Influence of juvenile and mature wood on anatomical and chemical properties of early and late wood from Chinese fir plantation

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Abstract
The proportion of juvenile wood affects the utilization of wood seriously, and the transition year of juvenile wood (JW) and mature wood (MW) plays a decisive role in the rotation and the modification of wood. To find out the demarcation of JW and MW, the tracheid length (TL) and microfibril angle (MFA) of early wood (EW) and late wood (LW) from four Chinese fir clones were measured by optical microscopy and X-ray diffraction. Then the data were analyzed by the k-means clustering method. The correlation and the differences among wood properties between JW and MW were compared. Results indicated that the LW showed better properties than that of EW, but the anatomical differences between EW and LW did not influence the demarcation of JW and MW. The cluster analysis of TL and MFA showed that the transition year was in the 16th year and the transition zone of EW and LW was different among clones. The MW has longer and wider tracheid, thicker cell walls, and smaller MFA. In terms of chemistry, MW had a higher content of holocellulose, α-cellulose, less content of extract, but no significant difference in lignin content compared with JW. The stabilization of chemical components was earlier than that of the anatomical properties. Correlation analysis showed that there were strong correlations between the chemical composition and anatomical characteristics in JW and MW. In general, compared with chemical components, anatomical indicators were more suitable for JW and MW demarcation. The differences and correlations between JW and MW properties provide a theoretical basis for wood rotation and planting.

Keywords: Cunninghamia lanceolata, Type of wood, Transition, Anatomical characteristics, Chemical composition

Introduction
The traditional wood production and the collection of wood derivatives products based on the logging of natural forests have led to the gradual exhaustion of natural resources, but also lead to soil erosion, drought, flood, and biological extinction. The fundamental solution to this problem is to adopt oriented cultivation, wood efficient utilization technology, and establish a high-quality industrial wood plantation. Planted forests are increasing rapidly in the world to alleviate deforestation and natural forest degradation, while providing a variety of goods and services [1]. Forest plantations have been supplying up to 33% of the total industrial roundwood in the world and are expected to reach 50% of the global industrial roundwood production by 2040 [2]. China has the largest area of plantation reserves in the world [3]. At the same time, softwood is one of the most important renewable resources on earth. It is mainly used as a material in the structural components of wooden structures [4]. Chinese fir (Cunninghamia lanceolata [Lamb.] Hook.), the most important conifer species for timber production and has wide geographic distribution in southern China,
accounting for 20–30% of the total commercial timber production which has important commercial significance for timber and pulp industry [5–8]. Chinese fir is a subtropical tree species, which is planted well and utilized widely in practice in south area of China. KAI13, DABA8, KAI13 and KAI24 are four groups of Chinese fir clones selected by the Kaihua Country Forest Farm, which have excellent growth conditions. We studied wood properties for rational processing and efficient utilization. At the same time, our research groups have studied Chinese fir mechanical properties and their correlation with microstructures [9], but the influence of juvenile and mature wood on anatomical and chemical properties of early and late wood has not been studied yet. Therefore, it is of great significance to study the wood properties of Chinese fir plantation. The quality of wood is determined by the properties of JW (juvenile wood) and MW (mature wood), the utilization of wood is closely related to the proportion of these two parts [10]. For example, a high JW proportion involves a reduced lumber strength, warping problems during the drying process and a reduction of yields in pulp production [11]. Plantation trees are typically harvested after only 30 years and this means that approximately half of the timber produced originates from the juvenile part of the stem [12]. Wood located near the pith is commonly referred to as juvenile wood, and wood found further from the pith as mature wood [13]. Analyzing the radial variation of wood anatomical properties within stems can determine the boundary between the juvenile and mature wood [14–16]. Only by understanding the anatomical structure of wood can it be possible to understand why specific wood characteristics are formed and how it affects the way wood is used [17]. Darmawan discussed a transition age between juvenile and mature wood in order to infer an age of harvestability [18]. Fiber length and microfibril angle are the anatomical traits that determine wood quality [19]. Other properties such as ring area and latewood percentage also can define the extent of the core wood zone [13]. Compared to MW, JW has different properties, such as lower wood density, shorter fiber length, higher lignin content, and higher compression wood content [20, 21]. Due to the strengthening of forestry practices and the promotion of more cost-effective production of raw materials, the increase in the proportion of JW in artificial forest resources is inevitable [13]. Methods that had received much attention to defining juvenile/mature wood are based on segmented regression analysis, piecewise regression model and k-means algorithm cluster analysis [22–28]. Modulus of elasticity/shear modulus also can be chosen as the index for determining the juvenile/mature wood boundary [29]. Studies to determine the chemical composition of juvenile and mature wood have revealed significant differences in the content of these components [30]. Chemical composition varies among and within tree species and also depend on the age and part of a tree [31]. The different species of wood and even its different areas are characterized by many physical and chemical features such as the shape of the tracheid, and the proportion of its chemical components [32].

