Fiber Morphology in Walnut Branchwood: Relation to the Branch Diameter, Branching Level, and Tension Wood

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The branchwood of fruit trees is being promoted to supplement the fiber material for paper manufacturing in China. This study was conducted to investigate the fiber morphology of walnut branchwood, and to highlight its potential utilizations in papermaking. The effects of the branch diameter, branching level, and tension wood on the fiber morphology were also investigated. The results showed that approximately 65% of the fibers were longer than 900 µm, 95% of the fibers had a slenderness ratio greater than 40, and 67% of the fibers had a Runkel ratio less than 1. It is evident from the results that the fiber morphology of walnut branchwood is reasonably good for paper manufacturing. However, the diameter of the branch does not provide reliable information to predict the fiber morphometrics. In addition, the branching level and tension wood are not very helpful in the screening of fiber raw materials.

DOI: 10.15376/biores.17.2.2214-2227

Keywords: Branch diameter; Branching level; Fiber morphology; Tension wood; Walnut branchwood

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INTRODUCTION

According to the report of the National Forestry and Grassland Administration, over the past decade, the forest resources in China have increased by more than 70 million hectares in the past 10 years, ranking first in the world (NFGA 2019). However, the forest coverage rate is low, the harvestable resources are insufficient, and the harvestable timber resources for the paper industry are even lower (Cui and Liu 2020). In forest developed countries, *e.g.*, Finland, Sweden, the United States, Canada, *etc.*, the wood used for papermaking accounts for more than 30% of the total wood production in the country. However, only 4% of the total wood production is used for papermaking in China (Zhong 2021). The severe shortage of raw wood fiber is the primary factor hindering the development of the paper industries in China (Zhao 2021). The development of alternative wood fiber materials is an important direction for the long-term development of the Chinese paper industry. The branchwood of economic tree species have great potential as alternate timber (Suansa and Al-Mefarrej 2020).

Presently, the planting area of non-timber forests in China is greater than 40 million hm^2, ranking first in the world (NFGA 2019). Among them, the total planting area of walnut trees (*Juglans regia*) is 4.05 million hm^2, which accounts for 15% of the total non-timber forests area in China and is also the largest planting area in the world (Xi 2015).

Under natural conditions, walnut trees can grow higher than 25 m tall (Li *et al.* 2021). To improve the nut fruit yield, walnut trees usually need to be dwarfed and reshaped, so the trunk height is generally 2 m to 10 m (Xi 2015). The life span of a walnut tree is very long and can exceed 500 years (Liu 1986). Due to economic needs, the general renewal cycle of walnut trees is 8 years to 10 years, and the renewal cycle of branches is 3 years to 4 years (Zhang 2021). Currently, greater than 20 million m^3 of branch residue is generated annually through pruning and regeneration. Even if only 30% of them are used, this will result in approximately 6 million m^3 of wood, which can considerably reduce the pressure of wood shortage.

Unfortunately, compared with the trunk, the branches of economic tree species have several disadvantages, including irregular shape, limited size, high bark proportion, and difficulty in removing bark from the branches. Due to this, they can hardly be used as structural timber (Shmulsky and Jones 2019). It should also be emphasized that tension wood is often formed in the branches of angiosperms under the induction of hormones and the synergistic effects of light, wind, and gravity (Tsai *et al.* 2012). Tensile wood is typically characterized by gelatinous fibers that are rich in cellulose (Ruelle 2009). There are no major differences between the dimensions of gelatinous fibers and normal fibers. Some researchers have reported differences in the fiber dimensions between tension and opposite wood, but the differences are generally not major (Lee *et al.* 1997; Yoshizawa *et al.* 2000; Niu *et al.* 2010). Due to the above factors, walnut branchwood is difficult to split, and farmers rarely use it as firewood. After each renewal, a large number of walnut branches are left scattered in the field, which leads to a waste of wood resources and also increases the risk of fire. Therefore, the management of these walnut branches has become a difficult problem for orchard operators (Zhang *et al.* 2019).

