

DANIELE DE FREITAS PARMA

**GROWTH CHARACTERIZATION AND CHEMICAL COMPOSITION OF THE  
CELL WALL OF NATIVE SPECIES OF BAMBOOS**

Dissertation presented to the Universidade Federal de Viçosa, as part of the requirements of the Graduate Program in Botany, to obtain the title of Magister Scientiae.

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## ABSTRACT

PARMA, Daniele de Freitas, M.Sc. Universidade Federal de Viçosa, February 2017. **Growth characterization and chemical composition of the cell wall of native species of bamboos.** Adviser: Ana Paula Santos Gonçalves. Co-Adviser: Adriano Nunes Nesi.

Bamboos are predominant plants in forest areas and it have become a favorite choice for carbon sequestration to absorb atmospheric CO<sub>2</sub> and restorer additive balance of the Earth's climate system, due to its high growth rate compared to most other plant species. Furthermore, the bamboos are used to produce pulp and paper or charcoal and active carbon for special purposes. Furthermore, it has ecological and environmental functions in soil erosion control, water conservation and land rehabilitation. Thus, the quantification of its biomass provide among other things, an estimate of cellulosic material provide a potential source of renewable energy and base for carbon sequestration studies. Thus, the aim of this study were (i) Estimate biomass and carbon storage is *Merostachys* species (*M. fischeriana*, *M. tatariana* and), in order to understand its distribution along different compartments (foliage, branches and culm). (ii) Evaluate biomass models according to biometric variables collected - circumference and diameter of the culm at the base and at the height of the breast, total height and number of nodes. (iii) Quantify moisture and carbon content. (iv) Identify and quantify the main components of the culm cell wall - cellulose, hemicellulose, lignin and ash. To quantify the productivity of *M. fischeriana*, *M. tatariana* and *M. ximenesii*, twenty individuals of each specie were collected in Atlantic forest area in Southeastern Brazil. The plants were measured, cut, weighed and dried. To quantify the chemical components of the culm cell wall, three samples (base, medium and apex) of each of the five individuals of the three species were lyophilized and homogenized to obtain fine powder (40-60 mesh). The design of nested factors comparing the areas with each other and within each species was used. The allocation of biomass in *Merostachys* species was higher in the culm, with detection of some interspecific differences. Individual dry biomass correlates strongly with biometric variables the culm base diameter, diameter at breast height and total height. Thus, simple regression models, considering only those independent variables fit well to express the individual total dry weight as the dependent variable. The productivity of *Merostachys* with culture spaced 3m x 3m is highly competitive with other species of bamboo cultures; even with other plants (i.e. *Pinus*). *Merostachys* has an average daily growth rate of 0.07 m, reaching its maximum height in four months and its full development, with leaves and branches, 6-9

months. The moisture content is decreasing from the base toward the apex, as well as in relation to age. We verified that there was no significant difference between the content of cellulose, hemicellulose, lignin and ash between species of *Merostachys* as well as between the base, middle and apex. However, only *M. tatarica* presented significant differences ( $p = 0.0026$ ) for the lignin concentration between regions. The average concentration of cellulose in *Merostachys fischeriana*, *M. tatarica* and *M. ximenesii* ranged from 78-82%. In *Merostachys* the value found for holocellulose was higher than 98%, being this value related to the cellulose concentration, since the observed hemicellulose content is congruent with that verified for other species of bamboo. The lignin content was 0.86-1.06%. The ashes concentration between 0.52-0.68% was similar to that observed for some species of *Eucalyptus*. Studies of quantification of the chemical components of bamboo cell wall are rare. Comprehensive knowledge of biomass and chemical components in the bamboo species will facilitate the use of materials in the forestry industrial sector and help to enhance their utilization in the chemical and biochemical related industry. In addition, these results can be supporting taxonomic and ecological studies.

## RESUMO

PARMA, Daniele de Freitas, M.Sc. Universidade Federal de Viçosa, fevereiro de 2017. **Caracterização do crescimento e composição química da parede celular de espécies nativas de bambus.** Orientador: Ana Paula Santos Gonçalves. Coorientador: Adriano Nunes Nesi.

Os bambus são plantas predominantes em áreas florestais e tornou-se uma escolha favorita para o sequestro de carbono, para absorver o CO<sub>2</sub> atmosférico e restaurar o equilíbrio aditivo do sistema climático da Terra, devido à sua alta taxa de crescimento em comparação com a maioria das outras espécies de plantas. Ademais, os bambus são utilizados na produção de polpa e papel ou carvão e carbono ativo. Além disso, tem funções ecológicas e ambientais no controle da erosão do solo, conservação da água e reabilitação de terras. A quantificação de sua biomassa fornece, dentre outras coisas, uma estimativa de material celulósico disponível para uma fonte potencial de energia renovável. Assim, os objetivos deste estudo foram (i) Quantificar a biomassa e o teor de carbono das espécies de *Merostachys* (*M. fischeriana*, *M. tatariana* e *M. ximenesii*), compreendendo a sua distribuição nos diferentes compartimentos (folha, ramos e colmo). (ii) Avaliar os modelos utilizados para quantificação de biomassa de bambus, de acordo com as variáveis biométricas coletadas - circunferência e diâmetro do colmo na base e na altura do peito, altura total do colmo e número de nós. (iii) Quantificar o teor de umidade (iv) Identificar e quantificar os principais componentes da parede celular do colmo - celulose, hemicelulose, lignina e cinzas. Para quantificar a produtividade de *M. fischeriana*, *M. tatariana* e *M. ximenesii*, vinte indivíduos de cada espécie foram coletados em área de Floresta Atlântica no Sudeste do Brasil. As plantas foram medidas, cortadas, pesadas e secas. Para quantificar os componentes químicos da parede celular do colmo, três amostras (base, meio e ápice) de cada um dos cinco indivíduos das três espécies foram liofilizadas e homogeneizadas para se obter pó fino. O desenho amostral utilizado foi baseado em fatores aninhados comparando as regiões entre si e dentro de cada espécie. A distribuição de biomassa em *Merostachys* foi maior no colmo, com detecção de algumas diferenças interespecíficas. A biomassa seca individual correlacionou-se fortemente com as variáveis biométricas, diâmetro da base do colmo, diâmetro na altura do peito e altura total. Assim, os modelos de regressão simples testados para as espécies explicam a biomassa total individual, sendo esta a variável dependente. A produtividade de *Merostachys*, em cultivo com espaçamento de 3m x 3m, é altamente competitiva com

outras espécies de bambu; mesmo com outras plantas (i.e. *Pinus*). *Merostachys* tem uma taxa média de crescimento diário de 0,07 m, alcançando sua altura máxima em quatro meses e seu pleno desenvolvimento, com folhas e ramos, em 6-9 meses. O teor de umidade diminui a partir da base em direção ao ápice, bem como em relação à idade. Verificou-se que não houve diferença significativa entre o teor de celulose, hemicelulose, lignina e cinzas entre as espécies de *Merostachys*, bem como entre a base, meio e ápice. No entanto, apenas *M. tatarica* apresentou diferenças significativas ( $p = 0,0026$ ) para a concentração de lignina entre as regiões. A concentração média de celulose em *Merostachys fischeriana*, *M. tatarica* e *M. ximenesii* variou de 78-82%. Em *Merostachys* o valor encontrado para a holocelulose foi superior a 98%, sendo esse valor relacionado à concentração de celulose, uma vez que o teor de hemicelulose observado é congruente com o verificado para outras espécies de bambu. O teor de lignina foi de 0,86-1,06%. A concentração de cinzas foi de 0,52-0,68% semelhante à observada para algumas espécies de *Eucalyptus*. Estudos de quantificação dos componentes químicos da parede celular de bambu são raros. O conhecimento abrangente da biomassa e dos componentes químicos nas espécies de bambu facilitará o uso de materiais no setor industrial florestal e ajudará a melhorar sua utilização na indústria química e bioquímica relacionada. Além disso, esses resultados podem apoiar estudos taxonômicos e ecológicos.

