Effect of Growth Period on Cell Wall Mechanical Properties of Elephant Grass

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Elephant grass (*Pennisetum purpureum* Schum.) is a fast-growing native African plant species that produces commercially useful lignocellulosic biomass. It has been used in many countries to replace wood for paper, particleboard, and fiberboard. There is a close relationship between the mechanical properties of elephant grass cell walls and the performance of its products. The objective of this research was to investigate the cell wall mechanical properties at different growth periods of five types of elephant grasses, *i.e.*, *P. americanum* cv. Tift 23A×*P. purpureum* cv. Tift N51 (HP), *P. purpureum* cv. Tift N51 (N51), *P. purpureum* cv. Huanan (Huanan), *P. purpureum* cv. Sumu No.2 (Sumu-2), and (*P. americanum*× *P. purpureum*) × *P. purpureum* cv. Guimu No.1 (Guimu-1). The hardness and elastic modulus of the cell walls were investigated by means of nanoindentation. The results showed that the hardness and elastic modulus of these elephant grasses increased as growth period increased. However, the rate of increase varied for the different types of elephant grass, which could help guide the evaluation of the properties of this kind of bio-fiber resource for the production of high-quality biocomposite products.

Keywords: Elephant grass; Growth age; Mechanical properties; Cell wall

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INTRODUCTION

Elephant grass (*Pennisetum purpureum* Schum.) is a plant species native to Africa that possesses high rates of growth and lignocellulosic biomass production (Aroeira *et al.* 1999; Xie *et al.* 2011). This plant was introduced to South America and Australia over a century ago (Strezov *et al.* 2008), and was also introduced to China in the twentieth century. Elephant grass is potentially suitable as a source of refined fuel products such as bio-oil, char, and combustible gases (Strezov *et al.* 2008), or for bioenergy production by direct biomass combustion (de Morais *et al.* 2009). In some countries, elephant grass also finds use as a food source for livestock (Mpairwe *et al.* 1998) and can be used to feed ruminants (Ajayi 2011).

Because of the interest in non-wood fibers as raw material for pulp and paper products, research has been conducted to evaluate elephant grass as a raw material for pulp and paper production (Madakadze *et al.* 2010). It has also been found to be a suitable raw material for the production of particle board, fiberboard, and composite products (Nguyen *et al.* 2010, 2011).

Raw material chemical composition, kraft pulp yield and properties, and fibre characteristics of elephant grass have been determined in an effort to evaluate them as raw materials for pulp and paper production. Elephant grass had α-cellulose and Klason lignin contents of 45.6 and 17.7%, respectively. Pulp yield, following a mild kraft process, was 50% for elephant grass. The weight-weighted fibre length averaged 1.32 mm. Pulp freeness was 139 mL for elephant grass. Elephant grass had a burst index above 5.85 kP.m^2 g^{-1}. These characteristics demonstrate the suitability of elephant grass for pulp production (Madakadze *et al.* 2010).

The pennisetum fiberboard made with urea-formaldehyde resin could be used as the core material for veneer overlaying. The mechanical property of pennisetum fiberboard was increased obviously by veneer overlaying on its surface (Nguyen *et al.* 2010; 2011). To utilize elephant grass more efficiently as a raw material for pulp, paper, particleboard, and fiberboard, it is important to understand the mechanical properties of the plant's cell wall, as these properties strongly affect the quality of biocomposite products produced from such feedstock (Liao *et al.* 2012).

The nanoindentation technique is useful for determining the elastic modulus and hardness of wood cell walls along longitudinal direction (Gindl *et al.* 2002; Gacitua *et al.* 2007; Jakes *et al.* 2008) and may be used to measure the cell-wall mechanical properties of the stalks of crops including cotton, soybean, rice straw, wheat straw (Wu *et al.* 2010), silver grass (Liao *et al.* 2012), bamboo (Wang *et al.* 2013a), hemp stalk (Li *et al.* 2013), reed stalk (Wang *et al.* 2013b), and castor stalk (Li *et al.* 2014).

Nanoindentation is widely used for measuring micro scale mechanical properties of materials that are relatively isotropic in their elastic properties, and addressing whether the modulus measured in an indentation test represents that of some specific crystallographic direction or some average value is not an issue. On the other hand, some materials have complex hexagonal crystal structures, and because of this, results from these materials can be used to provide some insight into the importance of elastic anisotropy (Oliver and Pharr 1992).

