Influence of Gender on the Mechanical and Physical Properties of Hemp Shiv Fiber Cell Wall in Dioecious Hemp Plant

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Hemp (*Cannabis sativa* L.) shiv has great potential for the production of bio-composites as a reinforcement material. To gain more information about hemp shiv, this research studied the influence of gender on the physical and mechanical properties of the fiber cell wall in the shiv of three dioecious hemp plant varieties by optical microscopy, image analysis software, WXRD, and nanoindentation. The results show that a hemp plant's gender greatly influences the properties of hemp shiv. While long and thin in female hemp shiv, the fibers are shorter with a larger diameter in male hemp shiv. In addition, the cell walls in female shiv are thinner than those in male shiv. The microfibril angle (MFA), relative degree of crystallinity, elastic modulus, and hardness values of fiber cell walls as well as the lignin content in male hemp plants are higher than those in female hemp plants. Besides, the relationship between mechanical properties and MFA do not align with those observed in previous research, which shows that the gender of an individual plant has a greater effect on the mechanical properties of the fiber cell wall than does its MFA. Thus, when the fiber from this dioecious plant is investigated or used, the sex of the plant should be known and considered.

Keywords: Different varieties; Gender; Hemp shiv; Fiber cell wall; Nanoindentation

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INTRODUCTION

Hemp (*Cannabis sativa* L.) is a plant that has been cultivated since prehistoric times. Now, as oil resources are being depleted, the synthetic fabric industry is being affected and more attention is being paid to natural fibers. Hemp has attracted attention due to its excellent antibacterial resistance, high fiber yield without fertilizer, and consistent growth under a wide range of agro-ecological conditions, especially in Europe and China (Cosentino *et al.* 2012). Hemp fiber is of high quality and has good mechanical properties. Textiles (Soljacic and Cunko 1994) and composites made from hemp also have good mechanical properties (Sawpan *et al.* 2011; Beaugrand *et al.* 2014).

The structure of hemp shiv resembles that of hardwood (shown in Fig. 1d). The hemp shiv is the largest part of the hemp core (Fig. 1c) and is a byproduct of hemp bast fiber production. Hemp shiv constitutes more than twice the weight of the bast fiber in a hemp plant. Thus, the question of how to use hemp shiv efficiently is key to promoting and developing the hemp fiber industry.

This study of the mechanical properties of the fiber cell walls of hemp shiv provides valuable information regarding potential uses.

Most varieties of the hemp plant are dioecious, while a few are monoecious. According to Faux *et al*. (2013), "flowering phenology and sexual dimorphism are two major features that affect stem and seed production in hemp (*Cannabis sativa* L.), a short-day naturally dioecious plant." Some surveys have studied the influence of plant gender on the quality, chemical properties, physical properties, and quantity of hemp fiber (Keller *et al.* 2001; Zhang *et al.* 2009), but there is little information about the influence of gender on the mechanical properties of the fiber cell wall in hemp shiv. Such research can provide useful information regarding the manipulation of hemp *via* genetic technology to obtain a material with ideal mechanical properties (Techen *et al*. 2010).

To study the mechanical properties of materials at the microscopic scale, the nanoindentation method is a strong tool. Since 1997 (Wimmer *et al.* 1997), it has been used to study the mechanical properties of fiber cell wall in woody materials. Until now, nanoindentation has also been widely used to research the mechanical properties of fiber cell walls in agricultural crop stalks (Wu *et al.* 2010; Wang *et al.* 2013), bamboo (Yu *et al.* 2007), hardwoods (Wu *et al.* 2009), and softwoods (Wimmer *et al.* 1997).

The objective of this study was to investigate the influence of gender on the mechanical properties (*i.e.,* hardness and elastic modulus) of the shiv fiber cell wall in three varieties of hemp, as well as to study the influence of gender on the physical properties (*i.e.,* microfibril angle, relative degree of crystallinity, and fiber size) and the lignin content of hemp shiv fiber in the three varieties of dioecious hemp.