## GENERAL INTRODUCTION

Bambusoideae are a lineage of perennial forest grasses (Poaceae) endemic to every continent except Europe and Antarctica (BPG 2012; Kelchner 2013). The Bambusoideae comprise 115 genera and approximately 1450 species of bamboos (BPG 2012). It is estimated that at least 40 million hectares of the earth's surface are composed of bamboo forests, accounting for approximately 1% of the world's forest cover (Lobovikov et al., 2007). In Brazil, where flows the greater diversity of bamboos in the Americas, there are 49 genus and 278 species (Flora of Brazil 2016).

Bambusoideae are divided into two morphologically distinct habits: woody and herbaceous bamboos. The woody bamboo syndrome includes strongly lignified culms, specialized culm leaves, complex vegetative branching, outer ligules on the foliage leaves, bisexual flowers, and gregarious monocarpy (BPG 2012). Therefore, as opposed to most grasses, bamboos present the  $C_3$  photosynthetic system (Larcher 2000; Düking et al., 2011). Thus, in the absence of limiting factors, they are able to increase their growth in biomass in response to an atmosphere enriched with  $CO_2$ .

The arboreal members of the subfamily Bambusoideae have small chromosomes, most of them poliploids ranging from tetraploids ( $2n = 48$ ) to octaploids ( $2n = 96$ ), unlike herbaceous ones that are diploids (Darlington & Wylie 1955; Ueda 1960; Soderstron, 1981). Thus, due to the great diversity in the basic number ( $x$ ), high number and small size of chromosomes, it is difficult to define the degree of ploidy in bamboo species, leading to the need to use specific cytogenetic techniques (Banik 1996).

The factor responsible for polyploidy may be related to geographic factors, since the number of chromosomes gradually increases from subtropical to tropical zone (Guang-zhu 1987). Depending on evolutionary trends, hybridization between polyploids occurs between closely related species, largely or poorly correlated or even between genera (Stebbins 1971). However, there is more chance of hybridization between varieties of the same species, occurring frequently between species of the same genus and neighboring habitats (Wright 1964).

The bamboos, some of which can quickly grow up to 45 m in height, serve as an economically important source of building materials, pulp and charcoal, besides has ecological and environmental functions in soil erosion control, water conservation and land rehabilitation (Zhou et al., 2005). Their potential for rapid establishment combined with their extensive vegetative reproduction also make bamboos important ecologically as they can serve as forest habitats of their own and can affect the survival of sympatric woody species (Lima et al., 2012).

Bamboos are predominant plants in forest areas and it have become a favorite choice for carbon sequestration to absorb atmospheric CO<sub>2</sub> and restore additive balance of the Earth's climate system, due to its high growth rate compared to most other plant species (Eliasch, 2008). Furthermore, in recent decades, bamboo has turned into a globally important biomass resource (Scurlock et al., 2000), mainly after the acceptance of bamboo in volunteer carbon finance mechanisms amplified its attractiveness as plantation species (Darabant et al., 2014).

Thus, the quantification of its biomass provide among other things, an estimate of cellulosic material provide a potential source of renewable energy and base for carbon sequestration studies (Popescu, 2007; Silveira et al., 2008). These studies are of great importance for decision making in the management of forest resources (Páscoa et al., 2004). However, studies on bamboo are scarce and most of these studies are carried out in natural plantations (Isagi et al., 1994; Shanmugavel & Fancis 1996; Veben et al., 1980; Embaye et al., 2005).

Biomass can be estimated by direct method (e.g. destructive techniques) or by indirect method (e.g. developing an allometric relationship). Destructive techniques for biomass estimation are time consuming and expensive due to the large dimensions and amounts of biomass that have to be processed (Verwijst & Telenius 1999). Allometric relationship yields a non-destructive and indirect measurement of biomass components and is often the preferred approach since it is less time consuming and less expensive than direct measurements (Nath et al., 2009). Therefore, the use of allometric relationships for biomass prediction is a prerequisite for productivity studies, carbon estimations, nutrient cycling of the forests (Nath et al., 2009; Subasinghe, 2016). Allometric functions have been developed for a number of bamboo species in order to estimate aboveground biomass stocks using easy-to-assess proxy variables, such as culm diameter at breast height (Singh & Singh 1999; Mognon et al., 2014, 2015; Sanqueta et al., 2015).

Bamboo has excellent mechanical properties, which are influenced by the moisture content of the culm (Liese 1998; Janssen, 2000; Lopez 2003). Another factor that influence the physical and mechanical properties of the culm is your age. The durability of the culms are related to its chemical composition and anatomical structure (Liese 2003; López 2003), especially lignin content and cell wall thickness (Liese 1998; López 2003). It can provide important information for taxonomical identification and seed selection. The total culm comprises about 50% parenchyma, 40% fibre, and 10% conducting tissues with some variation according to species (Liese & Grosser 1972). The structure of the bamboo in general can be viewed as being a composite material constituted roughly by bundles of long cellulose fibers,

the main element of its resistance, aligned longitudinally joined by a binder, lignin (Ghavami 2005).

The chemical composition of bamboo is similar to wood (Anokye et al., 2016). The main chemical constituents of the bamboo culms are cellulose, lignin and holocellulose and, to a lesser extent, the resins, tannin, waxes and organic salts (Liese 1985). After cellulose, lignin represents the second most abundant constituent in the bamboo and much interest has been focused on the chemical nature and structure (Liese 1985). Besides the aforementioned chemical components, parenchymal cells - the tissue surrounding fibrovascular bundles containing substances such as starch, protein and soluble carbohydrate, essential to maintaining plant (Mohanan 1997). However, the same elements are attractive to insects, fungi and drills that use as food and provide their development (Matoski 2005). A high moisture content of the bamboo culms also facilitates and accelerates the attack of various types of fungi (Liese 1980).

Despite many opportunities, the use of bamboo in Brazil is low, due to the lack of technological and scientific knowledge specifically developed in this area, as well as strategic vision of rational economic exploitation (Beraldo & Azzini 2004). The most cultivated genera in Brazil are: *Bambusa*, *Dendrocalamus* and *Phyllostachys*, all of Asian origin, brought by the first immigrants and due to good adaptation to the Brazilian tropical climate, were spread throughout the country (Teixeira 2006). Thus, this work was developed in a conservation area with native bamboo species in Brazil of genera *Merostachys* Sprengel.