In this paper, we found the demarcation of JW and MW of Chinese fir plantations by radial variation of TL (tracheid length) and MFA (microfibril angle) from EW (early wood) and LW (late wood), the less dense wood is named “early wood” or “spring wood”, outer regions of growth rings are denser and correspond to the wood formed in summer or autumn, also named “late wood” [32], and then the anatomical characteristics and chemical components differences between JW and MW were compared. The results provide a theoretical basis for the directional cultivation and improvement of Chinese fir.

Material and methods
Sample collection
The four 20-year-old Chinese fir clones (KAI3, KAI13, DABA8 and F24) were collected from a forest farm in Kaihua County, Zhejiang Province. Kaihua (118° 01′ east longitude, 28° 54′ north latitude) features subtropical monsoon climate with an average temperature of 16.4 °C, 1712.5-h sunshine, annual average rainfall of 1814 mm and 252-day frost-free period). 5 plants were prepared for each clone, a total of 20 plants. The basic information of experimental materials is shown in Table 1.

Sample preparation
A 70-mm-thick disc was cut from the trunk height of 1.3 m and cut the wood strips with a width of 1.5 cm outwards along the pith, and then cut the wood strips into small wooden blocks of 10 mm(R) × 10 mm(T) × 15 mm(L) according to different annual rings for the preparation of experimental specimens for anatomical characteristics (Fig. 1a). Samples were taken from the pith to the bark year by year according to the tree rings. Each year was divided into two parts: early and late wood; A disc with a certain thickness was cut at the height of 1.3 m for chemical components test, the disc was sawn into seven parts according to part A

Table 1 Details of four clones

| Clones  | Age (year) | Height (m) | DBH (cm) |
|---------|------------|------------|----------|
| KAI3    | 20         | 18.0       | 17.8     |
| KAI13   | 20         | 17.1       | 17.0     |
| F24     | 20         | 22.3       | 19.9     |
| DABA8   | 20         | 19.0       | 17.5     |
(1–4 years), part B (5–6 years), part C (7–8 years), part D (9–10 years), part E (11–13 years), part F (14–15 years), part G (16–20 years). After the raw material is crushed, 40–60 mesh samples were taken, and the chemical components were tested after water balance (Fig. 1b).

**The test method of wood anatomical properties**

For anatomical characterization, In accordance with the national standard, GB/T10336-2002 [33], wood macerations were conducted using glacial acetic acid and 30% hydrogen peroxide (1:1 ratio) at 80 °C for 4 h. 50 measurements were taken for the TL of both EW and LW. The samples were sliced by Leica RM2265 (Wetzlar, Germany), and the cross-section images with a ruler were taken by KEYENCE VHX-600E. The data were collected by ImageJ software. The thin slices of microfibril angle samples of each Chinese fir clone were determined by an X-ray diffractometer (XD-3). The average MFA of each wood sample of Chinese fir clones was calculated by the 0.6T method.

**The test method of wood chemical components**

The determination method mainly refers to the current national standards and other common methods, including GB/T 36055-2018 [34], GB/T 35816-2018 [35], GB/T 35818-2018 [36], GB/T 744-2004 [37]. The chemical composition content is based on the absolute dry wood, and the values were the average value of 5 samples in the group, with 3–5 parallel samples in each group.

