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RADIAL VARIATION IN CELL MORPHOLOGY OF *Melia azedarach* PLANTED IN NORTHERN VIETNAM

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ABSTRACT

The radial variation in cell morphology of ten-year-old *Melia azedarach* trees planted in northern Vietnam was experimentally investigated. The earlywood fiber lumen diameter and latewood fiber lumen diameter were almost unchanged from pith to 6th ring before significantly decreasing and remaining constant from 7th ring outwards. In contrast, fiber cell wall thickness in both earlywood and latewood increased from pith to 7th ring before becoming stable towards the bark. The maturation age of earlywood vessel lumen diameter estimated by segmented regression analysis indicated that wood of the *Melia azedarach* could be classified into core wood and outer wood, and the boundary between core and outer wood may be located at 7th ring from pith. This should be taken into account in wood processing using *M. azedarach* grown in northern Vietnam.

Keywords: Cell wall thickness, core wood, outer wood, specific gravity, vessel lumen diameter.

INTRODUCTION

Melia azedarach belongs to the *Meliaceae* family which produces many well-known timber trees such as *Swietenia macrophylla* King and *Cedrela odorata* L. in South America and Africa; and *Entandrophragma utile* (Dawe & Sprague) Sprague and *Entandrophragma cylindricum* Harms in tropical Africa. *M. azedarach* is native to northern Australia and Himalaya region of Asia, and is now naturalized in most subtropical and tropical regions of the world (Venson *et al*. 2008; Duong *et al*. 2017). Its wood has been used for manufacturing agricultural implements, furniture, plywood, boxes, poles, tool handles, and lightweight construction materials (Harrison *et al*. 2003; El-Juhany 2011). Currently, decreasing wood resources from native forests and the increase in wood processing costs have led to significant interest in wood sourced from plantations. Owing to the value of wood from other members of the *Meliaceae*, *M. azedarach* has recently received considerable attention given its relatively fine grain, durability, resistance to termites and insects, and ease of working (Duong 2018). In addition, with other fast-growing species, *M. azedarach* could contribute to the prevention of global warming owing to the ability to rapidly store carbon (Osei *et al*. 2018). *M. azedarach*, has become an important plantation species in Vietnam; however, further research is needed for effective utilization of wood from this species, such as the production of structural lumber.

There are some reports on *M. azedarach* wood properties with general agreement that wood of *M. azedarach* has a medium specific gravity (SG) (El-Juhany 2011, Trianoski *et al*. 2011, Duong *et al*. 2017) and medium dimensional stability (Venson *et al*. 2008, Duong and Matsumura 2018a). Mechanical properties of *M. azedarach* wood were also reported by some researchers (Matsumura *et al*. 2006, Venson *et al*. 2008, Duong and Matsumura 2018b, Duong *et al*. 2019) who suggested the possibility of using wood of *M. azedarach* as a new timber source. Within-tree variation of *M. azedarach* physical (Duong and Matsumura 2018a) and mechanical wood properties (Duong and Matsumura 2018b) have also been examined, and it was observed that wood beyond ring 7 from the pith displayed mature wood properties, i.e. comparatively long fibers, high specific gravity, and low microfibril angle (MFA) in the S_2 layer of the cell wall (Duong *et al*. 2017).

There are few published studies on the wood anatomy of *M. azedarach* (Lev-Yadun and Aloni 1993, Duong *et al*. 2017) and information on wood variability related to anatomical patterns of variation, which may have a large influence on processing and product performance (Walker 2006), is lacking. Further, no information regarding wood anatomical variation in relation to juvenile and mature wood or identification of when the transition occurs is available. Hence to better understand anatomical characteristics of *M. azedarach,* this study examined radial variation in cell morphology for plantation grown trees from northern Vietnam. Based on the results obtained, the process of xylem maturation, and the relationship between anatomical characteristics and wood properties are discussed.