The species can be differentiated by the following characteristics: hairiness, length and diameter of the internode; wall thickness; number of branches of the complement of branches and; nodes prominent or not. *Merostachys fischeriana* Rupr. ex Döll. presents internode with 36-22 cm long, yellowish green, glabrous at maturity, with 0.3-1.2 cm in diameter and wall with 1-3,2 mm thickness. The branch complement has 34-53 branches, nodes non-prominent.

*Merostachys tatiana*e Santos-Gonç., Carv.-Okano & Filg. presents internode with 20-77 cm long, yellowish green, hispid, covered by hairs, 1.1-2.7 cm diameter, 1-2 mm thick wall. The branch complement presents 10-12 branches, nodes non-prominent.

*Merostachys ximena*e Parma, Vinícius-Silva & Santos-Gonçaves presents 20-77 cm long, yellowish green, glabrous maturity, with 0.3-1.2 cm in diameter and 1-3.2 mm thick wall. The branch complement has 34-53 branches, nodes prominent.

Our general objective is to perform quantification and characterization of the biomass of *Merostachys* species. This genus has a wide distribution in the country and it is widely used in local crafts. Therefore, this work aims to provide data to extend the use of these species. This dissertation present key information that can help ecological and taxonomic studies.

Furthermore, it provides data that may be of interest in industry, mainly for the bioenergy industry.

Thus, this work provides information on the biomass quantification and carbon stocks for each of the compartments (culm, leaves and branches) for each of the five age classes, developing allometric models with easily accessible data, such as diameter at breast height and total height. Moreover, an approach is made of carbon stock and growth analysis of three *Merostachys* species, as the moisture content. In addition, it possible identifies and quantify the main constituents of the culm, cellulose, hemicellulose, lignin and ash. Moreover, a focus is given to different regions of the culm (base, middle and apex) of the three *Merostachys* species. This information with the anatomy can be used by industry, helps ecological and taxonomic studies.

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## CHAPTER 1

### ABOVE GROUND STANDING AND CARBON STORAGE IN A BAMBOO POPULATION IN THE BRAZILIAN ATLANTIC FOREST

#### ABSTRACT

*Merostachys* is one of most abundant woody bamboo genera in number of species and is distributed, mainly, in the Brazilian Atlantic forest. Basic properties can be used to reflect the quality of culms and suitability of different bamboo species for specific utilization. Since bamboo has become, nowadays, one of the most important raw material for construction and biofuels, it is of great relevance to investigate their basic characteristics such as biomass and chemical compounds. The purpose of this study was to evaluate biomass prediction and carbon storage capacity of three *Merostachys* species (*M. fischeriana*, *M. tatariana* and *M. ximenesii*), (foliage, branches and culm), as well as establish models for biomass production based on biometric variables. Twenty individuals of each species were collected in an Atlantic forest area in Southeastern Brazil to biomass analysis and three culm portions of each of the five replicates of each species to characterize and to analyze the composition and quantification of cell wall components. Different plant segments parts were measured, sectioned and weighed. As a result, the allocation of biomass in the species was higher in the culm and interspecific differences were observed. Individual dry matter correlated strongly with culm base diameter, diameter at breast height and total height. It was shown that simple regression models, considering only those independent variables were suitable to express the individual total dry weight as dependent variable. *Merostachys* presented a growth rate of 7 cm day<sup>-1</sup>, reaching its maximum height in four months and its full development, with leaves and branches, on 6 to 9 months. It was also observed that the moisture content decreases from the base toward the apex, as well as in relation to plant age. We verified that there were no significant difference amongst the content of cellulose, hemicellulose, lignin and ash between species of *Merostachys* as well as amongst the base, middle and apex. However, only *M. tatariana* presented significant differences ( $p = 0.0026$ ) for the lignin concentration amongst regions. The percentage of cellulose found for *Merostachys fischeriana*, *M. tatariana* and *M. ximenesii* vary between 78-82%. In *Merostachys* the value found for holocellulose was higher than 98% and for hemicellulose content around 20%. In studied species observed lignin content was 0.86-1.06% and ashes concentration vary between 0.52-0.68%. These results indicate that *Merostachys*

species have high potential for industry use as energy source. However, further research work is necessary for development of agronomic and biotechnological strategies for cultivation and production in large scale.

**Keywords:** Allometric equations, biomass, *Merostachys*

## 1 INTRODUCTION

Bamboos belong to subfamily Bambusoideae within the Poaceae family (McClure 1966). The subfamily comprises 1.2 thousand to 1.5 thousand species (Soderstrom & Ellis 1988) occurring naturally in all continents, except in Europe (Judziewicz et al., 1999). Bamboo resources in Brazil are amongst the richest in the world, where more than 280 species occur (20% of the total world) (Flora of Brazil, 2016). Due to their biological characteristics and growth habits, bamboo forests have ecological and environmental functions in soil erosion control, water conservation, land rehabilitation and carbon sequestration (Zhou et al., 2005).

The bamboos form a unique group of giant arborecent grasses. *Merostachys*, genus of Neotropical woody bamboo, have fast growth (5 to 20 cm day<sup>-1</sup>) and is a forest component that plays important ecological functions. Their flowering occurs gregariously in lengthy and irregular of 30 to 50 years (Guilherme & Ressel 2001; Liebsh & Reginato 2009; Guerreiro 2014). From a viewpoint of resource management of biomass, asexual reproduction, accompanied by high growth rate of the culm, is a very advantageous trait, whereas the culms can be harvest constantly, by thinning from a stand, without the need for re-planting (Isagi et al., 1993).

In addition, it is important to note that most of the tree species need decades to reach maturity, whereas bamboos usually mature in 7–10 years, which suggest that bamboos might have more potential to mitigate climate change (Nath et al., 2009). Although bamboos have long been thought to have good carbon sequestration abilities (INBAR, 2009a), little has been published to support this (INBAR, 2009b; Nath et al., 2009).

In recent years, the carbon cycle has become an important issue in the world. Plants perform an important role in the global carbon cycle, including accumulation and storage of carbon that limits the concentration of CO<sub>2</sub> in the atmosphere (Yen et al., 2009). Measurement of forest biomass provides an indication of carbon sequestration in trees and also an estimate of cellulosic material as a potential source of renewable energy (Popescu 2007). The use of allometric relationship for biomass estimation is a basic prerequisite in the study of a global carbon balance and carbon sequestration (Nath et al., 2009) and also for understanding ecological and evolutionary aspects of plant species (Páscoa 2004). Many studies have focused on the contributions of trees to carbon storage but few have focused on bamboo plants (Mognon et al., 2015).

*Merostachys* is one of most abundant bamboos genera in number of species, distributed in Brazilian Atlantic forest. Due to the lack of knowledge about the anatomical, physical and

mechanical properties of this bamboo, *Merostachys* is not widely used in Brazilian industry; currently, it was only used for traditional products such as handicraft. However, *Bambusa* Schreb., *Dendrocalamus* Nees and *Phyllostachys* Siebold & Zucc., genera introduced in the country, are more known and commercially used for civil construction and charcoal production (Vasconcellos, 2000; Ribeiro, 2005; Capello, 2008).