Wu (2010) investigated the nano-mechanical properties of crop-stalk cell walls, *i.e.* those of cotton (*Gossypium herbaceum*) stalk, soybean (*Glycine max*) stalk, cassava (*Manihot esculenta*) stalk, rice (*Oryza sativa* L.) straw, and wheat (*Triticum aestivum* L.) straw by means of nano-indentation and atomic force microscopy (AFM), in order to evaluate their potential as materials for reinforcement. The elastic modulus of wheat straw was found to be 20.8 GPa, which was higher than that of the other four crops. The highest hardness was observed in cotton stalk at 0.85 GPa. The elastic moduli of the crop stalks were lower than those of most of the hardwood species, but higher than that of some softwoods and of lyocell fiber. The mean value of the hardness of the five crop stalks' cell walls was higher than those of wood or lyocell fiber. Besides, nano-mechanical properties of switchgrass and cotton cellulose nanocrystals (CNCs) were measured using nano-indentation (Wu *et al.* 2013). Mechanical testing showed that the reduced modulus (E_r) and the hardness (H) of switchgrass CNC films were higher than those of the cotton CNC films. The objective of this research was to investigate the effect of growth stage and grass type on the cell-wall mechanical properties of elephant grass using the nanoindentation technique.

EXPERIMENTAL

Materials

Sample preparation

Recent Chinese research into the planting and applications of elephant grass is predominantly focused on five varieties of the plant: *P. americanum* cv. Tift 23A×*P. purpureum* cv. Tift N51 (HP), *P. purpureum* cv. Tift N51 (N51), *P. purpureum* cv. Huanan (Huanan), *P. purpureum* cv. Sumu No.2 (Sumu-2), and (*P. americanum* × P. purpureum) ×*P. purpureum* cv. Guimu No.1 (Guimu-1). Of these, HP and Sumu-2 are grown in China across a wide range of latitudes, while Huanan and Guimu-1are cultivated in southern China because of their unsuitability to the colder climate of the north. There are plans for the cultivation of N51 in the future. Their genotypes, evaluated with a description of their origin, are listed in Table 1. These five kind of air-dried elephant grass stalks were obtained from the Garden of Jiangsu Academy of Agricultural Sciences (China). They were sampled at three different intervals during their life cycle, 3-months, 4-months, and 6-months, with the 1st sampling taking place on August 2 (a growing time of three months), the 2nd on September 2 (a growing time of four months), and the last at approximately six months, at the time of the local first frost and the cessation of growth in the plants.

Table 1. Elephant Grass Genotypes with a Description of Their Origin

Cultivar	Species	Germplasm origin	Citation
Hybrid Pennisetum (HP)	*Pennisetum americanum* × P. *purpureum*	USA, 1981	Registered in 1989 in China (Wu1999)
N51	*P. purpureum* Schum. cv. N51	USA, 1985	Unregistered in 1989 in China (Wu1999)
Huanan	*P. purpureum* Schum. cv. Huanan	Indonesia, 1960	Registered in 1990 in China (Wu 1999)
Guimu-1	(*Pennisetum americanum* × *P. purpureum*) ×P. *purpureum* cv. Guimu No.1	Improved cultivar from hybrid between 'hybrid' and 'Mott', 2000, Institute of Animal Science, Guangxi Academy of Agricultural Sciences in China	Registered in 1990 in China (Forage Product Certification Committee,2001)
Sumu-2	*P. purpureum* Schum. cv. Sumu No.2	Improved cultivar from *P.purpureum*Schum.cv.N51by Institute of Animal Science, Jiangsu Agricultural Sciences and Zhejiang Shaoxing Baiyun Construction Co., LTD. in China	Registered in 2010 in China (Ma *et al.*2011)

Grass samples were cut into 4 to 5 blocks of 5 mm (L) by 2 mm (W) by 1 mm (T). The specimens were then sealed in FoodSaver polymer film, according to the process shown in previous research (Meng *et al.* 2013). Each small grass sample was placed between two films and pressed by an electric iron with the temperature set at 160 °C. The pre-sealed samples were then embedded in Spurr's resin (ChemAce Chemical Supply, USA) and cured in an oven at 70 °C for 8 h. The surfaces of the cured specimens were prepared with three kinds of knives to obtain a very smooth surface. The smoothed specimens were conditioned at 21 °C and 60% relative humidity in the nanoindentation test room for at least 24 h before testing.