**The method of k-means analysis**

k-means clustering represents one of the most popular clustering algorithms, which groups the unlabeled dataset into different clusters. With reference to Alboukadel Kassambara's suggestion, in this article we adopted the hierarchical k-means clustering method by Rstudio software. The algorithm is summarized as follows: (1) compute hierarchical clustering and cut the tree into k-clusters; (2) compute the center of each cluster; (3) compute k-means by using the set of cluster centers as the initial cluster centers. The code is available on DATA NOVIA in the following repository: https://www.datanovia.com/en/lessons/hierarchical-k-means-clustering-optimize-clusters/.

**Results and discussion**

**Variation in tracheid length from pith to bark**

As shown in Table 2, the TL of LW was slightly longer than that of EW. In the study of radial variation in anatomical features, if the mean of data is skewed distribution, then it cannot describe the overall sample situation, if there is a large value of outliers, the average may be invalid. When the value of outliers is very high, a large amount of valuable data is also lost with truncated means. Therefore, this paper uses box plots to describe the data distribution of Chinese fir TL and MFA from the pith to the bark, to better observe the inflection point of the curve change. As shown in Fig. 2, the TL of Chinese fir increased rapidly at first
and then slowly with the increase of growing years. The TL of LW is longer than that of EW, and the range is greater as well. The TL value from biggest to smallest was KAI3, KAI13, F24, DABA8. Among the four clones, the TL was an extremely significant difference between EW and LW.

The TL changes from the pith to the bark, curves have the similar tendency of a first rapidly increase, and then slowly, finally get stable with growth age. The blue dotted line marks the inflection point of rapid increase curve to slow increase curve and the slow increase curve to the stable line. In the EW part, the inflection points of the TL growth curves of the four groups of clones were 6th and 16th (Fig. 2a), 10th and 16th (Fig. 2c), 7th and 16th (Fig. 2e), 7th and 16th (Fig. 2g), respectively; In the LW part, the inflection points of the TL growth curves of the four groups of clones were 8th and 15th (Fig. 2b), 8th and 16th (Fig. 2d), 9th and 16th (Fig. 2f), 6th and 16th (Fig. 2h), respectively. In the first 6–8 years, the length of EW and LW tracheid increased rapidly, and the area of slow increase curve of EW tracheid was generally bigger than that of LW (e.g., KAI3, KAI13), there were also clones that get smaller than that of LW (e.g., DABA8), or no significant difference between EW and LW (e.g., F24). In general, the TL tends to stabilize around 16 years. The regression equation of TL \( y = a - b \times \ln(x + c) \) can be determined by nonlinear fitting, in which \( R^2 \) is greater than 0.97 (Table 3).

The radial variation of TL of LW is accorded with logarithmic function. Although there were significant differences in TL among clones, the pattern of tracheid growth and the angle of microfibril in early and late clones were consistent with the annual rings and were stable in about 16 years. This tendency is a characteristic of JW formation in the tree's early years [38]. Although there were significant differences in TL among the four groups, the TL tended to be stable in the 16th year. Even if the inflection point of wood tracheid growth was about the 16th year, the transition zone was different among clones, which indicated that there were some inaccuracies in the determination of the wood transition area.

### Variation in microfibril angle from the pith to bark

As shown in Table 4, the MFA of KAI3 EW and LW was the smallest. With the growth of tree age, except for LW of KAI3, there was no significant difference in MFA of EW and LW among different clones. The MFA of LW is smaller than that of EW, and the variation range of EW was larger than that of LW. Similarly, among them, the MFA of EW and LW from smallest to largest is KAI3, KAI13, F24 and DABA8. As shown in Fig. 3, the MFA of different clones had the same trend, and the fluctuation range of EW was significantly greater than that of LW. In the EW part, the MFA box of KAI3 (Fig. 3a), DABA8 (Fig. 3c), and F24 (Fig. 3d) dropped to the lowest point at 13-year-old, and KAI13 (Fig. 3e) at 12; In the LW part, the lowest points of each clone were: age 12 of KAI3 (Fig. 3b); 14 of DABA8 (Fig. 3d); 13 of KAI13 (Fig. 3f); 13 of F24 (Fig. 3h). The difference of clones had no significant effect on MFA. MFA of EW and LW showed a trend of first decreasing and then stabilizing, which consistent to Mario Vega's research results [39]. MFA declined to its lowest value around year 13 and then remained relatively stable with little fluctuation. Compared with tracheid length, MFA has lower transition ring, which was consistent with Darmawan's research results, confirmed that the determination of transition ring was dependent on the trait considered [18].