Since bamboo has become the most important raw material for construction and biofuels (Lobovikov et al., 2007, Capello, 2008), it is of great value to investigate in details its basic characteristics chemical compounds. Especially since it is known that there is variation in the percentage of chemical constituents among species of the same genus (Higuchi, 1957; Youdi et al., 1985). The anatomical structure of culm is the basis for understanding the physical, mechanical properties and its utilizations (Abd. Latif et al., 1993). Thus, a comprehensive knowledge of the chemical compounds in different organs of bamboo species will facilitate the use of this material in the forestry industrial sector and help to enhance their utilization in the chemical and bio-chemical related industry.

Basic properties can be used to reflect the quality of culms and suitability of different bamboo species for specific utilization. The bamboo cell walls consist of between 90 to 98% of hemicellulose, cellulose and lignin, while the other 2 to 10% of extractives, resins, tannins, waxes and inorganic salts (Liese, 1985, 1992).

In this study a complete and detailed study of biomass allocation, carbon concentration, moisture content, growth analysis and linear regression models for bamboo culm have been done for three bamboo species. As well as a chemical and anatomical analysis of the stem which is the most important part, economically, of the plant. The current study was performed at “Mata do Paraíso”, a forest reserve of Southeastern Brazil, in order to examine the stand biomass and carbon of common bamboo species from Brazilian Atlantic forest, *Merostachys fischeriana*, *M. tatariana* and *M. ximenesii*. The aims of this study were: (i) to estimate the aboveground biomass production of *M. fischeriana*, *M. tatariana* and *M. ximenesii* plants, (ii) to determine the moisture content and carbon stock in the culm, (iii) to develop regression models for estimation of biomass organic carbon content of these three species, (iv) to quantify the main constituents of culm, cellulose, hemicellulose, lignin and ashes, and (v) characterize the anatomical culm.

## 2 MATERIALS AND METHODS

### 2.1 Study area

Botanical material was collected in the Mata do Paraíso (20°48'07"S, 42°51'31"W), located in the Research Station, Training and Environmental Education (EPTEA) in Viçosa city, Minas Gerais State, Brazil. This forest fragment area of 194 ha, at 690 to 870 m of altitude (Valentin-Silva et al., 2015). The EPTEA area was classified as a Forest Semideciduous within Atlantic Rainforest and Atlantic Forest biome (Veloso et al., 1991) composing a mosaic in different succession stages and small marsh areas (Silva-Junior et al., 2004). The average annual precipitation and relative humidity are 1268.2 mm and 81%, respectively, and average annual temperature is 20 °C (Lorenzon et al., 2013).

The EPTEA soils are classified as red-yellow latossolos in areas with convex profiles, cambic on the tops of elevations due to the existence of B-horizon of thinness, ultisols in the areas of concave profiles and on the terraces and hydromorphic alluvial in larger bed (Alves et al., 2007). The region includes nine native species of bamboo (Parma et al., 2016). In this study, we used three bamboos species, *Merostachys fischeriana*, *M. tatariana* and *M. ximenesii*. These species present diagnostic morphological characteristics that are easy to observe in the field and thus to differentiate and delimit the species.

### 2.2 Species characterization

*Merostachys fischeriana* Rupr. ex Döll. presents internode with 36-22 cm long, yellowish green, glabrous at maturity, with 0.3-1.2 cm in diameter and wall with 1-1.92 mm thickness. The branch complement has 34-53 branches, nodes non-prominent (Fig. 1e-f).

*Merostachys tatariana* Santos-Gonç., Carv.-Okano & Filg. presents internode with 20-77 cm long, yellowish green, hispid, covered by hairs, 1.1-2.7 cm diameter, 1-2.23 mm thick wall. The branch complement presents 10-12 branches, nodes non-prominent (Fig. 1a-b).

*Merostachys ximenesii* Parma, Vinícius-Silva & Santos-Gonçalves presents 16-32 cm long, yellowish green, glabrous maturity, with 0.5-1.2 cm in diameter and 1-2.1 mm thick wall. The branch complement has 3-87 branches, nodes prominent (Fig. 1c-d).



Figure 1: Characterization of *Merostachys* species. A-B. *Merostachys tatiana*, a-Habit and b-Node. C-D. *M. ximena*, c-Node and d-Habit. E-F. *M. fischeriana*, e-Habit and f-Node.

## 2.3 Data collection

### 2.3.1 Biomass

Twenty bamboo samples for each species were obtained based on age considering the morphological characteristics used by Lin (1961), Fu (2000) and Ostapiv (2007), as well as a temporal monitoring of bamboo clumps since 2012. These bamboo samples were sectioned at base to measure the biomass and fresh weight of culms, branches, and leaves. These three organs were sampled at base, middle, and apex portion of the culm (Fig. 2) and weighted using

digital scale. The samples were dehydrated in drying oven with air circulation (CIENLAB, CE 220/480, Campinas, Brazil) at 70 °C for 3-5 days until acquiring constant weight, thus the absolute dry weight was obtained.

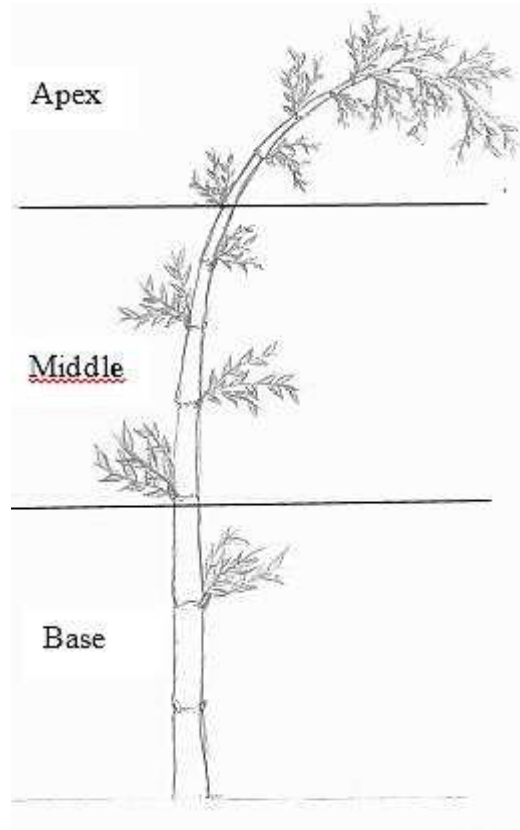


Figure 2: Division of culm in three portions: base, middle and apex.

Measurements diameter and circumference of the base (Db, Cb) and breast height (Dbh, Cbh), height (Ht), number of nodes in the culm (Nn) were collected were collected applying stratified sampling method (Mognon et al., 2014, 2015; Sanquetta et al., 2015). Moreover, some individuals of each clump, especially the youngsters, were marked in order to monitor its growth for a period of ten months.

### 2.3.2 Chemical analysis

We collected five culms of each species (*M. fischeriana*, *M. tatarica* and *M. ximenesae*) with three years old. From each culm was withdrawn three internode being the second corresponding to the base, another in the middle region and finally, the penultimate internode corresponding to the apex (Fig.3). The samples were dehydrated in drying oven with air circulation (CIENLAB, CE 220/480, Campinas, Brazil) at 70 °C for 3-5 days until constant

weight. Subsequently, the material was milled using the crusher 9FQ-400 Forage and knife mill Willey (TE-680/Tecnal).

