Profiling the Chemical Composition and Growth Strain of Giant Bamboo (*Dendrocalamus giganteus* Munro)

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The chemical composition of the wax layer and green epidermis at the surface of giant bamboo (*Dendrocalamus giganteus* Munro) culms were conveniently analyzed through the diffuse reflectance infrared Fourier transform (DRIFT) with Si-Carb sampling technique. Results from the radial lignin content profiling of giant bamboo showed that the lignin content in the middle layer was lower than the layers either from the inner or outer culms. As for the longitudinal depth profiling, the lignin contents of bamboo culms increased gradually from the top toward base portion. The distribution of growth strains in the radial direction of giant bamboo culm was investigated by the kerf method with strain gauges. The longitudinal tensile strains in various positions of giant bamboo culm were found to decrease in the order of the middle layer, the outer layer, and the inner layer. The tensile strains of different layers in the radial direction of giant bamboo culm correlate with their lignin content. The highest tensile strain on the middle layer of the bamboo culm was associated with the lowest lignin content. These results provided experimental evidence in the relationship between longitudinal tensile strain and lignin content of bamboo culm.

Keywords: Dendrocalamus giganteus; FTIR analysis; Growth strain; Lignin; Si-Carb sampling

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INTRODUCTION

Bamboo is an essential and renewable lignocellulosic resource, and the utilization of bamboo resources has increased tremendously. Due to the fast growing characteristics of bamboo, it has been recognized as a promising plant to sequester CO_2 and reduce the pressure on natural resources (Vogtlander *et al.* 2010; Cao *et al.* 2014; Escamilla and Habert 2014; Ren *et al.* 2014). Many researchers have reported the diverse properties of bamboo such as anatomical structure, physical and mechanical properties, chemical composition, and degradation (Li *et al.* 2007; 2010; Wang and Ren 2008; Rousset *et al.* 2011; Tomak *et al.* 2013; Wahab *et al.* 2013). Giant bamboo (*Dendrocalamus giganteus* Wallich ex Munro) belongs to the subfamily Bambusoideae of the family Poaceae (Gramineae). It is also known as dragon bamboo and is widely distributed in South-East Asia. Giant bamboo is the tallest among all the bamboo species and it is often used for food (edible bamboo shoot), construction, furniture, decoration materials, paper, and other raw materials (Ramanayake and Yakandawala 1997; Bonilla *et al.* 2010).

Growth stress accumulates in stem and twigs of plants during growth, and many biomechanical and biochemical assays have been applied to investigate the growth stress of plants and lignocellulosic materials (Yamamoto *et al.* 1991; Huang *et al.* 2001; 2005; 2010; Raymond *et al.* 2004; Clair *et al.* 2006; Tsai *et al.* 2012; Zhang *et al.* 2014). Most growth strain and growth stress investigations have focused on the reaction wood, including compression wood (*i.e.*, softwood, gymnosperms) and tension wood (*i.e.*, hardwood, dicotyledons). Less empirical research has been concerned with the growth strain and growth stress of the other woody plants.

A culm of the giant bamboo is thick-walled, with a thickness of 2.0 to 2.5 cm, and suitable for the analyses of chemical composition and growth strain of different bamboo culm sections. The study was performed to determine: (1) the surface chemical analysis of giant bamboo culm; (2) the radial and longitudinal depth profiling of lignin content of bamboo specimens by infrared analyses with the Si-Carb sampling technique; and (3) radial distribution of growth strain in giant bamboo culm.

EXPERIMENTAL

Materials

Fresh giant bamboo (*Dendrocalamus giganteus* Munro, 4 years of age) was collected from the Lienhuachi Research Center, Taiwan Forestry Research Institute, located in Nantou County in central Taiwan. The length of each bamboo node was 30 cm. For the abbreviation of bamboo specimens, the number IN denotes the ordinal number of the internodes with a length greater than 25 cm from the bottom (ground) to the top section of the bamboo plant.

Methods

DRIFT analysis with Si-Carb sampling

Diffuse reflectance infrared Fourier transform (DRIFT) spectra were obtained using a Fourier transform infrared spectrometer incorporating a Spectra Tech diffuse reflectance accessory unit (Bio-Rad FTS-40, USA). Depth profiles of specimens were analyzed by DRIFT spectroscopy with the Si-Carb sampling technique (Plonska-Brzezinska *et al.* 2011). The sampling method used Si-Carb abraders (Spectra-Tech Si-Carb Sample Prep Kit) to obtain sample powders from air-dried specimens. The scanning range was 600 to 4000 cm^{-1}, with a spectral resolution of 4 cm^{-1}. The number of specimen replications was three.