Branchwood is undesirable in any structural application, but it can be used for fiber production. This can play an important role in alleviating the shortage of raw materials in the paper industry. Research on the potential of pulp production from the branches of fruit trees has been started since 1930 (Crossey 1938), including the proportion of bark on branches (Song and Lee 2005), wood properties (Gryc *et al.* 2011; Zhao *et al.* 2018), mechanical properties (Passialis and Grigoriou 1999; Dadzie *et al.* 2016), and pulping characteristics (Cai 2001; Dong and Li 2010). Among many factors, the fiber dimensions might be the first to be considered if the branchwood is to be used as raw materials for paper making. Fibers with a length greater than 400 µm, a slenderness ratio greater than 40, and a Runkel ratio less than 1 are considered suitable for papermaking (Bao and Jiang 1998; Ohshima *et al.* 2005; Kiaei *et al.* 2014). It is controversial whether the quality of fibers from branchwood meets the requirements for pulping and papermaking. Many researchers found that branches have smaller wood fibers than the trunks for hardwood species, which could be one of the primary limitations for using branchwood in pulping (Ververis *et al.* 2004; Zhao *et al.* 2020). However, some studies also showed contradictory results. For example, Mendoza *et al.* (2019) found that the fiber dimensions of trunkwood and branchwood of *Pterocarpus indicus* were statistically the same, except for the fiber length. Dadzie *et al.* (2016) even found that the fibers in the branchwood of *Pterygota macrocarpa* were larger than the fibers in the trunkwood. The authors also found in a previous study on *Populus ussuriensis* that the fibers in the branchwood were slightly larger than those in the trunkwood (Zhao *et al.* 2018). These inconsistent results may be due to the different tree species, larger proportion of tension wood in the branches (Tsai *et al.* 2012), and the variable branch diameter, which is generally related to the age of the cambium and the distance from the apical meristem (Nicolini *et al.* 2001; Peterson *et al.*

2007).

The purpose of this exploration activity was to identify and characterize the fiber morphology of the pruned branches from walnut plantations, and to provide recommendations on which branches could have potential for pulping and papermaking. In addition, this study will determine whether the branches could be considered as an alternative to pulp logs for providing various wood raw materials, based on the branch diameter, branching level, and tension wood.

EXPERIMENTAL

Material

Branches were taken from an intensively-cultured walnut orchard situated in the western region of Luoyang City in Central China (112°31’E, 34°58’N. The orchard is even-aged (18-year-old) and pure. The branches were pruned from the walnut trees using renewal pruning at the end of the growing season (November 2020). The diameter and branching level were recorded for each branch. For the branching levels, they were labeled according to Zhao (2015). One 5 cm thick disc sample was taken from each branch, the tension and opposite wood zone were identified according to the eccentricity, and then small wood chips were cut from the tension and opposite wood zone (as shown in Fig. 1).

Fig. 1. The selection process of samples containing tension wood (TW) and opposite wood (OW)

Methods

The wood chips were macerated in a 1 to 1 ratio of 10% chromic acid to 10% nitric acid solution at a temperature of 60 °C (Jeffrey 1917). The macerated fibers were rinsed and placed on microscopic slides for taking photographs using a digital microscope (Mshot-MD50, Micro-shot Technology Limited, Guangzhou, China). The fiber dimensions were measured with an image computer analysis system (TDY, version 5.2, Beijing Tian Di Yu Technology Co. Ltd., Beijing, China), which included the fiber length, fiber width, lumen diameter, and double wall thickness. For this test, it was necessary to measure 100 fibers per chip sample. Two derived values, *i.e.*, the slenderness ratio (the fiber length to fiber width) and Runkel ratio (the double wall thickness to lumen diameter) were calculated using measured fiber dimensions (Ohshima *et al.* 2005).

Data Analysis

The fitting curves of the fiber dimensions and derived values distributions were developed using a normal distribution function, and the skewness and kurtosis were used to check the normality of the data sets. A linear mixed model was performed with a restricted maximum likelihood method to analyze the significance of the three fixed effects, *i.e.*, the branch diameter, branching level, and tension wood, and their interactions. Otherwise, random effects, *i.e.*, the tree individual level and residuals, were concluded. Statistical significance (a p-value equal to 0.05 or 0.01) of the effects was determined using the F-test (Goldstein 2011).