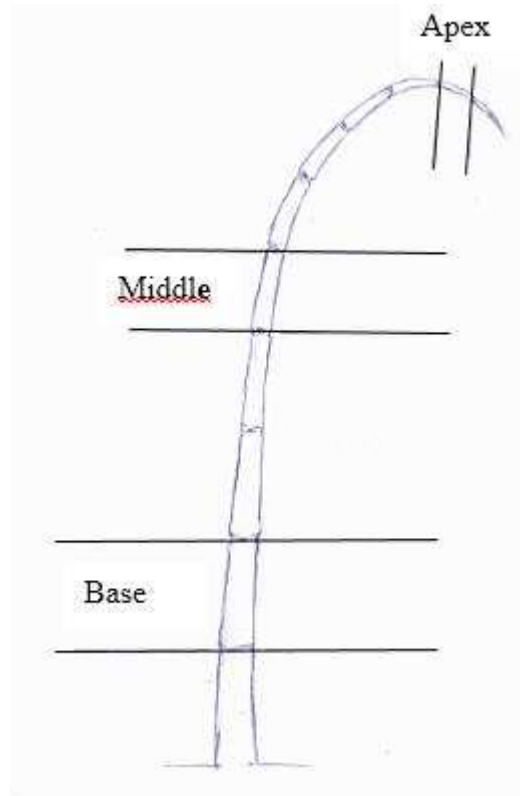


Figure 3: Selection of three internodes to chemical analysis.

#### 2.4 Allometric equations for biomass estimation

We perform simple linear correlation analysis for all measured growth parameters with dry biomass, considering each of the organs and the total biomass. For the indirect estimation of total dry biomass, different regression models of different works were tested (Zianis & Mencuccinni 2004; Nath et al., 2009; Yen et al., 2008; Yen et al, 2010; Darabant et al., 2014; Mognon et al., 2014, 2015; Sanquetta et al., 2015; Sohel et al., 2015) (Table 1A). The performance of the equation was evaluated by determination of regression coefficients (adjusted  $R^2$ ) and standard error in percentage ( $S_{yx}\%$ ).

#### 2.5 Ash and carbon content determination

Sub-samples of culm, branch and leaf from different culm age classes for the three species were grinded and carbon content was determined. About 1.0 g of oven-dried and grinded samples were taken in calcined pre-weighted crucibles. The crucibles were positioned

in the muffle oven (SPLABOR, SP-1200DM/B, São Paulo, Brazil) at 550 °C for five hours and were cooled slowly inside the same oven (Sohel et al., 2015) up to 200 °C and then placed in aluminum desiccators. After cooling, the crucibles with ash were weighted and the percentage of organic carbon was calculated as described by Allen et al. (1986).

$$Ash(\%) = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

$$C(\%) = (100 - Ash) \times 0.058$$

(Considering 0.58% carbon in ash free culm, branch and foliage materials, according to Allen et al., 1986)

where  $C$  is the organic carbon,  $W_1$  is the weight of crucibles,  $W_2$  is the weight of oven dried grind samples + crucibles,  $W_3$  is the weight of ash + crucibles.

The carbon storage in different culm tissues was determined by multiplying the biomass with the carbon concentration. The total  $C$  storage in the above ground standing biomass was obtained by summing the  $C$  concentration values for leaf, branch and culm tissues.

## 2.6 Moisture content of bamboo culms

The moisture content in the wood that matches the ratio of mass of water contained therein and the mass of dry wood was determined according to the ASTM D 143-06 (Standard Methods of Testing Small Clear Specimens of Timber).

$$U\% = [(m_o - m_i)/m_o] \times 100$$

where  $U$  is the humidity,  $m_i$  is the initial mass and  $m_o$  is the dry weight.

## 2.7 Soil analysis

Soil samples of each bamboo clump were collected at a depth of 0-20 cm, in a total of 20 samples. The samples were dried and passed through a 2 mm sieve, stored in plastic container and sent to the Departamento de Solos of the Universidade Federal de Viçosa for chemical analysis (pH in H<sub>2</sub>O – Relation 1:2.5; Ca<sup>2+</sup> - Mg<sup>2+</sup> - Al<sup>3+</sup> - Extractor: KCl – 1mol/L; SB= sum of exchangeable bases; T - cation exchange capacity in pH 7.0; m= aluminum saturation index; MO (Organic matter) = C. org. x 1.724 –Walkley-Black; S – Extractor - acetic

acid monocalcium phosphate; N – Ntotal- sulfuric acid digestion - Kjeldahl distillation P-Na-K-Fe-Zn-Mn-Cu-Cd-Pb-Ni-Cr- Mehlich-1 Extractor; H + Al - Extractor calcium acetate 0.5mol/L – pH 7.0; t - Cation exchange capacity; V= cation exchange capacity; P-rem= remaining phosphorus) (Table 2A) and physical analysis (coarse sand, fine sand, silt, clay) (Table 3A).

## **2.8 Cell wall extraction**

The cell wall was extracted sequentially in MilliQ water at 70 °C for 1 h, ethanol at 80 °C for 4 h, ethanol at 25 °C for 4 h and acetone at 54 °C for 1 h according to procedure suggested by Foster et al., (2010).

## **2.9 Lignin content**

Approximately 5 mg of cell wall residue was used to determine the lignin content according to Foster and collaborators (2010) with modifications as follow. Samples were incubated for 30 min at 70 °C in 100 µl 25% (v/v) acetyl bromide in glacial acetic acid and 4 mL of perchloric acid. The samples were cooled to room temperature and centrifuged (15 min, 14000 rpm). The supernatant was transferred to a new tube. The pellet was washed with 500 mL of acetic acid and centrifuged (3 min, 14000 rpm). The supernatants and washings were combined and transferred to a new tube containing 200 mL of NaOH (2M). The final volume was adjusted to 2 mL with glacial acetic acid. 100 µl of the solution was pipetted into a UV specific 96 well plate and read in a ELISA reader at 280 nm. The lignin concentration were calculated by means of the Bouguer-Lambert-Beer law (Chang et al., 2008).

## **2.10 Cellulose quantification**

Approximately 5 mg of cell wall residue was used to determine the amount of cellulose, a colorimetric assay (based on DuBois et al., 1956; Masuko et al., 2005) was performed according to Van Acker et al., (2013). The cell wall residue was incubated with 2 M Trifluoroacetic acid (TFA) and 20 µl inositol (5 mg ml<sup>-1</sup>) for 2 h at 99°C while shaking (750 rpm). After incubation, the remaining pellet was washed three times with water and twice with acetone and dried under vacuum. Concentrated sulfuric acid (150 µl) and 30 µl 5% (w/v) phenol (freshly made in water) were added to the dried pellet and incubated for 1 h at 90°C with gentle shaking (500 rpm). After centrifugation for 3 min at 23,477 g, a 50 µl aliquot of the supernatant

was diluted 20 times with MilliQ water (Millipore, Billerica, MA, USA) to measure the absorbance at 493 nm. The amount of cellulose was calculated back from a standard curve of Avicel® PH-101 (FMC BioPolymer, Philadelphia, PA, USA).