EXPERIMENTAL

Materials

Samples of three local industrial hemp (*Cannabis sativa* L.) varieties (*i.e.*, Yunma No. 2, Yunma No. 3, and Yunma No. 4), were used in this study. The time to maturity for Yunma No. 2 is 120 days; it grows to 2.4 to 3.0 m in height, is on average 1.3 to 1.7 cm in diameter, and the oil content of the seeds is approximately 33.1%. The THC (tetrahydrocannabinol) content is 0.059%, and the ratio of female plants to male plants is 2.2:1. For Yunma No. 3, the time to maturity is 120 days, its height averages 3.0 m, diameter averages 2.1 cm, and the seed oil content is 34.3%. The THC content is 0.13%, and the ratio of female plants to male plants is 1.8:1. The time to maturity of Yunma No. 4 is 130 days, it grows to 3.1 m in height, the seed oil content averages 33.2%, and the THC content is 0.16%. The ratio of female plants to male plants is 2.0:1.

These three hybrid varieties come from the same female and male parent plants, but the physiological and physical properties of the hybrids are different. The three varieties were harvested from the same field and were kindly provided by the Yunnan Industry Hemp Co., Ltd. (Kunming, Yunnan, China).

The male and female stalks of the three varieties were tested separately to study the influence of gender on the mechanical, physical, and chemical properties of the fiber cell wall. Six sample groups of different genders were obtained from the same stem location, about 100 cm above the base of the stems (Fig. 1b).

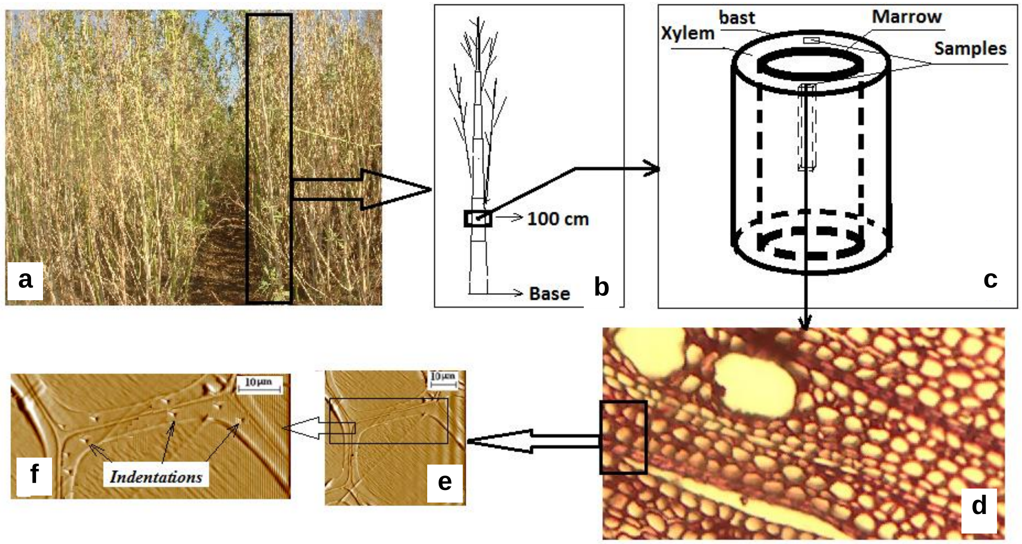

Fig. 1. Structure of hemp shiv: (a) Industrial hemp plant; (b) The location of samples based on the hemp shiv; (c) Cross-section of hemp shiv; d) The structure of xylem in the hemp stalk; (e) The structure of hemp stalk fiber cell wall; and (f) Indentations on fiber cell wall

Methods

Measuring fiber size

Small, matchstick-shaped samples (20 mm (length) X 1 mm (width) X 1 mm (thickness)) were cut from six sample groups and placed into boiling distilled water until they sank to the bottom of the container. The samples were then moved to another container and boiled in a 1:1 (v/v) mixture of 30% H_2O_2 (analytical reagent, Tianjin Binhai Fengchuan Potassium Hydroxide Reagent Co. Ltd., Binhai District, P.R. China) and glacial acetic acid (analytical reagent, Tianjin Binhai Fengchuan Potassium Hydroxide Reagent Co. Ltd., Tianjin, P.R. China) for 12 h at 60 °C. The samples were washed with distilled water until no acid remained in the fibers, with a final pH value approximately 7.0. The bundle was then separated into individual fibers. Fibers were stained with 1% Safranine solution and placed on temporary slides; the coverslips were placed over the fibers, and the fibers size were tested as those were dry. The length, diameter, and lumen diameter of the 200 fibers per sample was measured with a Motic Images Plus 2.0 image analysis system (China) in the Wood Science Laboratory, College of Materials Engineering, Southwest Forestry University (Kunming, Yunnan, China).