RESULTS AND DISCUSSION

Fiber Morphology

The total number of fibers measured was 6000. Most fibers were thick-walled and non-septate libriform fibers. Occasionally, separate fibers were present in the walnut branchwood (Fig. 2a). Gelatinous layers are found in many wood fibers, which are commonly referred to as gelatinous fibers. Collapse of the gels during macerating, resulting in very high fragmentation, is commonplace and has been observed easily in the lumen of gelatinous fibers (Fig. 2b). It is well known that gelatinous fibers are formed in the tension wood of angiosperms (Ruelle 2009). Tension wood is often formed in branches, so it is not surprising that a large number of gelatinous fibers were found in walnut branchwood (Tsai *et al.* 2012). The analysis in this study revealed no percentage difference in the number of any fiber type, although this result may also reflect variability in the sampling design.

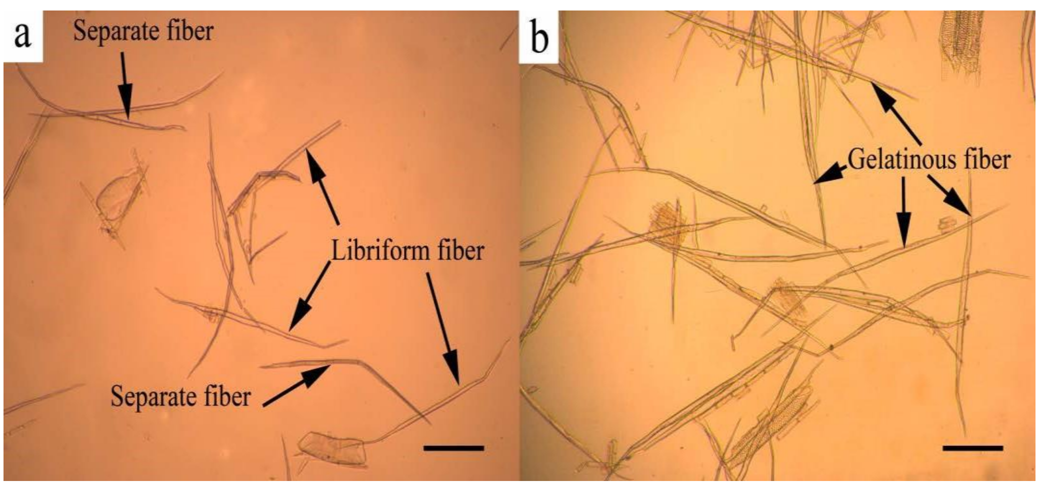

Fig. 2. Fiber morphology of walnut branchwood

The results for the fiber dimensions and their derived indices are summarized in Table 1. The average fiber length of 954 µm for walnut branchwood was larger than the value (893 µm) reported by Song *et al.* (2020) for walnut trunkwood and was not far from the 700 µm to 1600 µm values reported by Kong and Wei (2000) for hardwood sources. The average fiber length value met the intermediate standard stipulated by the International

Society of Wood Anatomy (Chalk *et al.* 1937). Fiber length is a key factor in selecting wood for the production of high-quality pulp. The strength properties of paper have been reduced by shortening the fibers, especially the tearing strength and folding endurance (Wimmer *et al.* 2002). The relationship between the fiber length and the mechanical strength of paper may be due to the large contact area between longer fibers, with the contact area also being related to the fiber width (Pulkkinen and Alopaeus 2012). The walnut branchwood had a narrower fiber width (average 20 µm) than the trunkwood reported by Song *et al.* (2020) and was similar to certain wood resources (Huang and Chen 2014) and non-wood resources (Ververis *et al.* 2004). The fibers are longer and narrower, resulting in a larger slenderness ratio (as shown in Table 1, sixth row). The average slenderness ratio of the fibers for walnut branchwood was 46.5, which was larger than the value of 39 reported by Song *et al.* (2020) for trunkwood and not far from the values of 32 to 58 reported by Kong and Wei (2000) for other various hardwood species. The slenderness ratio is also an important parameter reflecting the performance of the paper. For example, there is a positive relationship between the slenderness ratio and the folding endurance (Ona *et al.* 2001). Xu *et al.* (2006) suggested an approximate value of 33 as an acceptable value for the slenderness ratio in papermaking, while Bao and Jiang (1998) and Zhao *et al.* (2020) suggested an average value greater than 40. In any case, the computed slenderness ratio implies the fibers of walnut branchwood are suitable for papermaking.