### **2.11 Hemicellulose quantification**

The chemical contents of the bamboo cell walls is constituted of cellulose, hemicellulose, lignin and ash. Thus, we calculated the hemicellulose content indirectly, based on the obtained values of cellulose, lignin and ash.

$$Hemicellulose(\%) = 100 - (C + L + A)$$

where *C* is the cellulose content, *L* the lignin content and *A* the ash content.

### **2.12 Anatomy**

Samples were collected in three culm internodes (Fig. 3), which will be fixed in FAA 50% and stored in 70% alcohol. Afterwards, they underwent a cooking process in order to soften the supporting tissues and facilitate the transversal anatomical cuts by hand free with steel sheet.

The obtained sections were selected for thickness and the thickest ones were discarded; the finest were clarified by immersion in 5% sodium hypochlorite under white light for 30-40 minutes. The slides were then washed in distilled water and stained with Safranin O and Astra Blue, differentiated in distilled water, mounted between slide and cover in glycerinated gelatin. Subsequently, fought with colorless enamel. The slides were analyzed and photographed on a Microscope Axio Scope.A1 Zeiss in increments of 100 and 400x. The anatomical structure of the culm was described according to Liese (1998).

### **2.13 Statistical analysis**

Data were tabulated using the descriptive statistical analysis, Teste t for independents samples and Tukey test. A significance level of 5% was adopted.

The experimental design was based on nested anova to compare chemical analysis results. This test (also known as a hierarchical anova) is used when we have one measurement variable and two or more nominal variables (McDonald, 2014). The nominal variables are nested, meaning that each value of one nominal variable (subgroups) is found in combination with only one value of the higher-level nominal variable (groups) (McDonald, 2014). All of the

lower level subgroupings must be random effects (model II) variables, meaning they are random samples of a larger set of possible subgroups (McDonald, 2014).

The analysis was performed in two stages: (1) for cellulose and lignin, five replicates were used, however, a sample of each compound, due to its low concentration, was not read by ELISA reader, and the analysis was done with four replicates. It is important to note that the unread samples in each case are different replicates. (2) For hemicellulose and lignin, three replicates were used. We compared the concentration of each compound (cellulose, lignin, hemicellulose and ashes) amongst the three species (*Merostachys fischeriana*, *M. tatariana* and *M. ximenes*) and amongst the three regions (base, middle and apex).

### 3. RESULTS

#### 3.1 Culm density and stand biomass

In local conditions, culm densities calculated in presumed plantations of *Merostachys fischeriana*, *M. tatariana* and *M. ximenes* were 11.685 culm ha<sup>-1</sup>, 6.555 culm ha<sup>-1</sup> and 10.545 culm ha<sup>-1</sup>, respectively, with a planting spacing of 7 x 5 m. Considering the spacing of 3 x 3 m the number of culms per hectare increases (Table 1).

Table 1: Characterization of bamboo planting in different plant densities

Characteristic	<i>M. fischeriana</i>	<i>M. tatariana</i>	<i>M. ximenes</i>
Number of culm/ clump	41 ± 4.16 <sup>a</sup>	23 ± 1.78 <sup>b</sup>	37 ± 1.15 <sup>a</sup>
Clump area (m <sup>2</sup> )	0.93 ± 0.06 <sup>a</sup>	1.01 ± 0.16 <sup>a</sup>	3.14 ± 0.33 <sup>b</sup>
Number of culms in spacing 3 m x 3 m	45551 ± 1372.54 <sup>a</sup>	25553 ± 221.93 <sup>b</sup>	41107 ± 41.06 <sup>c</sup>
Biomass (3 m x 3 m) t ha <sup>-1</sup>	3825 ± 1.14 <sup>a</sup>	33.70 ± 0.29 <sup>b</sup>	14.88 ± 0.01 <sup>c</sup>
Number of culms in spacing 7 m x 5 m	11685 ± 260.55 <sup>a</sup>	6555 ± 167.56 <sup>b</sup>	10545 ± 181.10 <sup>a</sup>
Biomass (7 m x 5 m) t ha <sup>-1</sup>	9.81 ± 0.80 <sup>a</sup>	8.64 ± 0.33 <sup>a</sup>	3.82 ± 0.01 <sup>b</sup>

Means ± Standard Mean Error.

Means of species, into each characteristic, followed by different letters (ab) in the column differ (p < 0.05) by Tukey test.

### 3.2 Biomass structure and allometric equations

Biomass structure for the different components (culm, foliage and branches) among the different culm age classes for the three species revealed that the culm components shares the highest proportion of biomass (30-58%), followed by foliage (22-40%) and branch (17-30%) (Table 2). In addition, the *Merostachys* species have a higher productivity in the fourth year (Table 3).

*M. fischeriana* presented leaves, in the first year, with a higher proportion of biomass (42%), followed by the culm (32%) and branches (26%) (Table 2). From the second year, the distribution of biomass remains practically constant with 53-60% in the culm, 22-26% in the leaves and 16-23% in the branches. The total biomass of a complete culm ranged from 0.62 kg in the first year to 1.35 kg in the fourth year and then began to decline. Thus, in the fifth year the individual biomass was 0.72 kg (Table 3).

*M. tatarica* showed a pattern in the distribution of biomass; in the culms a percentage of 50% was found, and the leaf and the branches was 25% each (Table 3). Biomass had two higher peaks, one in the second year with 1.60 kg the other one and in the fourth year with 1.73 kg. The lowest peak was in the fifth year with 1.23 kg. This species present habit erect to scandent, a larger diameter of the culm and height compared to the other species; additionally it also has the biggest wall thickness (Table A4).

In *M. ximenes* culms display 33-51% of the biomass, followed by leaves (31-40%) and branches (17-30%) (Table 2). Unlike other species of *Merostachys*, the culm in *M. ximenes* had its most representative on the first year (51%); although the total biomass was lower (0.063 kg). The highest biomass was reached at the fourth year with 0.68 kg (Table 3). This species has a small diameter and a wall slightly thickened in the middle region (2.1 cm). In addition, due to present leaves larger than the others studied species, this organ also has more expressive in all over the years.

Adjusted equations by the method of linear regression to estimate the total biomass of *Merostachys* were satisfactory, being the highest determination coefficient value 0.81 for *M. fischeriana*, 0.84 for *M. tatarica* and 0.90 for *M. ximenes* (Tables 9A, 10A and 11A).