### Demarcation of juvenile and mature wood

The hierarchical k-means algorithm was used for cluster analysis of Chinese fir MFA and TL and the k value was set as number 4. The clustering diagram of four sets of clones in different years was obtained as shown in Fig. 4. According to the diagram, the cluster analysis results of TL and MFA of EW and LW showed that they were divided into four parts: blue, yellow, green and red, corresponding to color labels 1, 2, 3 and 4. In EW part, tree ages were divided into 3th–5th, 6th–9th, 10th–15th, 16th–20th (Fig. 4a) four categories; In LW part, tree ages were divided into three categories, 5th–8th, 9th–14th and 15th–20th (Fig. 4b), among which the blue part was not getting reasonably clustered.

Understanding the transition between JW and MW is an important first step in understanding wood quality and the modeling of fiber attributes [18]. According to the radial variation of TL and MFA, the inflection points of curves were located, which was subjective. Therefore, we use statistical classification (k-means clustering) to analyze TL and MFA. The demarcation of juvenile and

### Table 2 Tracheid length of early wood and late wood from four clones

| Clone | Early wood (μm) | Late wood (μm) |
|-------|-----------------|----------------|
| KAI3  | 6622.93 ± 634.26 | 6262.55 ± 591.84 |
| DABA8 | 2662.04 ± 829.97 | 2717.80 ± 853.31 |
| KAI13 | 3037.65 ± 686.83 | 3045.84 ± 635.96 |
| F24   | 2865.94 ± 649.08 | 2898.65 ± 558.42 |

Statistical differences between clones were denoted using lowercase letters, with different letters corresponding to significant differences (p < 0.05)
Fig. 2 The radial variation of tracheid length versus cambial age of Chinese fir. a TL of KAI3 EW, b TL of KAI3 LW, c TL of DABA8 EW, d TL of DABA8 LW, e TL of KAI13 EW, f TL of KAI13 LW, g TL of F24 EW, h TL of F24 LW
Late wood: 2173.88 μm for MW, 2772.28 μm for JW.
Early wood: 2101.16 μm for MW, 1904.73 μm for JW.

The results were consistent with the inflection points above. This is slightly different from the results of Jian L's study on the demarcation of Chinese fir plantation [41], which may be caused by the differences in tree planting area. The variation of MFA indicating that the maturity year of MFA was earlier than that of TL. Therefore, determining the transition year by MFA was not accurate.

Table 3: Regression equation of tracheid length

| Position | Equation | y = a - b*ln(x + c) |
|----------|----------|---------------------|
|          |          | Plot                |
|          |          | KAI3 | DABA8 | KAI13 | F24    |
| Early wood | a        | 2101.16 | -147.34 | 1634.38 | 1421.56 |
|           | b        | -577.88 | -1193.40 | -700.51 | -684.92 |
|           | c        | -1.52  | 1.67    | -1.50  | -0.40  |
|           | R²       | 0.99   | 0.98    | 0.99   | 0.97   |
| Late wood | a        | 2173.88 | -58.61  | 1842.55 | 1914.96 |
|           | b        | -550.32 | -1196.50 | -611.24 | -487.35 |
|           | c        | -1.4146 | 1.1831  | -1.4405 | -0.6470 |
|           | R²       | 0.99   | 0.98    | 0.99   | 0.97   |

Table 4: Microfibril angle of early wood and late wood among clones

| Clone | Early wood (°) | Late wood (°) |
|-------|---------------|---------------|
| KAI3  | 13.41 ± 4.81ab| 12.82 ± 2.43ab|
| DABA8 | 14.89 ± 3.42ab| 14.32 ± 2.93ab|
| KAI13 | 14.49 ± 3.97ab| 14.08 ± 3.37ab|
| F24   | 14.46 ± 3.45ab| 13.42 ± 3.01abc|
| F     | 2.13          | 7.83          |
| Sig   | 0.100         | 0.000         |