Table 1. Descriptive Statistics of the Fiber Morphology

Variables	Mean	Minimum	Maximum	Standard Deviation	Skewness	Kurtosi
Fiber length (µm)	954.307	461.590	1509.320	139.583	0.155	-0.476
Fiber width (µm)	20.764	9.873	36.027	3.173	0.879	1.244
Lumen diameter (µm)	10.894	4.859	26.446	2.662	-0.659	0.825
Double wall thickness (µm)	9.870	3.520	17.760	1.456	-0.193	1.617
Slenderness ratio	46.526	18.941	87.390	6.825	1.430	2.274
Runkel ratio	0.945	0.146	1.868	0.201	-0.859	0.541

The average double wall thickness of the walnut branchwood fibers was 9.87 µm (Table 1, fifth row) which was similar to the double wall thickness of trunkwood (10.02 µm) (Song *et al.* 2020), as well as *Populus ussuriensis* branchwood (10.16 µm) (Zhao *et al.* 2018). The thicker wall of the fibers resulted in greater flexibility of the fibers during the pulp refining process, but a greater void volume in manufactured paper (Kiaei *et al.* 2014). The average lumen width of the walnut branchwood fiber was 10.9 µm (Table 1, seventh row) which was lower than the lumen width of the walnut trunkwood (12.9 µm) fiber (Song *et al.* 2020). The average lumen width of a fiber affects the beating of the pulp. The larger the lumen width, the easier the beating of the pulp, since liquids can penetrate into the empty spaces of the fibers (Kiaei *et al.* 2014). From this aspect, the fibers of walnut branchwood are adequate for making paper. The average Runkel ratio of the walnut branchwood fibers was 0.945, which was less than 1 (Table 1, eighth row). According to previous studies by Ohshima *et al.* (2005) and Kiaei *et al.* (2014), fibers with a Runkel ratio less than 1 would collapse and provide a large surface area for bonding during papermaking. Therefore, the calculated Runkel ratios for the fibers from walnut branchwoods are suitable for papermaking.

Distributions of the Fiber Dimensions and their Derived Indices

The fiber dimensions showed comparatively high standard deviation values (as shown in Table 1, fourth column), which indicated large variations within the population. Since wood is an inhomogeneous material, the fiber dimensions vary considerably (Zobel and Buijtenen 1989). The distributions of the fiber dimensions and their derived indices can be seen in Fig. 3. When comparing histograms plotted with the expected normal frequency curves, it appeared that the fiber dimension data was adequately represented by the normal distribution (Fig. 3). Referring to West *et al.* (1995), the data seemed to satisfy the normality assumption (an absolute skewness less than 2.1 and an absolute kurtosis less than 7.1), despite the fact that the histogram of the parameters did not appear to have a symmetrical bell shape but was often slightly skewed. The fiber length data seemed to fit more to a rectangular distribution, with numerous values around the mean (approximately 20%) but had a sharp decrease in the measured frequencies (lower than 12%) at some length (50 µm) from the mean (Fig. 3a). Almost all fibers were longer than 600 µm, while approximately 65% of them were longer than 900 µm. Walnut branchwood had a positively skewed width distribution of the fibers (Fig. 3b), which could be the primary factor for the negatively skewed slenderness distribution (Fig. 3c). In addition, 97% of the fibers had a slenderness ratio greater than 33 and 95% of the fibers had a slenderness ratio greater than 40.