Table 2: The proportion of foliage, branches and culms to aboveground biomass for each age range

Year	<i>M. fischeriana</i>			<i>M. tatariana</i>			<i>M. ximenes</i>		
	Foliage	Branches	Culm	Foliage	Branches	Culm	Foliage	Branches	Culm
1	42	26	32	23	24	53	31	17	51
2	26	21	53	25	26	49	40	26	33
3	22	18	60	25	25	50	36	30	33
4	26	16	58	23	24	53	32	25	42
5	23	23	54	28	29	43	36	26	36

Table 3: Characteristics of *M. fischeriana*, *M. tatariana* and *M. ximenes* samples mean  $\pm$  standard error. Dbh: diameter at breast height, Ht: total height

Species	Age	Dbh (cm)	Ht (m)	Biomass (kg)			
				Foliage	Branches	Culms	Aboveground
<i>M. fischeriana</i>	1	2.12 $\pm$ 0.14	6.99 $\pm$ 1.34	0.41 $\pm$ 0.10	0.26 $\pm$ 0.07	0.30 $\pm$ 0.05	0.62 $\pm$ 0.09
	2	1.92 $\pm$ 0.10	7.91 $\pm$ 0.51	0.19 $\pm$ 0.01	0.15 $\pm$ 0.02	0.37 $\pm$ 0.04	0.73 $\pm$ 0.04
	3	2.00 $\pm$ 0.27	10.65 $\pm$ 0.92	0.17 $\pm$ 0.05	0.14 $\pm$ 0.05	0.44 $\pm$ 0.05	0.74 $\pm$ 0.12
	4	3.15 $\pm$ 0.39	10.08 $\pm$ 1.39	0.41 $\pm$ 0.18	0.23 $\pm$ 0.04	0.91 $\pm$ 0.26	1.35 $\pm$ 0.36
	5	2.15 $\pm$ 0.13	9.37 $\pm$ 0.49	0.17 $\pm$ 0.04	0.16 $\pm$ 0.03	0.38 $\pm$ 0.04	0.72 $\pm$ 0.11
<i>M. tatariana</i>	1	2.57 $\pm$ 0.10	9.34 $\pm$ 1.34	0.29 $\pm$ 0.01	0.29 $\pm$ 0.05	0.66 $\pm$ 0.06	1.26 $\pm$ 0.10
	2	3.12 $\pm$ 0.26	10.62 $\pm$ 1.24	0.35 $\pm$ 0.12	0.42 $\pm$ 0.13	0.82 $\pm$ 0.07	1.60 $\pm$ 0.19
	3	2.80 $\pm$ 0.15	10.61 $\pm$ 0.80	0.29 $\pm$ 0.04	0.39 $\pm$ 0.05	0.78 $\pm$ 0.11	1.46 $\pm$ 0.14
	4	3.07 $\pm$ 0.42	11.34 $\pm$ 1.98	0.35 $\pm$ 0.06	0.41 $\pm$ 0.07	0.95 $\pm$ 0.21	1.73 $\pm$ 0.27
	5	2.3 $\pm$ 0.21	9.92 $\pm$ 0.97	0.32 $\pm$ 0.11	0.35 $\pm$ 0.10	0.54 $\pm$ 0.11	1.23 $\pm$ 0.30
<i>M. ximenes</i>	1	0.65 $\pm$ 0.02	3.24 $\pm$ 0.24	0.02 $\pm$ 0.002	0.01 $\pm$ 0.002	0.03 $\pm$ 0.003	0.063 $\pm$ 0.006
	2	0.9 $\pm$ 0.12	4.09 $\pm$ 0.51	0.06 $\pm$ 0.02	0.04 $\pm$ 0.009	0.05 $\pm$ 0.008	0.15 $\pm$ 0.04
	3	1.77 $\pm$ 0.29	7.31 $\pm$ 0.38	0.22 $\pm$ 0.10	0.18 $\pm$ 0.03	0.20 $\pm$ 0.06	0.60 $\pm$ 0.19
	4	2.17 $\pm$ 0.10	7.83 $\pm$ 0.32	0.25 $\pm$ 0.03	0.20 $\pm$ 0.02	0.33 $\pm$ 0.02	0.68 $\pm$ 0.11
	5	1.1 $\pm$ 0.21	5.17 $\pm$ 0.46	0.11 $\pm$ 0.03	0.08 $\pm$ 0.02	0.11 $\pm$ 0.03	0.30 $\pm$ 0.08

### 3.3 Growth curve and accumulation of dry weight

*Merostachys* present rapid growth, around seven cm daily (Fig. 4). In the field, we found that in the early days, the culm has a higher rate of growth (20 cmday<sup>-1</sup>), but after reaching the height of 5 to 6 m, the culm begins to grow more slowly, reaching up to 5 cm/day. After the culm stop growing, branches and leaves begin to emerge, which occurs in *Merostachys*, in most cases, from the apex to the base. Thus, between 60 to 90 days the culm already has developed

with branches and leaves. During maturity occurs a thickening of the cell walls of fibers decreasing the internal spaces (lumen diameter).

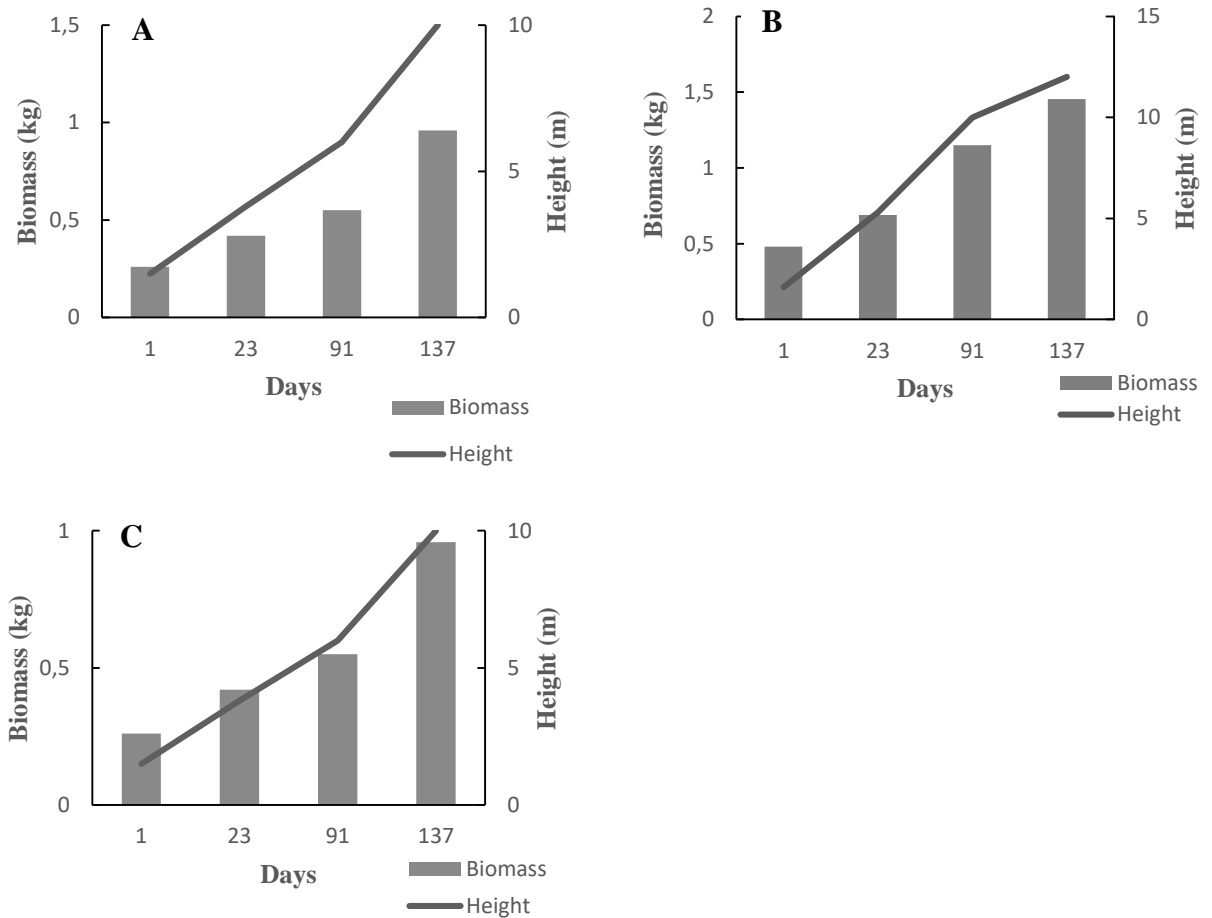


Figure 4: Growth curve indicating the growth and development of *Merostachys* species. A. *Merostachys fischeriana*. B. *M. tatiana*. C. *M. ximena*.