Measurement of growth strain

Growth strain is measured by the kerf method using a sensitive strain gauge device attached to different layers of giant bamboo culms (Huang *et al.* 2010; Tsai *et al.* 2012). The middle part of the culm internode was cut for a window approximately 20 cm long and 15 cm wide. The strain gauges were then glued onto the inner, middle, and outer layer of the culm internode in the radial direction. Each strain gauge was parallel to the bamboo fiber orientation. The strain gauge length used in this study was 5 mm, and the electrical resistance was 120 Ω (Kyowa KFW Type, Japan). The bridge output was delivered through a strain amplifier (Kyowa DPM 603A Model) to a data logger (Kyowa UCAM 10B Model and Yokogawa HP1300 Model, Japan), and the longitudinal growth

strains were recorded. Strain is defined as the elongation or contraction ratio. The unit of growth strain is expressed as micro strain ($\mu\varepsilon$) defined by Eq. 1,

$$\text{Micro strain } (\mu\varepsilon) = (L_0\text{-}L)/L_0 \times 10^6 \quad (1)$$

where L_0 is the original length of strain gauge and L is the final length of strain gauge.

Statistical Analysis

The Scheffe's multiple comparison procedure from the SAS 9.3 statistical program (Cary, NC USA) was employed to evaluate differences for the specimens and get results with a 95% confidence interval.

RESULTS AND DISCUSSION

Surface Chemical Composition Analysis of Giant Bamboo Culm

Plant waxes are hydrophobic hydrocarbons, including alkanes, esters, fatty acids, aldehydes, and alcohols (Koch and Ensikat 2008). Li *et al.* (2012) analyzed the leaf wax of 23 different bamboo species of three subgenera, *Dendrocalamus*, *Bambusa*, and *Dendrocalamopsis*, by gas chromatography-mass spectrometry (GC-MS) and concluded that the dominant waxes of three subgenera bamboos were much different. The main waxes of the *Dendrocalamus* species are C27 and C29 *n*-alkanes, and their average chain length (ACL) is 28.3. Leaf wax of giant bamboo contained 42.7% C27 *n*-alkanes and 39.8% C29 *n*-alkanes. Most authors have discussed the foliar cuticular waxes of plants, and few have investigated the composition of bamboo culm waxes by GC-MS or FTIR (Dubis *et al.* 1999; 2001; Li *et al.* 2012; Kitagami *et al.* 2013).

White fine powders of waxes were scraped from the surface of a giant bamboo culm by the Si-Carb abrader and analyzed by DRIFT spectroscopy. Figure 1a shows the FTIR spectrum of culm wax. The culm waxes displayed absorption bands at 805 cm^{-1} (alkene C-H), 968 cm^{-1} (alkene C-H), 1150 cm^{-1} (C-O), 1371 cm^{-1} (CH_3), 1422 cm^{-1} (CH_2), 1458 cm^{-1} (CH_2), 1508 cm^{-1} (aromatic ring), 1654 cm^{-1} (ketone C=O), 1725 cm^{-1} (ester/acid C=O), 2920 cm^{-1} (CH_2), and 3392 cm^{-1} (OH). According to the related FTIR spectrum of plant waxes (Dubis *et al.* 1999; 2001; Luz 2006; Athukorala *et al.* 2009), giant bamboo culm waxes are composed of saturated and unsaturated hydrocarbons, including alkanes, alcohols, ketones, aldehydes, fatty acids, and minor aromatic compounds.

After removing the wax layer by use of sand paper, light green powders were obtained from green epidermis of bamboo culm. Light green powders included the chlorophyll pigments and bamboo fibers. Accordingly, the FTIR spectrum (Fig. 1b) of green epidermis was similar to those of outer light yellow bamboo fibers (Fig. 2c), which were light yellow. The characteristic absorption bands of chlorophyll as stated in related work (Wang *et al.* 2007; Mecozzi *et al.* 2011) are 1650 cm^{-1} (amide C=O), 1540 cm^{-1} (amide C-N), 1230 cm^{-1} (amide N-H), and 1710-1740 cm^{-1} (ester C=O) in the FTIR spectrum. In comparison, the band at 1654 cm^{-1} observed in the spectrum of green epidermis (Fig. 1b) was more intense than the corresponding band in the outer bamboo fibers spectrum (Fig. 2c). The band of amide C=O of chlorophyll at 1654 cm^{-1} overlapped with the conjugated carbonyl (aryl ketone) band of lignin in the bamboo fiber (Table 1).