Measuring microfibril angle (MFA) and relative degree of crystallinity

To investigate the influence of gender on the physical properties of the fiber cell walls, the microfibril angle (MFA) and relative degree of crystallinity of the six groups of samples were tested by wide angle X-ray diffraction (WXRD) using a DX-200 Materials Research Diffractometer (Dandong Fangyuan Instrument Co. LTD. China) with a Cu-Kα radiation source. Details of the procedure are described in the work of Li *et al.* (2014).

Nanoindentation

The six sample groups for nanoindentation testing were cut into small pieces with dimensions of about 2 mm x 2 mm x 6 mm following oven-drying. The embedding medium added into the plastic mold was epoxy resin (Electron Microscopy Sciences, 1560 Industry Road Hatfield, PA 19440), which was composed of cycloaliphatic epoxide resin (ERL-4221) (2.5 parts), polycoldieposide (DER-736) (1.5 parts), nonenyl succinic anhydride (NSA) (6.5 parts), and dimethylaminoethanol (DMAE) (0.1 parts) (Meng *et al.* 2013; Li *et al.* 2014). Only one sample group was used for each hemp shiv because the mechanical properties of different hemp shiv do not differ greatly (Beaugrand *et al.* 2014). The embedded samples were placed in an oven under vacuum for 30 to 40 min to remove the air from the samples before being cured in the same oven at 70 °C for 8 to 10 h. The details of this method are described in Li *et al.* (2013; 2014). The cured samples were trimmed to remove the epoxy resin and reveal the face of the hemp stalk surface. Thick sections of the sample were cut with a glass knife to smooth the face, and thin sections were then cut with a diamond knife. The thin sections were put into a special container to protect their surfaces. All nanoindentation experiments were performed at the Center for Renewable Carbon using a TriboIndenter nanomechanical test instrument (Hysitron, Inc., USA). A Berkovich indenter (a three-sided pyramid with an area-to-depth function) was used for all experiments, and the indentation was performed in a displacement-controlled mode in three discrete segments. In the first segment, loading force was applied at a constant displacement rate of 5 nm/s until the intended indentation depth of 200 nm was reached. In the second segment, the maximum force was held at a constant depth for 10 s. In the third and final segment, the sample was unloaded at a constant displacement rate of 10 nm/s until 90% of the loading force was removed. This process is described in detail by Wu *et al.* (2009) and Li *et al.* (2014). Forty to 60 indentations were made on cross-sections of six to ten cell walls for each sample (Figs. 1e and 1f). Finally, the hardness (H) and the elastic modulus (E) were calculated from the load-displacement data *via* the methods described by Oliver and Pharr (1992).

RESULTS AND DISCUSSION

Physical Properties of Fiber Cell Walls

The physical properties of hemp shiv fiber include the fiber size, the relative degree of crystallinity, and the MFA (Tables 1 and 2). According to the data in Tables 1 and 2, the gender of a plant greatly influences the physical properties of the material. In the three varieties examined in this study (Yunma Nos. 2, 3, and 4), the fiber length of the male hemp plant was shorter than that of the female hemp plant (p-value was < 0.01 with SAS software), while the diameter and lumen diameter of the male hemp plant were larger than those of the female hemp plant (p-value was <0.01 with SAS software). The thickness of the male hemp plant was observed to be greater than that of the female hemp plant (p-value was <0.05 with SAS software).