Methods

Nanoindentation

Nanoindentation tests were performed on a TriboIndenter® system manufactured by Hysitron, Inc. (Minneapolis, MN, USA). A Berkovich nanoindenter tip with a three-sided pyramidal shape and an area-to-depth function (Oliver and Pharr 1992) was used for all experiments. The single indentation procedure included four steps, as described by Liu *et al.* (2006). A total of 45 indentations were performed and were checked by rescanning the image (Fig. 1), as described in a previous manuscript (Zhang *et al*. 2012). Scanning probe microscopy was performed to measure the physical shape of the indentation and confirm the nanoindentation position.

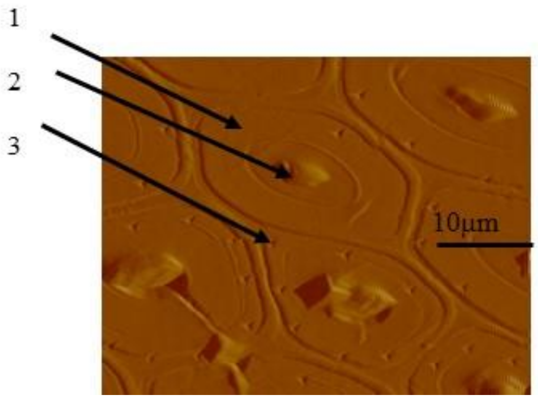

Fig. 1. Post indentation scanning probe microscope image (Note. 1. Secondary cell walls; 2. Cell lumen; 3. indentation mark)

From the indentation showed in Fig. 1, the load-displacement curve can be attained. Consequently, hardness and reduced modulus were calculated from valid data according to Oliver and Pharr (1992) and Wu *et al.* (2009). The reduced modulus Er (*i.e.*, the composite modulus for indenter and sample combination) was calculated from the nanoindentation measurements using Eq. 1 (Oliver and Pharr 1992):

$$E_{\gamma} = \frac{\sqrt{\pi}\left(\frac{dP}{dh}\right)_{unloading}}{2\sqrt{A_{hc}}} \tag{1}$$

where *P* is the indentation load, *h* and *hc* are the penetration and contact depths, respectively, and A_{hc} is the projected contact area, which is a function of the contact depth. The Meyer hardness (*H*) is given by Eq. 2,

$$H = \frac{P_{\max}}{A_{hc}} \tag{2}$$

where P_{max} is the maximum indentation load.

Determination of holocellulose and lignin contents

The specimens were smashed and screened to 60 to 80 mesh. The contents of holocellulose and cellulose were determined according to the standard GB/T 2677.10 (1995), and lignin contents were determined according to the standard GB/T747 (2003).

Statistical analysis

A one-way analysis of variance (ANOVA), linear regression, and parameter estimation analysis were conducted using Statistic Analysis System (SPSS; USA) 16.0 version software. Statistical significance was at the 0.05 level.