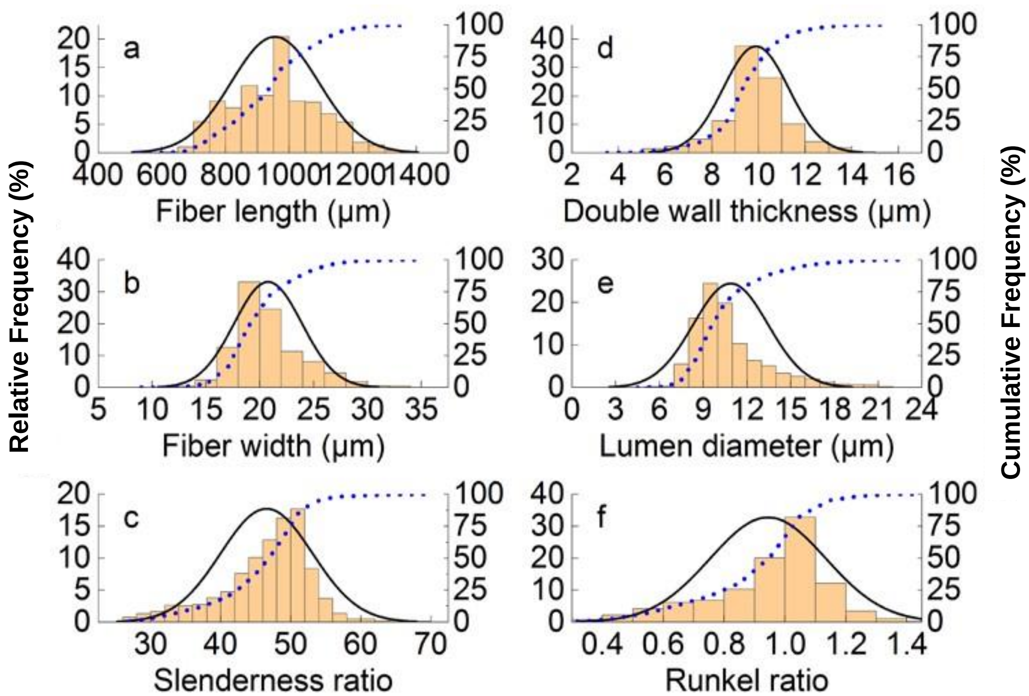

Fig. 3. Relative frequency (histogram), cumulative frequency (dotted line), and fitted normal distribution (solid line) of the wood fiber dimensions (note: Sk is skewness and Ku is Kurtosi)

It is shown that the normal double wall thickness frequency curves were similar to the corresponding measured histograms (Fig. 3d). The spread and the skewness of the fiber wall thickness distribution can considerably affect the hygroexpansivity (Pulkkinen *et al.* 2008). The frequency distributions of the lumen diameter showed a clear right skewed curve (as shown in Fig. 3e), which resulted in left skewed wall-lumen ratio distributions with one distinct peak (a skewness of -0.859 and a kurtosis of 0.541). In addition, 67% of the fibers had a Runkel ratio less than 1 (as shown in Fig. 3f). The distributions of the fiber dimensions are probably more important than their mean values in controlling pulp quality, because in the production of pulp, the ideal fiber mixture should contain fibers of different lengths and thicknesses at the same time (Pulkkinen *et al.* 2006). The percentage of fibers with different dimensions is often different for different quality paper. For example, in high-quality paper, long and thin fibers are more prominent, while in low-grade paper, short and thick fibers are more represented. The measurement results of the fiber dimension distribution show that walnut branch wood is more suitable for providing raw materials for high-quality paper (*e.g.* copy paper).

The large variations in the fiber morphological parameters were analyzed using a linear mixed model (as shown in Table 2). In the random effect, in addition to the inevitable residual, the selection of the sample tree may also lead to morphological variation in the fibers. Analysis of variance confirmed that the effect of the tree level on the parameters was not significant (as shown in Table 2, ninth row), primarily due to the same site and silviculture. The effects of the branch diameter, branching level, and tension wood on most parameters were significant.