Differences of chemical composition between juvenile wood and mature wood

The content of lignin in MW was 33.34%, 33.22% in JW, 49.25% in α-cellulose and 46.41% in JW, 73.58% in holocellulose and 72.57% in JW, 1.28% in extractive of MW and 2.08% in JW (Table 6). Wood was divided into seven parts along the radial direction, and part G was the MW part. The contents of lignin, holocellulose and α-cellulose in MW were higher than those in JW, which consistent with Funda research result [31]. Along the direction from pith to bark, the content of lignin increased slightly at site G, there was no significant difference among the sites, but the content of α-cellulose increased gradually, and there was no significant difference between site G and site E. The content of holocellulose had no significant difference at A–D, there was no significant difference in the content of holocellulose in part E and part G. There was no significant difference in the content of extract in A–C, but a significant decrease in part D. There was no significant difference in extract content between part E and part G (Fig. 5). In addition to the extractive content, the cellulose content and lignin content of MW were higher than that of JW. There was little difference in chemical composition between different clones,
Fig. 3 The radial variation of microfibril angle versus cambial age of Chinese fir. 

- **a** MFA of KAI3 early wood
- **b** MFA of KAI3 late wood
- **c** MFA of DABA8 EW
- **d** MFA of DABA8 LW
- **e** MFA of KAI13 EW
- **f** MFA of KAI13 LW
- **g** MFA of F24 EW
- **h** MFA of F24 LW
which may be due to the same growing environment and tree species [31, 51]. The same lignin content may make the gap of mechanical strength between JW and MW narrowed.

There was no significant change of lignin in different parts along the radial direction, indicating that there was no significant difference in lignin content between JW and MW. There was no significant difference between extract, holocellulose and α-cellulose in part E compared with part G (mature wood part), indicating that the chemical components in part E (11–13 years) had reached the mature level [52].

**Table 5** Differences of anatomical characteristics between juvenile wood and mature wood

| Clone  | TL (μm) | TD (μm) | DWT (μm) | MFA (°) |
|--------|---------|---------|----------|---------|
|        | J       | M       | J        | M       | J       | M       | J       | M       |
| KAI3   | 3075.93 ± 598.38 | 3725.28 ± 345.94 | 29.09 ± 5.96 | 32.02 ± 6.49 | 7.14 ± 2.23 | 7.41 ± 2.37 | 13.12 ± 4.32 | 12.06 ± 1.75 |
| DABA8  | 2440.58 ± 794.28 | 3414.56 ± 477.92 | 27.93 ± 4.60 | 30.37 ± 4.17 | 6.40 ± 2.26 | 6.61 ± 2.26 | 14.82 ± 3.29 | 14.08 ± 2.90 |
| KAI13  | 2848.20 ± 640.50 | 3582.85 ± 343.98 | 27.10 ± 6.17 | 29.14 ± 5.63 | 6.66 ± 2.24 | 7.04 ± 2.85 | 14.48 ± 3.92 | 13.53 ± 2.82 |
| F24    | 2724.40 ± 590.56 | 3341.22 ± 367.74 | 28.38 ± 6.15 | 30.21 ± 4.74 | 7.02 ± 2.61 | 7.45 ± 2.60 | 14.36 ± 3.57 | 12.75 ± 1.96 |
| Average| 2772.28 ± 351.98 | 28.13 ± 30.44 | 6.81 ± 7.13 | 14.20 ± 13.11 |

TL: tracheid length, TD: tracheid diameter, DWT: double cell wall thickness, MFA: microfibril angle, J: juvenile wood, M: mature wood