To summarize, the fibers are long and thin in female plants, but shorter with a larger diameter in males. This may be because the primary function of the female hemp plant is to produce seed, whereas the primary function of the male hemp plant is simply to produce the blossom. For two given female and male plants of the same height, there will be fewer fiber cells in the female plant than in the male plant. In addition, the cell wall of the fiber will be thicker in the male hemp plant because the female hemp plant does not need to expend a lot of energy and material building the fiber cell wall, but rather it can expend that energy and matter on seed production.

Table 1. Fiber Size in Male and Female Hemp Plants

Variety	Gender	Length (mm)		Diameter (μm)		Lumen Diameter (μm)		Cell Wall Thickness (μm)	
		Average	Range	Average	Range	Average	Range	Average	Range
Yunma No. 2	Female	0.53 (0.12)	0.25 to 0.79	29.3 (8.37)	11.9 to 56.6	21.2 (6.31)	7.80 to 40.0	4.03 (1.23)	0.97 to 9.50
	Male	0.43 (0.11)	0.22 to 0.80	33.5 (8.19)	13.5 to 66.8	24.9 (5.81)	10.0 to 60.0	4.31 (1.09)	0.61 to 9.95
Yunma No. 3	Female	0.49 (0.13)	0.24 to 0.80	29.82 (7.63)	14.0 to 65.0	21.1 (6.04)	9.40 to 46.0	4.38 (1.28)	0.25 to 14.1
	Male	0.48 (0.11)	0.20 to 0.80	36.3 (8.45)	15.4 to 75.0	26.9 (5.93)	11.0 to 60.1	4.67 (1.07)	0.14 to 10.6
Yunma No. 4	Female	0.49 (0.11)	0.18 to 0.80	27.2 (8.02)	13.5 to 60.0	18.9 (5.98)	7.80 to 9.0	4.14 (1.21)	0.15 to 9.00
	Male	0.48 (0.10)	0.22 to 0.80	30.7 (7.87)	12.6 to 50.0	21.4 (6.18)	9.40 to 45.0	4.64 (1.44)	0.69 to 10.00

Note: The standard deviations in parentheses represent variations in the length, diameter, lumen diameter, and cell wall thickness, respectively

Table 2. Chemical, Physical, and Mechanical Properties of the Fiber Cell Wall

Variety	Gender	Lignin Content (%)	MFA (°)	Relative Degree of Crystallinity (%)	Elastic Modulus (GPa)	Hardness (GPa)
Yunma No. 2	Female	20.8 (0.07)	10.2 (0.07)	38.8 (0.21)	8.28 (1.01)	0.37 (0.05)
	Male	21.5 (0.06)	12.5 (0.05)	49.3(0.29)	16.02 (1.89)	0.45 (0.03)
Yunma No. 3	Female	19.8 (0.05)	8.70 (0.04)	48.6(0.29)	15.24 (1.35)	0.40 (0.06)
	Male	20.9 (0.06)	11.1(0.03)	50.1 (0.32)	16.54 (1.48)	0.48 (0.07)
Yunma No. 4	Female	20.3 (0.04)	9.75 (0.06)	46.5 (0.28)	11.44 (1.26)	0.42 (0.05)
	Male	21.2 (0.05)	12.8(0.04)	48.6(0.31)	16.62 (1.73)	0.51 (0.06)

Note: The standard deviations in parentheses represent variations in the lignin content, MFA, relative degree of crystallinity, elastic modulus, and hardness, respectively

The cell wall is composed of cellulose, hemicellulose, lignin, and other compounds. These chemical constituents are distributed throughout different layers of the cell wall, such as the primary wall (P) and secondary wall (which is further divided in to the S1 layer, the S2 layer, and the S3 layer). Cellulose forms the cytoskeleton of the fiber cell wall, and the basic structural unit of cellulose is the microfibril, which consists of a crystalline region and an amorphous region. The ratio of the crystalline region to the amorphous region is called the relative degree of crystallinity. The relative crystallinity is one important indicator of the properties of the fiber; if the relative crystallinity of fiber is high, then the mechanical strength of the fiber is also likely to be high. When the relative crystallinity of a fiber is low, the fiber can more easily form interfaces with other substances when used in composites, and there will be more exposed groups that can form new chemical bonds with other materials (Yang 2001). Of the secondary wall layers, the S2 layer is the thickest, and the spiral angle of cellulose fibrils in this layer, the microfibril angle (MFA) (which was measured in this experiment), is considered to be the single most influential factor in understanding a fiber's mechanical properties (Page *et al.* 1977).