Table 2. Linear Mixed Model of the Analysis of Variance and the F Value for the Fixed Effects and Variance 190 Components Rate for The Random Effects

Dependent Variables		Fiber Length	Fiber Width	Lumen Diameter	Double Wall Thickness	Slenderness Ratio	Runkel Ratio
Fixed effects	BL	57.561**	10.331**	28.409**	24.059**	89.377**	49.320**
	BD	89.331**	38.930**	2.285	135.611**	321.757**	32.401**
	TW	0.507	31.675**	8.119**	59.854**	32.100**	0.706
	BL×BD	60.856**	3.862*	9.224**	63.862**	133.169**	51.541**
	BL×TW	4.037*	17.844**	12.914**	83.613**	25.737**	45.912**
	BD×TW	0.014	52.531**	2.320	193.884**	77.184**	32.574**
	BL×BD×TW	2.310	25.784**	16.984**	123.077**	46.832**	62.998**
Random effects (%)	TL	3.451	11.297	14.680	11.561	23.139	23.440
	Residuals	96.549 **	88.703**	85.320 **	88.439 **	76.861 **	76.560 **

Note: BL is branching level; TW is tension wood; BD is branch diameter; and TL is tree individual level; * is a *p*-value less than 0.05 and ** is a *p*-value less than 0.01

The Effect of the Branch Diameter

The relationship between the fiber morphology and branch diameter was explored, revealing an increase in the fiber dimension and a decrease in the derived indices as the branch diameter increased (Table 3). Compared to thick branches, thin branches had smaller fibers, which may be due to the effects of the cambial age and the control of the meristem at the top of the branches (Nicolini *et al.* 2001; Peterson *et al.* 2007). The branch diameter is an important tree variable in plantation management because it has considerable influence on the tree growth, timber quality, fruit yield, and important physiological processes (Dong *et al.* 2016; Royer-Tardif *et al.* 2017; Jin *et al.* 2019). Although significant correlations exist between the fiber morphometrics and the diameters of the branches at *p*-value less than 0.05 or *p*-value less than 0.01 levels, the branch diameter may not be adequate to forecast fiber morphometrics, due to the absolute value of Pearson's correlation coefficients (r is less than 0.13).

Table 3. Correlation Analysis between the Branch Diameter and the Fiber Morphological Parameters

	Fiber Length	Fiber Width	Lumen Diameter	Double Wall Thickness	Slenderness Ratio	Runkel Ratio
r	0.028*	0.118**	0.125**	0.030*	-0.077**	-0.085**
p	0.032	0.000	0.000	0.021	0.000	0.000
Number	6000	6000	6000	6000	6000	6000
Note: r is the Pearson's correlation coefficient; and p is the significance level; * is a *p*-value less than 0.05 and ** is a *p*-value less than 0.01						

The Effect of the Branching Level

The difference in the fiber morphology among the walnut branching levels was analyzed. The research results are presented in Table 4.

Table 4. Multiple Comparisons of the Fiber Morphology among the Branching Levels

Branching Level	Fiber Length (µm)	Fiber Width (µm)	Lumen Diameter (µm)	Double Wall Thickness (µm)	Slenderness Ratio	Runkel Ratio
Primary branch	970.247 ± 143.641a	21.352 ± 3.407a	11.369 ± 3.010a	9.982 ± 1.490a	46.109 ± 7.115b	0.925 ± 0.215b
Secondary branch	943.321 ± 134.392b	20.191 ± 3.052c	10.53 ± 2.493c	9.661 ± 1.511b	47.256 ± 6.562a	0.954 ± 0.204a
Tertiary branch	949.353 ± 139.199b	20.749 ± 2.936b	10.784 ± 2.369b	9.965 ± 1.338a	46.213 ± 6.731b	0.956 ± 0.181a
Note: means ± standard deviation with different letters within a column were significant at a *p*-value less than 0.05						

The average fiber length extended up to 970, 943, and 949 µm in the primary, secondary, and tertiary branches, respectively (Table 4, second row). Although the average fiber length of the secondary and tertiary branches was significantly lower than the average fiber length of the primary branch, it was also greater than 900 µm, which meets the length requirement in pulping and papermaking (Chalk *et al.* 1937). The primary branch had the longest fibers. However, the shortest fibers were not in the tertiary branch, but in the secondary branch. Different levels of branches varied greatly, including the branch morphology, leaves, flowering, fruit bearing, *etc.* (Suzuki 2002; Kawamura and Takeda 2006; Jarčuška and Milla 2012). In general, the length and diameter of the cells decreased as the branching level increased (Sellin *et al.* 2008; Zhao 2015). The authors suggested that the reason for this was that crown characteristics of the trees growing in timber forests differed from those of growing in non-timber forests due to selective pruning regimes (Ustin *et al.* 1991; Meng and Zhang 2016; Zhang 2021). The results of this study showed that the fiber dimensions of all branching levels were within the acceptable range for papermaking. For instance, the average slenderness ratio of the primary branch fiber was the smallest, but it was greater than 40 (Table 4, sixth row). The average Runkel ratio of the primary branch fiber was the largest, but it was less than 1 (Table 4, seventh row). Thus, the screening of fiber raw materials is hardly supported by the branching leveling.