RESULTS AND DISCUSSION

Reduced Modulus of Cell Walls for Elephant Grass at Different Growth Stages

The reduced cell wall moduli for the five kinds of elephant grass at different growth stages are shown in Fig. 2. The growth periods of elephant grass are about 6 months in most areas of China. The elephant grass grows more quickly during the first 3-months or 4-months than other growth periods. From this graph, it can be seen that the reduced modulus increased with the progression of elephant grass growth stage. When the growth period was 3 months, 4 months, and 6 months, the reduced modulus of HP were 16.9 GPa, 20.2 GPa, and 20.2 GPa, N51 were 15.4 GPa, 16.3 GPa, and 21.0 GPa, Huanan were 14.4 GPa, 15.7 GPa, and 17.9 GPa, Sumu-2 were 15.0 GPa, 18.4 GPa, and 20.7 GPa, and Guimu-1 were 9.93 GPa, 13.2 GPa, and 16.9 GPa. The differences, however, were not significant at the 0.05 level. One of the reasons for the changes was that the chemical components of elephant grass changed between the growth periods of 3 months to 6 months. A relationship exists between plant age and the chemical composition of the plant, and this composition influences the cell wall mechanical properties. The primary components of plant cell walls are holocellulose (*i.e.,* cellulose, hemicellulose) and lignin (Thorstensson *et al.* 1992). It has been shown that the composition of holocellulose varies among different elephant grasses (Herrera *et al.* 1995). Among these components, cellulose dominates the longitudinal properties of cell walls (Bergander and Salmén 2002). For this reason, the cellulose content of elephant grass was tested to understand why the reduced moduli of cell walls varied. Table 2 lists the contents of holocellulose, cellulose, and lignin of the five elephant grasses in their different growth stages. Total content of holocellulose and lignin were not equal to 100% due to the extractives. The deduction may be made that there exists a relationship between growing time and the micro-mechanical properties of elephant grass stalks since both cellulose content and reduced cell modulus increased with plant age, although the rates at which the reduced modulus increased differed greatly among different types of plants. Table 3 shows the rate of increase of the reduced modulus for the third to the fourth month and the fourth to the sixth month of growth. The order, from the highest to lowest rate of increase, was as follows: Guimu-1 > Sumu-2 > HP >Huanan> N51 for the third to the fourth months of growth, and N51 > Guimu-1 >Huanan>

Sumu-2 > HP for the fourth to the sixth months of growth. Bergander and Salmen (2002) summarized values for the longitudinal Young modulus of wood cell wall components: 2.0 GPa for lignin, 7.0 GPa for hemicelluloses, and 167.5 GPa for cellulose. Since the stiffness of cellulose is more than 80 times that of lignin, a slight increase of cellulose should noticeably alter the stiffness of the composite cell wall. This assumption is supported by the finding that changes of the elastic constants of lignin in a model for cell wall stiffness have only small effects on the overall longitudinal stiffness (Bergander and Salmen 2002). And findings (Fujino and Itoh 1998; Donaldson and Singh 1998; Hafren *et al.* 1999) suggested that the unlignified cell wall consists of cellulose microfibrils organized as cellulose-hemicellulose strands embedded in a hemicellulose sheath. The spaces between individual cellulose-hemicellulose strands is occasionally bridged by hemicelluloses. During lignification, the spaces between cellulose-hemicellulose strands and possibly also part of the hemicelluloses sheath covering the cellulose are filled with lignin, thus filling, sealing, and hydrophobising the structure. The filling of existing spaces with lignin certainly increases the overall stiffness of the structure.