In this research, the MFA and relative degree of crystallinity of fiber cell walls were found to be larger in male hemp plants than in female plants (p-value was <0.01 with SAS software). The MFA values of male plants (11.1 to 12.8°) were higher than those of female plants (8.70 to 12.2°), representing changes of about 22% to 31%. The relative degree of crystallinity of male plants (48.6% to 50.1%) was higher than those of female plants (38.8% to 48.6%), representing changes of about 3% to 27% (Table 2). The results show that the sex of the plant has a great influence on the MFA and relative degree of crystallinity of the fiber cell wall; thus, when these properties of the fiber cell walls in dioecious plants are studied, the gender of the plant should be considered and mentioned in the research.

The Mechanical Properties of Fiber Cell Wall

The mechanical properties (including the elastic modulus and hardness) of the fiber cell walls in male hemp plants were found to be greater than those of the fiber cell walls in female hemp plants (p-value was <0.01 with SAS software) (Table 2). The elastic modulus values of fiber cell wall in male hemp plants (16.02 MPa to 16.62 MPa) were higher than those in female hemp plants (8.28 MPa to 15.25 MPa), which is a change of about 8 percent to 93 percent, and the hardness values of fiber cell wall in male hemp plants (0.45 MPa to 0.51 MPa) were higher than that in female hemp plant (0.37 MPa to 0.42 MPa), which would be a change of about 20 percent (Table 2). The two main influencing factors on the mechanical properties (including the elastic modulus and hardness) of fiber cell walls are the MFA and chemical characteristics (mainly the lignin content). In addition, a positive correlation exists between the lignin content and the mechanical properties of the fiber cell wall, especially the hardness (Gindl *et al.* 2004) and a negative correlation exists between the MFA and the mechanical properties of the hemp fiber cell wall (Konnerth *et al.* 2009).

However, in this research, the MFA of fiber cell walls in male hemp plants was determined to be larger than the MFA of female plants' fiber cell walls. If the influence of the MFA on the mechanical properties is the only factor considered, then it is difficult to explain why the mechanical properties of the fiber cell walls in male hemp plants were higher than those of the female plants (The correlation coefficients (r) between MFA and elastic modulus and hardness of cell wall were 0.47 and 0.76, respectively). At the same time, the lignin content of male hemp stalks (20.9% to 21.5%) was greater than that of female hemp stalks (19.8% to 20.8%) (a change of about 3.37 percent to 5.56 percent; Table 2), and male hemp plants' fiber cell walls' mechanical properties should be higher than those of the female plants. Thus, the sex of a plant has a great influence on the lignin content of the hemp shiv, causing sex-based differences between the mechanical properties of fiber cell walls in dioecious hemp plants.

CONCLUSIONS

1. As dioecious plants, female and male hemp plants play different roles in the survival and reproduction of the species. These differing roles cause significant differences (p-

values <0.05) in the physical, chemical, and mechanical properties of hemp shiv fiber cell walls in male and female hemp plants.

2. Fibers are thin and long in female hemp plants and shorter with a larger diameter in male hemp plants.
3. The MFA, relative degree of crystallinity, lignin content, and mechanical properties (including the elastic modulus and hardness) of hemp shiv fiber cell walls in male hemp plants are higher than those of the fiber cell walls in female hemp shiv.
4. The results of this study do not follow the relationship seen between the MFA and the mechanical properties of fiber cell walls in previous research (The correlation coefficients (r) between MFA and elastic modulus and hardness of cell wall were 0.47 and 0.76, respectively).
5. Finally, this research seems to indicate that the sex of plant has a more significant influence on the lignin content and the mechanical properties of fiber cell wall than previously understood. Alternatively, the gender of the plant can be controlled by gene manipulation technology to obtain a consistent material with better properties to satisfy commercial demand.

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