MATERIALS AND METHODS

Sample preparation

Ten-year-old *Melia azedarach* L. trees were sampled from a state-owned plantation in Thai Binh province, Vietnam (20°38′33″N, 106°12′16″E). As seedlings, the trees were planted at a spacing of 4 m × 3 m. Three trees were chosen for destructive sampling based on straightness, normal branching, and absence of any disease or pest symptoms (Table 1). The north and south sides of the sample trees were marked before felling. A cross-sectional disc 30 mm thick was cut from each sample tree at a height 1,3 m above the ground. From each disc, pith-to-bark strips

[Radius × 10 (Tangential) × 10 (Longitudinal) mm] and [Radius × 30 (T) × 15 (L) mm] were cut from the south side to examine cell morphology and wood SG, respectively. All strips were conditioned (temperature 20 ºC and relative humidity 60 %) to a constant weight before commencing experiments.

Table 1: Diameter and height of the sampled trees.

Tree no.	DBH (cm)	H (m)
1	30,0	15,6
2	25,2	14,5
3	22,8	16,3

Note: DBH, diameter at breast height (at 1,3 m above the ground); H, tree height.

Cell morphology

Radial variation of cell morphology was investigated by the method described by Ishiguri *et al*. (2012). Transverse sections (20 µm in thickness) were obtained from each ring with a sliding microtome. The sections were stained with safranin and then dehydrated and mounted in biolite (Wako Pure Chemical Industries, Ltd.). Digital images of transverse sections were taken using a digital camera (CAMEDIA C5050ZOOM, Olympus) attached to an optical microscope and analyzed with ImageJ software (2012). For each ring earlywood vessel lumen diameter (EVLD), earlywood fiber lumen diameter (EFLD), and earlywood fiber cell wall thickness (ECWT) were measured on the first 5 rows of earlywood cells, while latewood vessel lumen diameter (LVLD), latewood fiber lumen diameter (LFLD), and latewood fiber cell wall thickness (LCWT) were measured on the outermost latewood cells. Average tangential and radial lumen diameters were determined by measuring 30 vessels and fibers in each ring. Double wall thickness of 30 fiber cells was measured, and one half of the double wall thickness was defined as the fiber wall thickness in each ring. Average tangential and radial fiber diameter was determined by summing average lumen diameter and wall thickness (× 2).

Specific Gravity

Specific gravity (SG) was measured as described by Duong *et al*. (2017). Due to distinct growth rings, radial strips were then cut into individual rings for measurement of SG in air-dry condition. SG was measured by an electronic densimeter MD-300S. Measurement time per sample was about 10 seconds. All experiments in this study were conducted at Kyushu University Wood Science Laboratory, Japan.

Maturation age estimation

We observed that the changes in EVLD with increasing cambial age followed a nonlinear pattern with an upper asymptote. Thus, a segmented regression model with quadratic equation and a plateau was adopted to describe this relationship (Tsuchiya and Furukawa 2009b). The model was fitted using the function *nls* in R version 3.3.2 (R Core Team 2016). The maturation age (M) and a plateau (P) of EVLD were then calculated from coefficient estimates of the quadratic segment in the model as follows (Eq. 1, 2):

$$M = \frac{-\beta_1}{2\beta_2} \qquad (1)$$

$$P = \beta_0 - \frac{{\beta_1}^2}{4\beta_2} \qquad (2)$$

In which, β_0 is the intercept and β_1 and β_2 are the first and the second coefficient estimates for the quadratic segment of the model.

RESULTS AND DICUSSION

Radial variation in cell morphology

Descriptive statistics (means and standard deviations) in cell morphology and SG of the sampled *M. azedarach* trees planted in northern Vietnam are shown in Table 2. Average EVLD was 137,79 μm varying between trees from 133,67 μm to 144,96 μm; and average LVLD was 90,37 μm varying from 82,83 μm to 94,52 μm. Lumen diameter of earlywood fibers averaged 7,15 μm (range 6,93 μm to 7,35 μm) and 4,38 μm (range 4,15 μm to 4,73 μm) in latewood, while fiber wall thickness varied from 1,04 μm to 1,08 μm in earlywood and from 1,74 μm to 1,79 μm in latewood (Table 2). Palakit *et al*. (2018) reported vessel diameters for *M. azedarach* grown in northeastern Thailand that ranged from 120 μm to 210 μm but to the best of our knowledge, there have been no previous reports of lumen diameters of fibers, and fiber wall thickness. Thus, the present study experimentally documents these properties of *M. azedarach* for the first time. Anoop *et al*. (2014) reported the anatomical properties of *S. macrophylla,* and showed that the values of vessel diameter in earlywood, fiber lumen diameter and fiber wall thickness were 167,6 μm, 12,8 μm, and 1,9 μm, respectively, which are similar to what we report for *M. azedarach*.