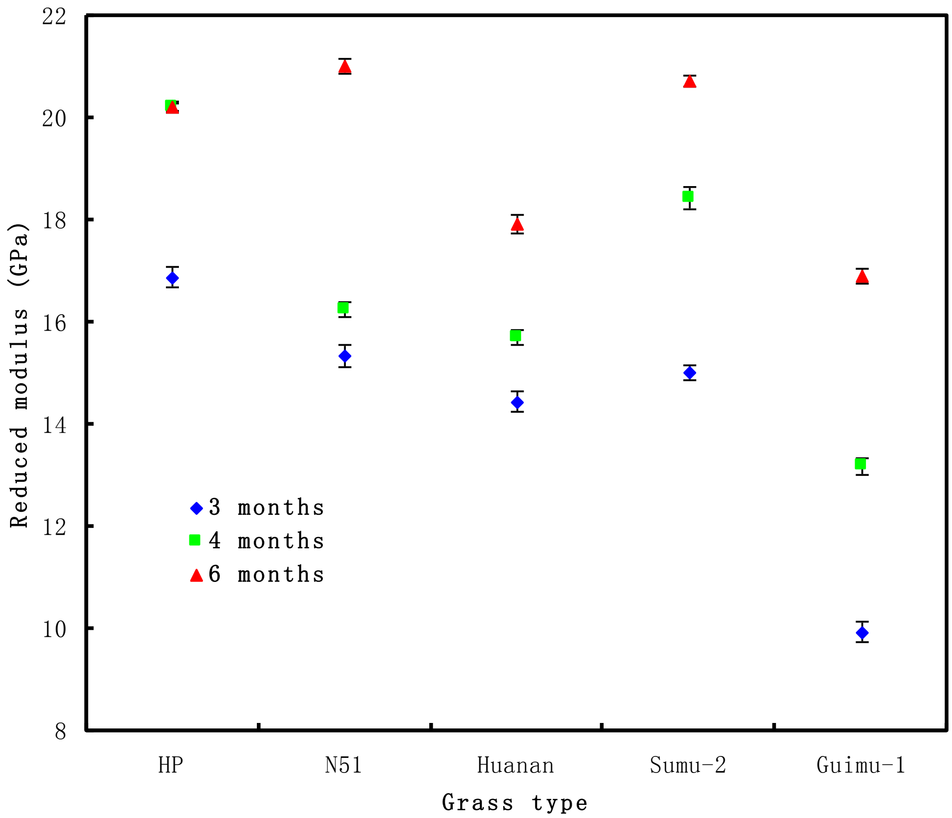

Fig. 2. Reduced modulus of cell walls for the five kinds of elephant grass in different growth stage. Note: The error bar of the y-axis is the standard deviation.

Table 2. Holocellulose, Cellulose, and Lignin Contents of Five Types of Elephant Grass (%)

Growth stage (months)	HP			N51			Huanan			Sumu-2			Guimu-1		
	H	C	L	H	C	L	H	C	L	H	C	L	H	C	L
3	69.0	33.1	10.0	64.5	35.5	15.1	68.0	35.2	15.7	68.4	29.8	13.4	69.1	33.8	12.4
4	67.7	36.0	14.2	66.3	35.6	17.3	68.5	36.5	17.8	67.5	37.2	19.4	68.9	34.5	13.0
6	77.0	36.4	19.7	75.8	37.8	20.2	71.0	36.9	21.6	71.2	38.3	21.3	77.0	34.6	17.3

H: holocellulose; C: cellulose; L: lignin

Table 3. Increasing Rate of the Reduced Modulus for Five Types of Elephant Grass (%)

Growth period	HP	N51	Huanan	Sumu-2	Guimu-1
From 3 to 4 months	19.7	5.8	8.8	22.8	32.9
From 4 to 6 months	0.07	29.3	14.0	12.5	28.1

Hardness of Cell Walls in Elephant Grass at Different Growth Stages

A positive relationship was found between the hardness of plant cell walls and their lignin content. From Fig. 3, it can be seen that the hardness of cell walls for different kinds of elephant grass increased as they grew.

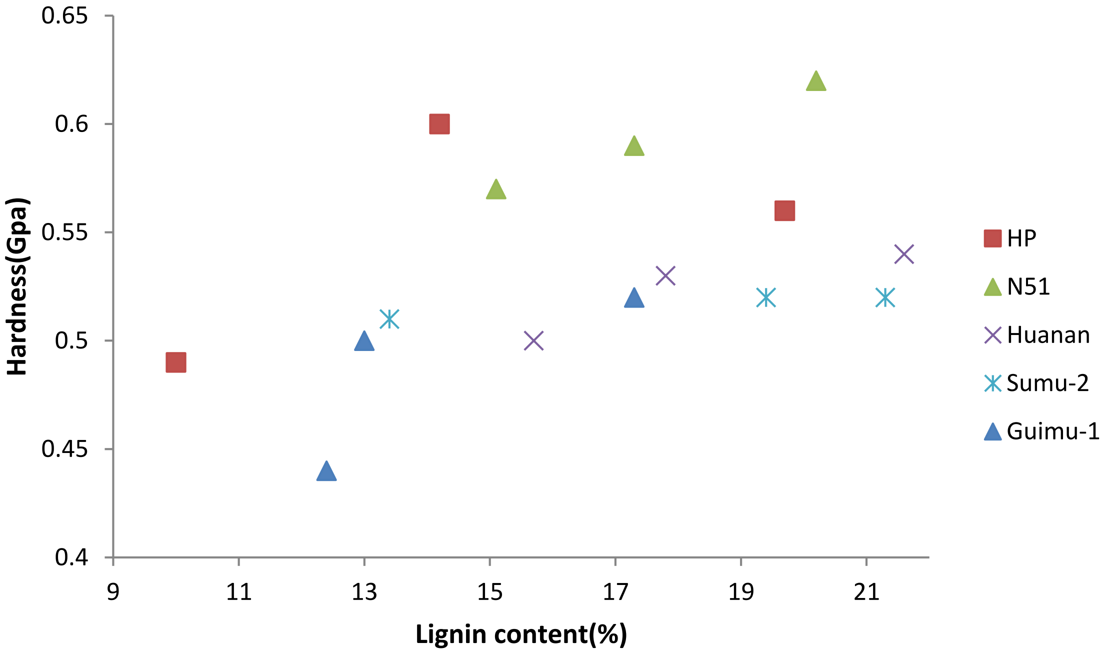

Fig. 3. Hardness of cell walls for the five kinds of elephant grass in different growth stage
Note: from left to right, it represents 3 months, 4 months, and 6 months separately