The addition of two kinds of C=O bands from chlorophyll and lignin of green epidermis led to an increase in the intensity of the band at 1654 cm^{-1} in the spectrum. These results illustrated that Si-Carb sampling technique is a quick and convenient method applied to the chemical analysis of lignocellulosic materials.

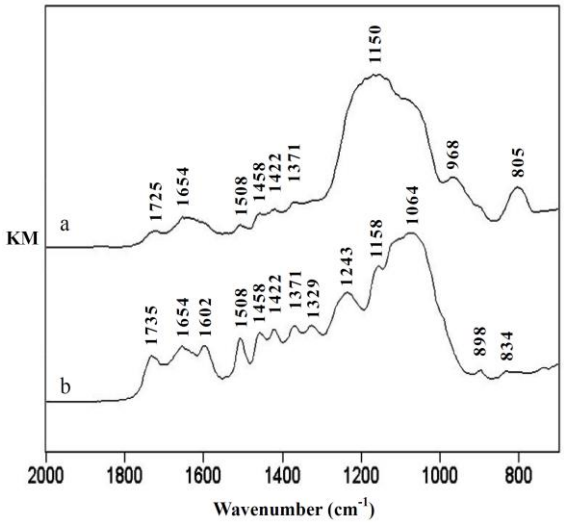

Fig. 1. FTIR spectra of the surface of giant bamboo culm IN40. (a) wax layer and (b) green epidermis

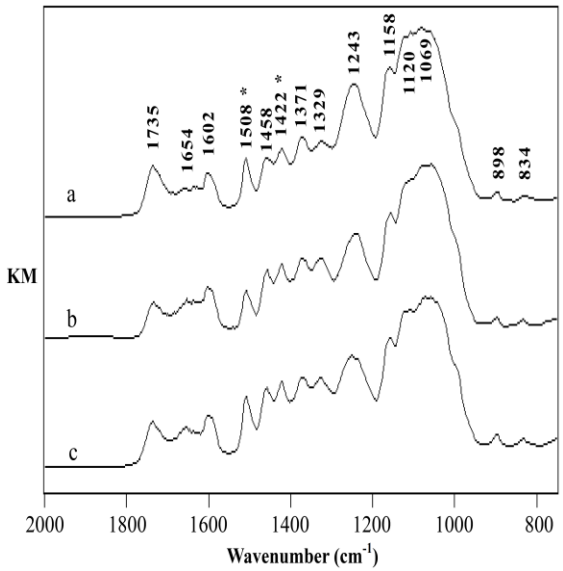

Fig. 2. FTIR spectra at various positions of giant bamboo culm IN40. (a) inner layer, (b) middle layer, and (c) outer layer

Radial and Longitudinal Depth Profiling of Lignin Content of Giant Bamboo

The Si-Carb samplings combined with DRIFT were used to profile the lignin variation of giant bamboo vertically and radially. Peak assignments of FTIR spectra of main compositions of bamboo were adapted considering the spectral data from related studies (Faix 1992; Li *et al.* 2010; Qi *et al.* 2013; Tomak *et al.* 2013; Peng *et al.* 2014), as shown in Table 1.

Table 1. Diffuse Reflectance FTIR Peak Assignments of Giant Bamboo

Wavenumber (cm^{-1})	Peak Assignments
3356	O-H stretching
2928	C-H and CH_2 stretching
1735	Nonconjugated carboxylic acid and their ester
1654	Conjugated carbonyl (aryl ketone)
1602	Lignin aromatic skeletal (ring) vibrations
1508	Lignin aromatic skeletal (ring) vibrations
1458	C-H deformation (asymmetric) & aromatic skeletal vibrations
1422	Methylene groups of both holocelluloses and lignin, C-H in-plane deformation of CH_2
1371	C-H in-plane deformation (symmetric) for polysaccharides
1329	Syringyl ring breathing and C-O stretching
	CH_2 wagging
	Guaiacyl ring breathing and C-O stretching
1243	Guaiacyl ring breathing and C-O stretching
1158	C-O-C antisymmetric bridge stretching vibration in cellulose & hemicelluloses
	p-Hydroxyphenyl ring breathing and C-O stretching
1120	C-O-C symmetric stretching & aromatic C-H in-plane deformation & glucose ring vibration
1069	C-O stretching in cellulose & hemicelluloses
898	C_1-H deformation of cellulose (β-anomeric linkage)
834	Lignin C-H out of plane deformation
	Glucomannan & aromatic C-H

Figures 2 and 3 show the FTIR spectra of the various layers of giant bamboo culms IN40 and IN8. The lignin content in lignocellulosic materials is estimated principally from the absorption intensity at 1508 cm^{-1}, which is the characteristic absorption of the lignin aromatic ring. The intensity of the 1508 cm^{-1} peak of the spectrum at the middle layer (Fig. 2b) was weaker than those of the inner and outer layers (Figs. 2a and 2c), indicating that the lignin content in the middle layer of bamboo culm was lower than the other layers.