The overall value of wood SG was 0,52, varying between trees from 0,51 to 0,54, and this finding is in agreement with our previous work (Duong *et al*. 2017), in which we showed that wood SG values of 17 to 19-year-old *M. azedarach* planted in northern Vietnam ranged from 0,52 to 0,57 between trees. Other studies have shown that *M. azedarach* SG varies considerably. For example, for 17-year-old *M. azedarach* grown in Japan, Matsumura *et al*. (2006) found that SG ranged from 0,43 to 0,52, while Bolza and Kloot (1963) report a density of 445 kg/m^3 for *M. azedarach* var *australasica* (age was not specified for the sampled trees). Later studies by Nasser (2008) and Nasser *et al*. (2010) examined wood properties of 9-year-old *M. azedarach* grown in Egypt and Saudi Arabia respectively. SG's of trees irrigated with sewage effluent in Egypt (0,60) and primary treated sewage-effluent in Saudi Arabia (0.65) were higher than those of trees irrigated with municipal water (0,55 and 0,59 respectively). The higher SG's reported by Nasser (2008) and

Nasser *et al*. (2010) can likely be explained by the use of irrigation in these studies which reduced water stress permitting an extended period of latewood production.

Table 2: Characteristics of cell morphology and wood property in the sample *Melia azedarach* trees.

Property	**n**	**Tree 1**		**Tree 2**		**Tree 3**		**Total**	
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Cell morphology									
Earlywood vessel lumen diameter (µm)	10	133,67	31,81	134,72	34,61	144,96	36,70	137,79	33,41
Latewood vessel lumen diameter (µm)	10	92,54	17,73	82,83	14,42	94,52	20,00	90,37	18,25
Earlywood fiber lumen diameter (µm)	10	7,17	1,34	7,35	1,21	6,93	1,54	7,15	1,38
Latewood fiber lumen diameter (µm)	10	4,73	1,14	4,26	0,86	4,15	0,81	4,38	0,95
Earlywood fiber cell wall thickness (µm)	10	1,07	0,20	1,04	0,21	1,08	0,22	1,06	0,21
Latewood fiber cell wall thickness (µm)	10	1,74	0,29	1,79	0,31	1,74	0,39	1,76	0,34
Wood property									
Specific gravity	10	0,51	0,06	0,54	0,06	0,52	0,06	0,52	0,06

Note: n, number of rings; SD, standard deviation.

Images showing variation in vessels and fibers for rings of different ages are shown in Figures 1 and 2 respectively, while radial patterns of variation in cell morphology from pith to bark in earlywood and latewood of *M. azedarach* planted in northern Vietnam are shown in Figure 3. Vessel lumen diameter of *M. azedarach* trees tended to rapidly increase up to 7th ring from the pith before becoming constant towards the bark both in earlywood and latewood (Fig. 3a-b). The EFLD and LFLD, on the contrary, showed increasing and decreasing trends respectively from pith to 6th ring before significantly decreasing and then remaining constant from 7th ring outwards (Fig. 3c-d). The radial pattern in ECWT and LCWT was similar to that of the vessel lumen diameter (Fig. 3e-f). The radial pattern of variation from pith to periphery of these anatomical properties has been reported for other ring-porous species. For *M. dubia* (up to age 5) observed trends for vessel diameter and fiber wall thickness were consistent with our findings, while fiber lumen width decreased (15,9 µm to 9,4 µm) (*Saravanan et al*. 2013). It should be noted that Saravanan *et al*. (2013) did not distinguish between earlywood and latewood, if our earlywood and latewood observations for fiber lumen width were averaged then a similar trend would be observed. In *S. macrophylla*, Anoop *et al*. (2014) indicated that the vessel diameter gradually increased from pith to bark, while fiber wall thickness increased from pith to a peak, and declined towards the periphery in the radial direction. Tsuchiya and Furukawa (2009b) found that vessel lumen diameter in earlywood increased in size for up to 20 years before stabilizing in the ring-porous hardwoods *Acanthopanax sciadophylloides* and *Evodiopanax innovans*.