Comparison of Different Cell Wall Mechanical Properties

The reduced moduli of cell walls in five crop stalks (Wu *et al.* 2010) and the five types of elephant grass are shown in Fig. 4. Wheat straw had the highest reduced modulus (20.8 GPa) among the five crops. However, it was marginally smaller than that of N51, whose reduced modulus was 21.02 GPa. The rice straw and cassava stalk presented values of 19.4 and 19.0 GPa, respectively. The soybean stalk and cotton stalk both exhibited the lowest values of 16.3 GPa among the five crop stalks. The mean value of the five crop stalks was 18.4 GPa, which was also slightly smaller than the mean value of the five elephant grass stalks (19.4 GPa).

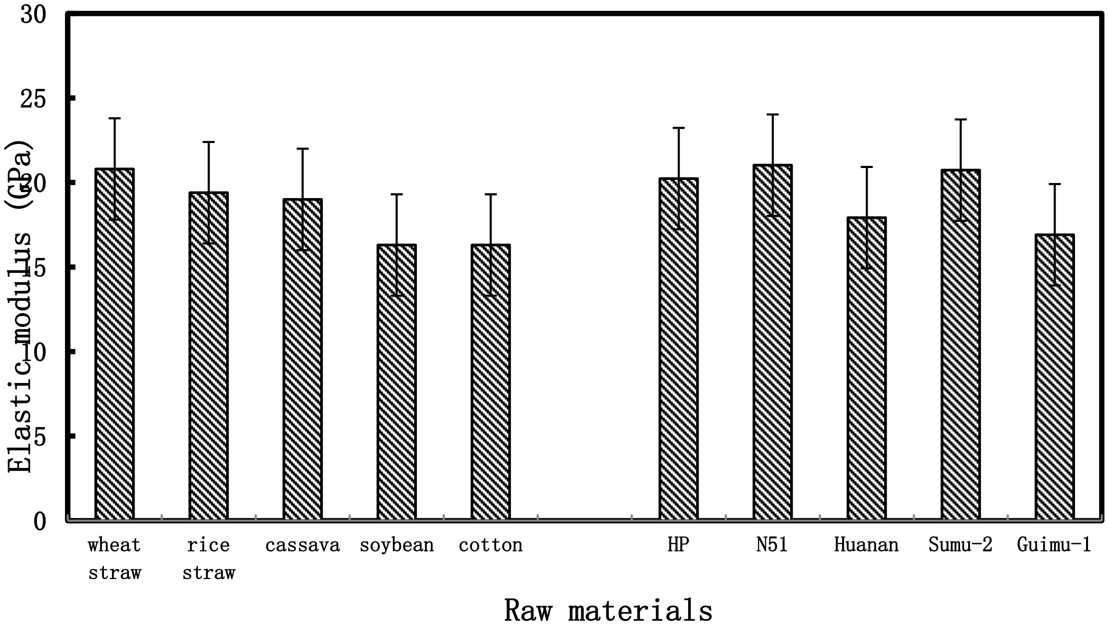

Fig. 4. Reduced modulus of cell wall in five crops and five elephant grass stalks with a growing time of six months (Note: the reduced modulus of cell wall in five crops was from a cited paper (Wu *et al.* 2010), and the reduced modulus of five elephant grass stalks was from the present study)

CONCLUSIONS

The following conclusions can be drawn from this study. The reduced moduli and hardness of five kinds of elephant grasses increase as they mature. Different types of elephant grass showed differing rates of increase in reduced modulus and hardness. The elephant grass has similar cell wall mechanical properties by comparing to other crop stalks, which are from 16 GPa to 22 GPa. These results may prove useful as a guide by which the appropriate type and age of elephant grass may be chosen for the production of pulp, paper, particle board, and fiberboard.

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