**Table 6** Differences of chemical composition between juvenile wood and mature wood

| Clone  | Lignin (%) | α-Cellulose (%) | Holocellulose (%) | Extract (%) |
|--------|------------|-----------------|-------------------|-------------|
|        | J          | M               | J                 | M           | J            | M             | J            | M            |
| KAI3   | 33.72 ± 1.63 | 33.95 ± 1.60 | 45.78 ± 3.15 | 49.03 ± 0.97 | 72.24 ± 2.36 | 73.10 ± 1.92 | 2.32 ± 1.44 | 1.34 ± 0.54 |
| DABA8  | 32.96 ± 1.34 | 33.81 ± 1.32 | 47.27 ± 2.50 | 48.20 ± 3.04 | 72.84 ± 1.77 | 73.08 ± 1.45 | 1.95 ± 0.98 | 0.91 ± 0.20 |
| KAI13  | 32.77 ± 1.35 | 31.95 ± 0.41 | 47.40 ± 2.68 | 51.56 ± 0.96 | 73.32 ± 1.75 | 74.90 ± 2.63 | 1.96 ± 1.11 | 1.36 ± 0.81 |
| F24    | 33.43 ± 1.27 | 33.66 ± 1.73 | 45.21 ± 2.20 | 48.19 ± 1.86 | 71.89 ± 1.76 | 73.25 ± 1.94 | 2.08 ± 0.81 | 1.51 ± 0.62 |
| Average| 33.22 ± 1.27 | 33.34 ± 1.30 | 46.41 ± 2.15 | 49.25 ± 1.85 | 72.57 ± 1.92 | 73.58 ± 1.94 | 2.05 ± 1.02 | 1.28 ± 0.62 |

J: juvenile wood, M: mature wood

**Fig. 4** k-means clustering analyses of MFA and TL. a Clustering plot of early wood; b clustering plot of late wood. Number 1–4 represent four different cluster groups

Correlation of wood anatomical properties and chemical composition between juvenile and mature wood

For JW (Fig. 6a), the TL and TD were positively correlated with the extractive content, while for MW (Fig. 6b), they were negatively correlated; For JW, the DWT was negatively correlated with α-cellulose (R = -0.617), and for MW, it was positively correlated (R = 0.599); For JW, holocellulose was negatively correlated with α-cellulose, but for MW, they were positively correlated (R = 0.535); The double wall thickness of JW was significantly positively correlated with the extract (R = 0.422), while there was no significant relationship in MW. That can be
explained by the existence of heartwood. Heart wood accumulates a significant number of secondary metabolites (woody known as extractives) [53, 54].

The correlation heatmap was analyzed to provide a theoretical basis for the comprehensive improvement of wood properties. Many scholars have studied the correlation between wood properties, such as the correlation...
coefficients between physical and chemical properties and correlation of wood anatomical properties [16, 55].

Conclusion

TL and MFA are two important anatomical indexes for the demarcation of JW and MW; the wood properties of coniferous wood are significantly different between EW and LW. Although LW properties were better than EW, the radial variation trends of different clones in the same area were basically the same, so there was no need to study separately. Through the study of the anatomical characteristics, it can be concluded that the transition year of Chinese fir was the 16th year. Cluster analysis is an appropriate method for the classification of wood properties. Compared with complex regression curves and subjective visual observations, the results were more scientific, reasonable and reliable. However, in chemical composition, the differences between the two types of wood were less than anatomical characteristics, and the stable time of chemical composition content was earlier than anatomical characteristics, so it was not appropriate to define the transition year of Chinese fir clones by chemical composition.

Abbreviations

JW: Juvenile wood; MW: Mature wood; EW: Early wood; LW: Late wood; TL: Tracheid length; MFA: Microfibril angle; TD: Tracheid diameter; DWT: Double cell wall thickness; LC: Lignin content; α-CC: α-Cellulose content; HC: Holocellulose content; EC: Extractive content.

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Authors' contributions

Conceptualization: C.L., and S.L.; methodology: C.L.; software, C.L.; validation: C.L. and Y.Y.; formal analysis: C.L.; investigation: Q.J. and S.L.; resources: C.L., YY, Q. J and S.L.; data curation: Y.L.; writing—original draft preparation: C.L.; project administration: S.L.; funding acquisition: C.L. and S.L.; All authors have read and approved the final manuscript.

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Availability of data and materials

Available upon reasonable request.

Declarations

Competing interests

The authors declare no conflict of interest.

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