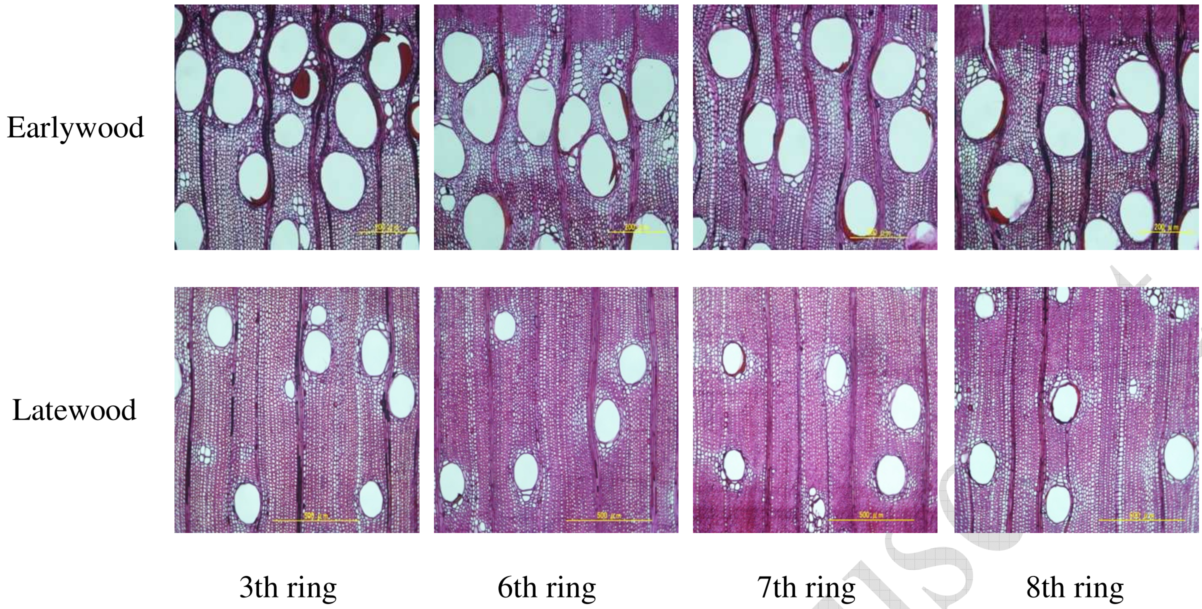

Figure 1: Images of vessels in different growth rings from pith of *Melia azedarach* (tree number 1). Scale bar = 500 µm.