The trend of lignin content in the three layers of the bamboo culm IN8 (Fig. 3) is similar to that of bamboo culm IN40. In addition, the stronger peak is at 1735 cm^{-1} in both FTIR spectra at the inner layer of bamboo culms N40 and N8 (Figs. 2a and 3a). The peak at 1735 cm^{-1} is theoretically assigned to nonconjugated carboxylic acids and ester groups (Table 1), originating from acetyl groups that bind to hemicelluloses such as glucomannan and xylan in lignocellulosic materials. It might reveal that the hemicelluloses content in the inner layer of bamboo is higher than the other positions.

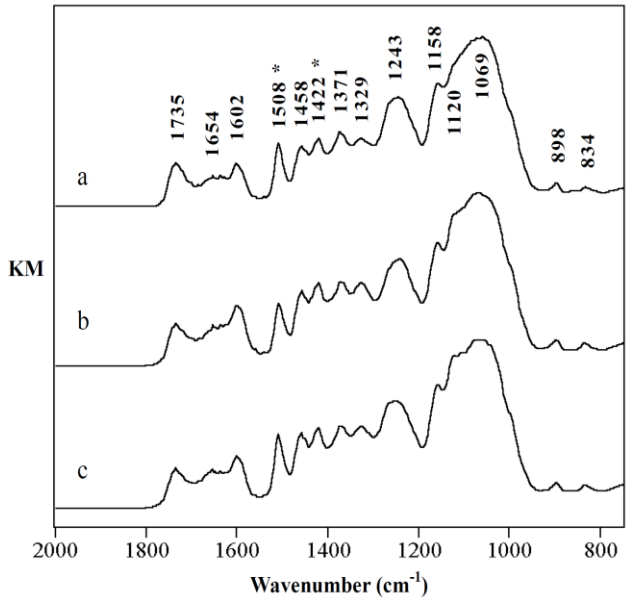

Fig. 3. FTIR spectra at various positions of giant bamboo culm IN8. (a) inner layer, (b) middle layer, and (c) outer layer

For the radial depth profiling of lignin content of giant bamboo, the relative peak intensity ratio of 1508 cm^{-1} (lignin aromatic skeletal vibration, Table 1) over 1422 cm^{-1} (methylene groups of both holocelluloses and lignin, Table 1, an internal standard) was used to estimate the position variation of bamboo lignin contents (Table 2). The intensity ratios from the inner layers of bamboo culms (2.44 to 2.89) showed the highest values followed by that from the outer (2.04 to 2.61) and the middle layers (1.74 to 2.11) of bamboo culms with the same height, indicating that the lignin contents in the middle layers were lower than the layers either from the inner or outer culms. The average relative lignin peak intensity ratios of three layers were 2.70 (inner layer), 1.84 (middle layer), and 2.34 (outer layer), with a statistical difference. The averaged intensity ratio from the middle layers was only 68% of that from the inner layers and 78% of that from the outer layers. The low lignin contents in the middle layer of bamboo culm were consistent with those in the genus *Gigantochloa* (Wahab *et al.* 2013).

As the longitudinal depth profiling of lignin content of giant bamboo, bamboo powders were sampled at six internodes of giant bamboo from IN8 to IN43 (base toward top). In each layer of bamboo culm, the intensity ratios gradually increased from the bamboo top toward the base, and the increased trends were most obviously in the inner and the outer layers of bamboo culms. The highest ratio was 2.89 from the inner layer of bamboo base (Table 2), indicating that the lignin contents of bamboo culms increased gradually from the top toward the base portion. Rousset *et al.* (2011) reported the Klason lignin contents of bamboo (*Bambusa vulgaris*) at a bottom, middle, and top location were 27.22%, 26.88%, and 26.93%, respectively. The lignin content was slightly higher at the bottom section without the statistical significance.

In bamboo shoots, the lignification of bamboo culms proceeding downward from top to base is more conspicuous. Chang *et al.* (2013) found an increased lignin deposition in basal portion of bamboo shoots from other bamboo species.

