction of distance to vessels   |                                      |
| Fibres distant (>5 cells) from vessel in RD | 1075.0                          |
| Fibres distant (>2 cells) from vessel in TD | 1004.0*                      |

** = significantly different at the 0.01 level; * = at the 0.05 level.
RD = radial direction; TD = tangential direction.
of variation in fibre length. We assume that about five fibres in RD are formed by the same fusiform initial or xylem mother cell in the cambial zone, and we hypothesize that vessel maturation affects the length of those fibres. Fibre dimensions are determined in part by the processes that occur during differentiation (Bailey 1920; Rao & Dave 1981; Ridoutt & Sands 1993). Honjo et al. (2006) emphasized that the final dimensions of wood fibres are also affected by the state of maturation of other xylem elements in the vicinity.

Fibre length also varied as a function of the distance to vessels in tangential direction (TD) as shown in Figure 4. Average fibre length increased from 610 µm in fibres directly adjoining vessels to 1070 µm, three fibres removed from the vessel. Average fibre length is then relatively constant up to 10 fibres from the vessel (970 µm). Length of the first two fibres near a vessel were significantly shorter than more distant fibres (Table 1).
The length of vessel-adjacent fibres in RD were not significantly different from those in TD; however, the length of fibres distant from a vessel in the RD differed significantly from those in TD (Table 1) and their increase with distance from a vessel showed different patterns (Fig. 3 & 4). Fibres in radial and tangential rows are derived from different fusiform initials, and fibre length is partly determined by the length of the fusiform initials from which they are derived (Bailey 1920; Rao & Dave 1981; Ridoutt & Sands 1993). This may explain the different patterns observed in radial and tangential directions away from the vessel.

**Length of fibre contacts with rays**

Figure 5 shows the procedure to measure contact (a, c and e) and non-contact zones of fibres with rays. ANOVA did not detect differences for fibre length in different percentage of fibres with rays (Table 2).

Figure 5. Fibre contact (a, c & e) and non-contact (b, d & f) with rays from a data set of serial cross sections.
We used also ANOVA to analyze the effect of the combination or interaction between distance from vessel and percentage of contact with rays to fibre length. Interactions between sources of variation were also not statistically significant (Table 2).

Turgeon and Beebe (1991) introduced the symplastic pathway via plasmodesmata for transportation of photosynthate from phloem to differentiating xylem. In symplastic transport, contact axial cells obtain their nutrients directly from the rays, whereas the non-contact axial cells obtain their nutrients via the pit pairs between axial elements. Zhang et al. (2005) found that the distance of fibres from the rays does not affect the transport of photosynthate; therefore, even the wood fibres far away from the rays are supplied with nearly the same amount of photosynthetic products, which are the source of raw materials for cell wall biosynthesis, and intrusive fibre elongation. These physiological considerations are in overall agreement with our result that there is no effect of the percentage of contact with rays on wood fibre length.

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**REFERENCES**