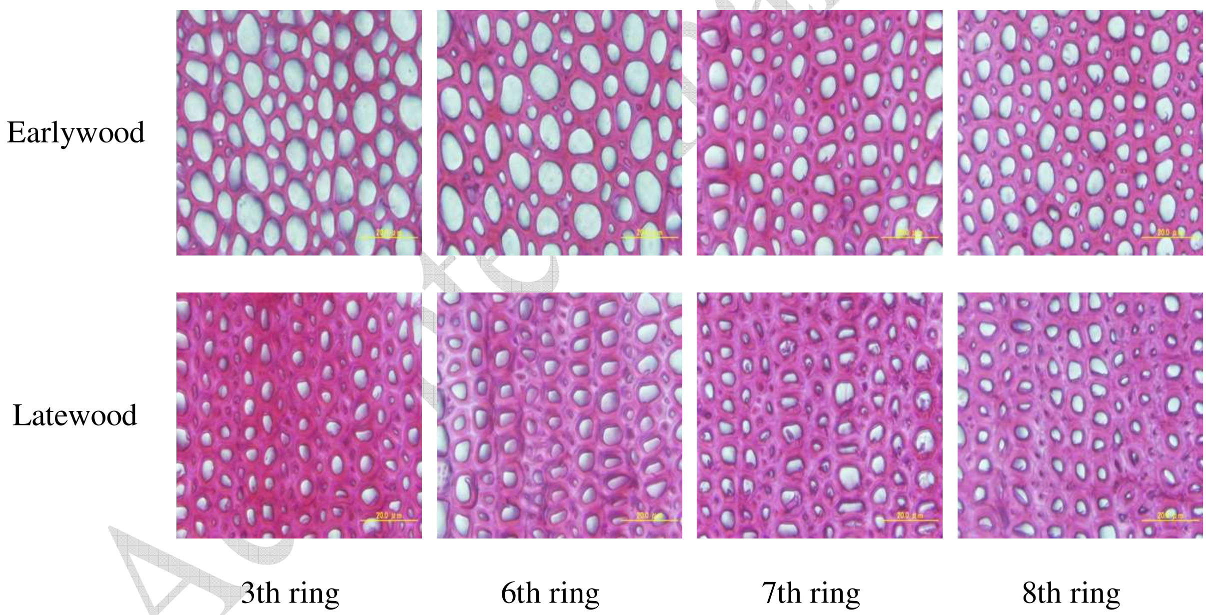

Figure 2: Images of wood fibers in different growth rings from pith of *Melia azedarach* (tree number 3). Scale bar = 200 µm.

Various wood anatomical properties can be used to differentiate between juvenile and mature wood (Bhat *et al*. 2001; Tsuchiya and Furukawa 2009b). Juvenile wood is formed by young cambium in which anatomical structure such as cell length and cell width changes rapidly with cambial age, while mature wood is formed when length of fusiform cambial cells becomes more or less constant or increases much more slowly with cambial age (Tsuchiya and Furukawa 2009a). In hardwoods, vessel elements in diffuse-porous wood and earlywood vessel elements in ring-porous wood have approximately the same length as the fusiform cambial cells from which they are derived, and wood fibers constitute the dominant component (Kitin *et al*. 1999, Tsuchiya and Furukawa 2009a). Radial variations of various anatomical properties, especially wood fiber length, vessel element length and vessel lumen diameter have been frequently considered for age demarcation between juvenile and mature wood (Lei *et al*. 1996, Gartner *et al*. 1997, Bhat *et al*. 2001, Honjo *et al*. 2005, Tsuchiya and Furukawa 2009a,b).

EVLD is one of the properties used to differentiate between juvenile and mature wood. EVLD generally shows an increase from pith to the bark, where EVLD is smaller near the pith, and gradually increases in size radially before leveling off in the outer part of the stem (Bhat *et al*. 2001, Tsuchiya and Furukawa 2009a,b) and as observed in Figure 3a-b. To estimate EVLD maturation age in *M. azedarach* we used a segmented regression model with quadratic equation and a plateau. We found that EVLDs increased rapidly in the inner part of the stem, and these values tended to be unchanged with an estimated plateau of 156 μm from cambial age of 7,4 towards the periphery of the tree (Fig. 4). The obtained result is comparable with those reported by Duong *et al*. (2017) showing that wood of *M. azedarach* beyond ring number 7 from pith had comparatively long fibers, high SG, and low MFA in the S_2 layer of fiber cell walls. These findings suggest that wood of the *M. azedarach* could be classified into core wood and outer wood based on EVLD, and the boundary between core and outer wood may be located at 7th ring from pith. This should be taken into account in wood processing using *M. azedarach* grown in northern

Vietnam. In other studies of plantation grown *M. azedarach* higher stocking rates have been employed, for example 2 m × 2 m in Nasser *et al*. (2010) and spacing's ranging from 1 m × 1 m to 3 m × 2 m in Leles *et al*. (2014). The influence of different planting densities on the maturation of *M. azedarach* is unknown and requires further investigation.