Table 2. Relative Peak Intensity Ratio (I_{1508} / I_{1422}) Values of Various Positions of Giant Bamboo Culms (Average ± Standard Deviation)

Specimen	Inner layer	Middle layer	Outer layer
IN43	2.44 ± 0.04	1.76 ± 0.02	2.04 ± 0.11
IN40	2.59 ± 0.03	1.74 ± 0.05	2.20 ± 0.09
IN38	2.74 ± 0.02	1.79 ± 0.03	2.32 ± 0.04
IN35	2.76 ± 0.03	1.77 ± 0.02	2.35 ± 0.03
IN18	2.81 ± 0.01	1.89 ± 0.05	2.47 ± 0.04
IN8	2.89 ± 0.04	2.11 ± 0.04	2.61 ± 0.02
Average	2.70 ± 0.16 [a]	1.84 ± 0.14 [c]	2.34 ± 0.19 [b]

Different letters (a, b, c) are statistically different at $p < 0.05$ according to the Scheffe's analysis

Radial Distribution of Growth Strain in Giant Bamboo Culm

Table 3 shows the longitudinal growth strains of different layers in the radial direction of giant bamboo culms at the bottom location (IN1 and IN3). The growth strain ranged from -24 to -210 με. A negative strain value represents a compressive strain from tensile stresses (Huang *et al.* 2001; 2005; Clair *et al.* 2006; Tsai *et al.* 2012). The growth strains of culm IN1 at the inner, middle, and outer layer were -24 με, -210 με, and -75 με, respectively. The trend of radial distribution of growth strain in culm IN3 was similar to culm IN1. The absolute value of the negative growth strain at the middle layer was greater than those of strains at the inner and outer layer. It indicated that greater tension stress occurred in the middle layer of bamboo culm. The order of longitudinal tensile strains in three layers of bamboo culm was the middle layer > the outer layer > the inner layer.

Table 3. Growth Strain of Various Positions of Giant Bamboo Culms

Specimen (Culm thickness)	Inner layer (με)	Middle layer (με)	Outer layer (με)
IN3 (2.2 cm)	-24	-158	-72
IN1 (2.2 cm)	-24	-210	-75

Lignin swelling theory and cellulose tension theory have been the main theories used to account for growth stress in tension wood (Bamber 1979; 1987; Boyd 1972; 1985). Bamber (2001) proposed an additional theory that lignin plays a unique role in the transmission of growth stress through the wood due to its adhesive function of binding the cellulose fibrils. And in tension wood, the low content lignin, especially in the gelatinous fiber bundles, seemed to facilitate the contraction of microfibrils and increase the longitudinal tensile stress. Kojima *et al.* (2012) suggested that the growth stress of plants is regulated by anatomical and chemical factors such as microfibril angle,

cellulose, and lignin. In their study, the increase in cellulose content and decrease in lignin content and microfibril angle were correlated with the high longitudinal tensile stress in stems of *Eucalyptus grandis.*

The longitudinal tensile strains of three layers in the radial direction of giant bamboo culm are experimentally related to their lignin contents. The highest tensile strain occurs in the middle layer of bamboo culm in correlation with the lowest lignin content (Table 2). The lower tensile strains occurring in the outside and inside of the bamboo culm are also associated with higher lignin contents. The close correlation between tensile strain and lignin content in giant bamboo is consistent with the findings of previous research on tension wood (Bamber 2001; Kojima *et al.* 2012). To our knowledge, these results are the first experimental evidence revealing the relationship between longitudinal tensile strain and lignin content in the radial direction of bamboo culm.

In this study, we analyzed the radial distribution of growth strain in the bottom culms of giant bamboo. The thickness of top culms is too narrow to measure the growth strain with strain gauges. The relationship between growth strain and lignin content in the longitudinal direction of bamboo culm warrants further investigation.

CONCLUSIONS

1. The Si-Carb sampling technique combined with DRIFT is a convenient way to obtain FTIR spectra of the wax layer, green epidermis, and underlying layers of bamboo culms.
2. Results from the longitudinal depth profiling of lignin content of giant bamboo show that the lignin contents gradually increased from the bamboo top toward the base, and the increased lignification trends were statistically significant in the inner and the outer layers of bamboo culms.
3. As the radial depth profiling of lignin content of giant bamboo, the lignin content in the inner layers of bamboo culms showed the highest values followed by that from the outer and the middle layers of bamboo culms at the same height.
4. Analyzing the radial distribution of growth strain in giant bamboo culm, it is evident that the highest longitudinal tensile strain occurred in the middle layer of bamboo culm, followed by the outer layer and the inner layer. The longitudinal tensile strains of the three layers in the radial direction of giant bamboo culm correlate with their lignin contents.

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