The Effect of the Tension Wood

The averages of the fiber morphological parameters of the tension and opposite wood are presented in Table 5.

Table 5. Difference of the Fiber Morphology between the Tension and Opposite Wood

Samples position	Fiber Length (µm)	Fiber Width (µm)	Lumen Diameter (µm)	Double Wall Thickness (µm)	Slenderness Ratio	Runkel Ratio
Tension wood	956.983 ± 138.217a	20.663 ± 3.155b	10.888 ± 2.657a	9.774 ± 1.499b	46.874 ± 6.799a	0.938 ± 0.204a
Opposite wood	951.631 ± 14.907a	20.865 ± 3.189a	10.900 ± 2.667a	9.965 ± 1.405a	46.178 ± 6.834b	0.852 ± 0.197b
Note: means ± standard deviation with different letters within a column were significant at a *p*-value less than 0.05						

No statistical differences were seen between the tension and opposite wood in terms of the fiber length (Table 5, second row), but there was a significant difference in the fiber width (Table 5, third row). Thus, the fibers of the tension wood were slenderer than the fibers of the opposite wood, which was confirmed by the slenderness ratio of the tension and opposite wood (Table 5, sixth row). The average double wall thickness of the tension wood was 9.774 µm ± 1.499 µm, whereas the average double wall thickness of the opposite wood was 9.965 µm ±1.405 µm. Therefore, the fibers of the tension wood were significantly thinner than the fibers of the opposite wood (Table 5, fifth row), while the difference in the fiber lumen diameter was not significant (Table 5, fourth row). The morphological difference in the fibers between the tension and opposite wood studied here agreed in general terms with previous descriptions of other hardwoods, *e.g.*, *Quercus mongolica* (Lee *et al.* 1997), *Populus* × *euramericana* (Niu *et al.* 2010), *Magnolia obovate*, and *Magnolia kobus* (Yoshizawa *et al.* 2000). As previously mentioned, tension wood is rich in gelatinous fibers, and the gelatinous layer has a high cellulose content, which is a favorable factor for kraft pulping and papermaking (Aguayo *et al.* 2012). It should be mentioned that in this study, which deals with walnut branchwood, the fiber morphology was not measured in other xylem, *i.e.*, lateral wood, apart from the tension and opposite wood. Nevertheless, most studies dealing with tension wood of the angiosperms reported that the lateral wood also contained gelatinous fibers, but the number of gelatinous fibers was less than that of the tension wood (Yue *et al.* 2014). The fiber dimension of the lateral wood was rather intermediary between that of the tension and opposite wood (Yoshizawa *et al.* 2000; Yue *et al.* 2014). From the measurement results of the fiber morphology, it can be concluded that the tension and opposite wood of walnut branches meet the requirements for pulping performance and can all be used as pulping wood.

CONCLUSIONS

1. The morphology of the fibers of walnut branchwood is quite good for the purpose of paper manufacturing.
2. The diameter of the branches does not provide adequate data from which to forecast the fiber morphometrics.
3. The branching level and tension are of little help in the screening of fiber raw materials.
4. The presence of stress wood is a favorable factor for walnut branches to be used as pulping and papermaking wood.

ACKNOWLEDGMENTS

The authors acknowledge the National Science Foundation of China (Grant No. 32171701) for financial support. The authors thank the students of the Henan University of Science and Technology for harvesting and collecting the branch samples.

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Article submitted: January 5, 2022; Peer review completed: February 20, 2022; Revised version received and accepted: February 22, 2022; Published: February 24, 2022.
DOI: 10.15376/biores.17.2.2214-2227