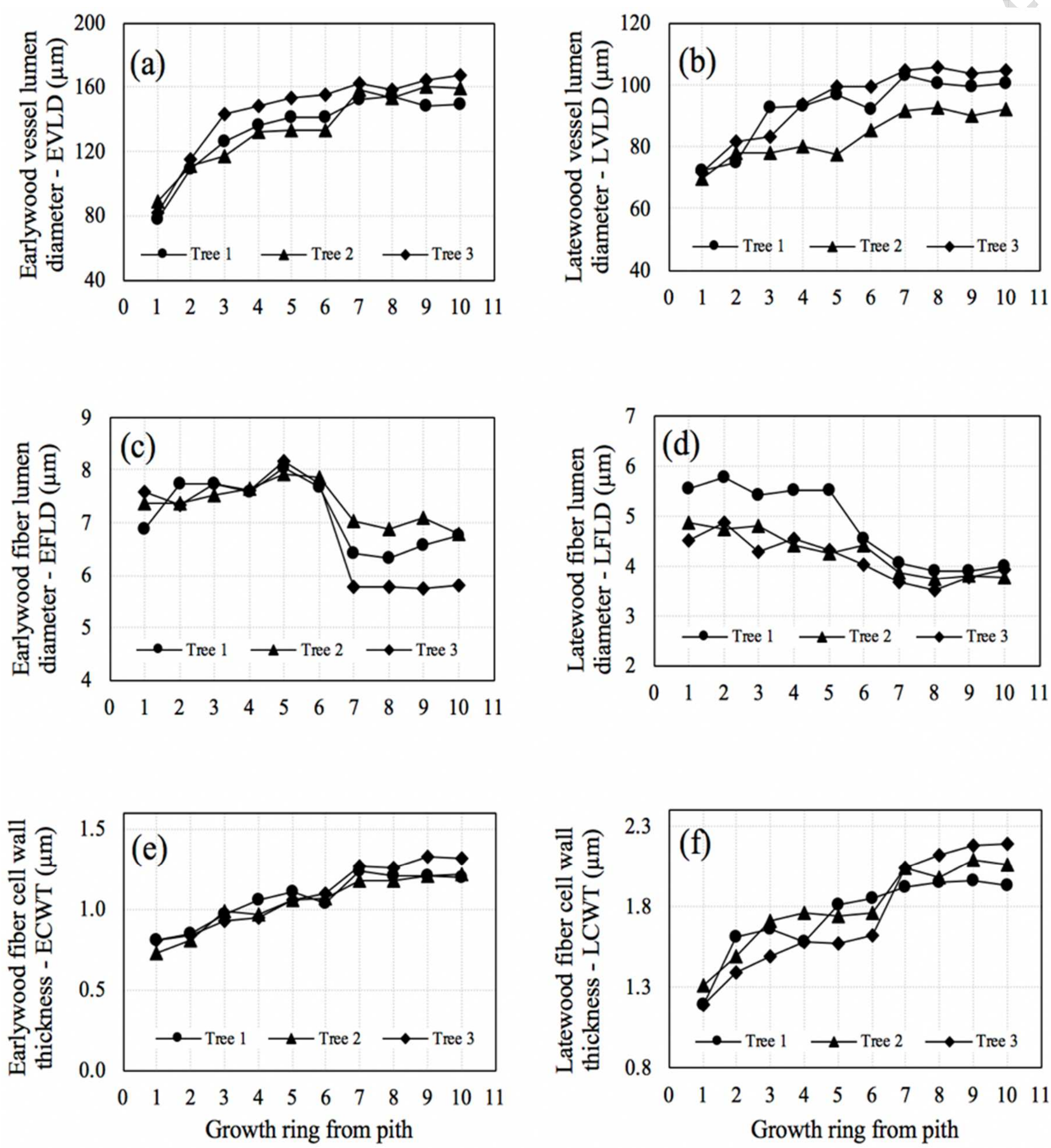

Figure 3: Radial variation in cell morphology from pith to bark in earlywood and latewood of *Melia azedarach* planted in northern Vietnam.

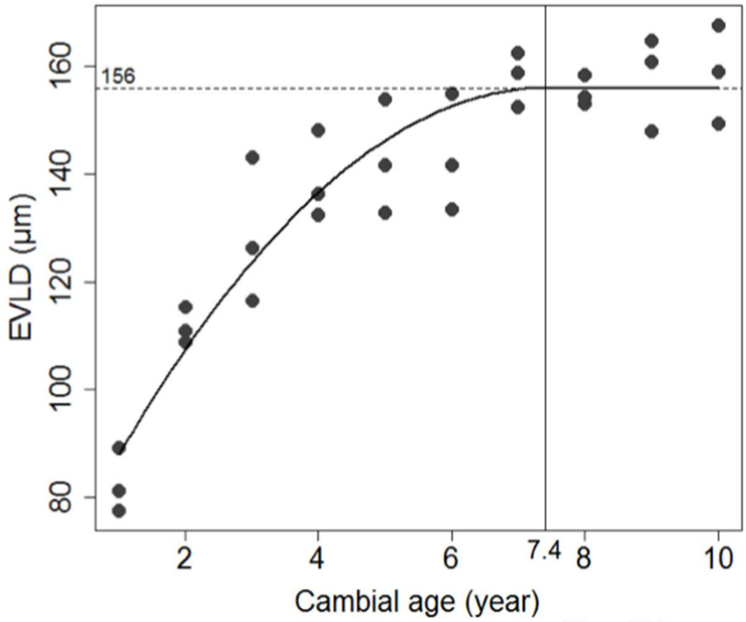

Figure 4: Changes in EVLD with increasing cambial age in *Melia azedarach* planted in Vietnam. A quadratic model with a plateau was fitted to show the maturation age. Coefficient estimates of the quadratic model, β_0, β_1 and β_2, are 65,20; 24,39 and 1,64 respectively. Points showed observed values in sample trees.

Relationship between anatomical characteristics and wood density

It has been shown by many investigators that wood properties are closely related to anatomical structure, and a detailed analysis of wood structure has been considered necessary to sufficiently explain wood property variation (Ifju 1983, Zhang and Zhong 1992). Wood density is a key indicator of wood quality because it is closely correlated with many physical and technological properties (Miranda *et al*. 2001). The density of wood depends on the size of cells, the thickness of the cell walls, and the interrelationship between the two features (Panshin and DeZeeuw 1980, Ishiguri *et al*. 2012). Assuming a constant cell wall density, wood density will be mainly determined by the voids in the wood mass, i.e., by the size of lumens in the wood cells (predominantly fibers and vessels in hardwoods). The size and density of vessels therefore have a major effect on wood density and tend to be inversely proportional to this property (Savidge 2003).

In this study, as shown in Table 3, a significant positive correlation was found between vessel lumen diameter and SG both in early and latewood (Pearson's correlation coefficient *r* = 0,79 in earlywood, *r* = 0,62 in latewood). While contradictory to those reported by Savidge (2003) a positive correlation between vessel lumen diameter and wood density was also found in *Casuarina equisetifolia* (Chowdhury *et al*. 2012). A probable explanation is that average vessel lumen diameter increased, while vessel frequency decreased from the pith to bark. Further experiments related to variation in vessel area of *M. azedarach* in radial direction will clarify the relation of wood density with vessel lumen diameter. There was a significantly positive relationship between wood fiber cell wall thickness and SG (*r* = 0,78 in earlywood, *r* = 0,79 in latewood) while wood fiber lumen diameter was negatively correlated with SG. The present results are in line with those of Ishiguri *et al*. (2009) and Chowdhury *et al*. (2012) for *Paraserianthes falcataria* and *C. equisetifolia*, respectively.

Table 3: Pearson's correlation coefficients between anatomical characteristics and wood specific gravity in *Melia azedarach.*

Anatomical characteristics	**Specific gravity**
Earlywood vessel lumen diameter (μm)	0,79***
Latewood vessel lumen diameter (μm)	0,62***
Earlywood fiber lumen diameter (μm)	- 0,28^{ns}
Latewood fiber lumen diameter (μm)	- 0,64***
Earlywood fiber cell wall thickness (μm)	0,78***
Latewood fiber cell wall thickness (μm)	0,79***

Note: *** $P < 0,001$; ns, not significant.

CONCLUSIONS

We investigated the radial variations in cell morphology and wood property of ten-year-old *M. azedarach* planted in northern Vietnam. Our results indicated that the vessel lumen diameter of the *M. azedarach* trees rapidly increased up to 7th ring from the pith before becoming constant towards the bark both in earlywood and latewood. EFLD and LFLD showed similar values from pith to 6th ring; however, EFLD and LFLD demonstrated increasing and decreasing trends respectively, before significantly decreasing and remaining constant from 7th ring outwards. ECWT and LCWT increased gradually with cambial age up to 7th ring before being less or more stable to the bark. Our data provide evidence that wood of the *M. azedarach* could be classified into core wood and outer wood based on EVLD, and the boundary between core and outer wood may be located at 7th ring from pith. Significant correlation coefficients were found between anatomical characteristics and SG except for EFLD and SG.

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