### 3.4 Moisture content of bamboo culms

In all compartments, *Merostachys* showed decreasing moisture content towards the apex (Table 4). The lowest level was presented by the culm, followed by branches and leaves. In general, the culm and branches presented in the first year the higher moisture content and the second year the lowest. In contrast, the leaves had the higher moisture content in the 4-5 year and lower in the early years.

Table 4: Moisture content of culm, foliage and branches from *Merostachys* species

Species	Year	C <sub>B</sub> (%)	C <sub>M</sub> (%)	C <sub>A</sub> (%)	F <sub>B</sub> (%)	F <sub>M</sub> (%)	F <sub>A</sub> (%)	B <sub>B</sub> (%)	B <sub>M</sub> (%)	B <sub>A</sub> (%)
<i>M. fischeriana</i>	1	33	34	41	62	50	45	44	51	41
	2	35	41	27	59	42	50	49	41	46
	3	51	49	38	51	55	41	48	52	49
	4	46	43	42	66	62	55	49	55	52
	5	52	51	56	64	50	65	71	60	55
<i>M. tatarica</i>	1	43	46	35	53	45	47	68	55	55
	2	30	44	37	57	45	39	52	62	48
	3	36	34	33	57	43	50	47	44	41
	4	38	38	43	59	56	53	52	53	50
	5	40	35	40	63	66	53	46	47	47
<i>M. ximenes</i>	1	67	45	48	56	55	57	56	42	44
	2	45	60	28	64	50	52	50	58	50
	3	42	39	45	64	64	61	51	48	39
	4	50	46	46	60	48	59	48	49	48
	5	52	45	46	55	52	51	56	57	58

C<sub>B</sub>: Culm base; C<sub>M</sub>: Culm middle; C<sub>A</sub>: Culm apex; F<sub>B</sub>: Foliage base; F<sub>M</sub>: Foliage middle; F<sub>A</sub>: Foliage apex; B<sub>B</sub>: Branches base; B<sub>M</sub>: Branches middle; B<sub>A</sub>: Branches apex.

### 3.5 Carbon content determination

The carbon content (57%) observed for *Merostachys* species was homogeneous amongst the different organs and species. Therefore, based on this study, the total carbon sequestration for *M. fischeriana*, *M. tatarica* and *M. ximenes* were 5.58-21.66 t ha<sup>-1</sup>, 4.90-19.20 t ha<sup>-1</sup> and 2.16-8.43 t ha<sup>-1</sup>, respectively.

### 3.6 Culm chemical content determination

The design of nested factors comparing the areas to each other and within each species was used. Thus, we verified that there was no significant difference amongst the content of cellulose, hemicellulose, lignin and ash amongst species of *Merostachys* as well as amongst the regions, base, middle and apex. (Table 5). However, *M. tatarica* presented significant differences for the lignin concentration amongst regions.

Table 5: Average lignin, cellulose, hemicellulose and ash content of *M. fischeriana*, *M. tatarica* and *M. ximenes*

Character <sup>1</sup>	Species <sup>2</sup>	Portion <sup>3</sup>											
		A			M			B					
Lignin (%)	XIM	1.06	± 0.09	<i>ax</i>	1.06	± 0.20	<i>ax</i>	1.27	± 0.08	<i>ax</i>	0.83	± 0.11	<i>ax</i>
	TAT	1.05	± 0.23	<i>ax</i>	0.97	± 0.19	<i>bx</i>	0.45	± 0.10	<i>abx</i>	1.72	± 0.53	<i>ax</i>
	FIS	0.86	± 0.12	<i>ax</i>	0.66	± 0.05	<i>ax</i>	0.69	± 0.14	<i>ax</i>	1.22	± 0.26	<i>ax</i>
Cellulose (%)	XIM	79.01	± 3.10	<i>ax</i>	77.95	± 5.94	<i>ax</i>	80.38	± 7.83	<i>ax</i>	78.70	± 2.80	<i>ax</i>
	TAT	77.90	± 4.18	<i>ax</i>	79.47	± 6.27	<i>ax</i>	80.95	± 9.87	<i>ax</i>	73.29	± 6.71	<i>ax</i>
	FIS	81.85	± 0.82	<i>ax</i>	84.84	± 1.11	<i>ax</i>	81.25	± 0.79	<i>ax</i>	79.47	± 0.81	<i>ax</i>
Hemicellulose (%)	XIM	19.71	± 3.83	<i>ax</i>	20.43	± 0.60	<i>ax</i>	18.29	± 7.26	<i>ax</i>	20.43	± 3.73	<i>ax</i>
	TAT	20.98	± 9.23	<i>ax</i>	19.48	± 7.74	<i>ax</i>	18.53	± 10.59	<i>ax</i>	24.92	± 9.35	<i>ax</i>
	FIS	17.63	± 0.89	<i>ax</i>	14.44	± 2.11	<i>ax</i>	17.99	± 0.10	<i>ax</i>	19.26	± 0.47	<i>ax</i>
Ash* (%)	XIM	0.52	± 0.01	<i>ax</i>	0.56	± 0.01	<i>ax</i>	0.60	± 0.01	<i>ax</i>	0.40	± 0.01	<i>ax</i>
	TAT	0.68	± 0.01	<i>ax</i>	0.72	± 0.01	<i>ax</i>	0.60	± 0.01	<i>ax</i>	0.62	± 0.01	<i>ax</i>
	FIS	0.57	± 0.01	<i>ax</i>	0.60	± 0.01	<i>ax</i>	0.60	± 0.01	<i>ax</i>	0.50	± 0.01	<i>ax</i>

<sup>1</sup> Means ± Standard Mean Error.

<sup>2</sup> Means of Species, into each characteristic, followed by different letters (ab) in the column differ (P < 0.05) by Tukey test.

<sup>3</sup> Means of Position, into each characteristic, followed by different letters (xy) in the row (Species) differ (P < 0.05) by Tukey test.

\* Standard Mean Error < 0.01

The average concentration of cellulose in *M. fischeriana*, *M. tatarica* and *M. ximenes* ranged from 78-82% (Table 5). Thus, the concentration of holocellulose, which corresponds to the cellulose content plus hemicellulose is higher than 98%. In addition, *Merostachys* presented lignin content between 0.86-1.06% (Table 5).

### 3.7 Culm anatomy description

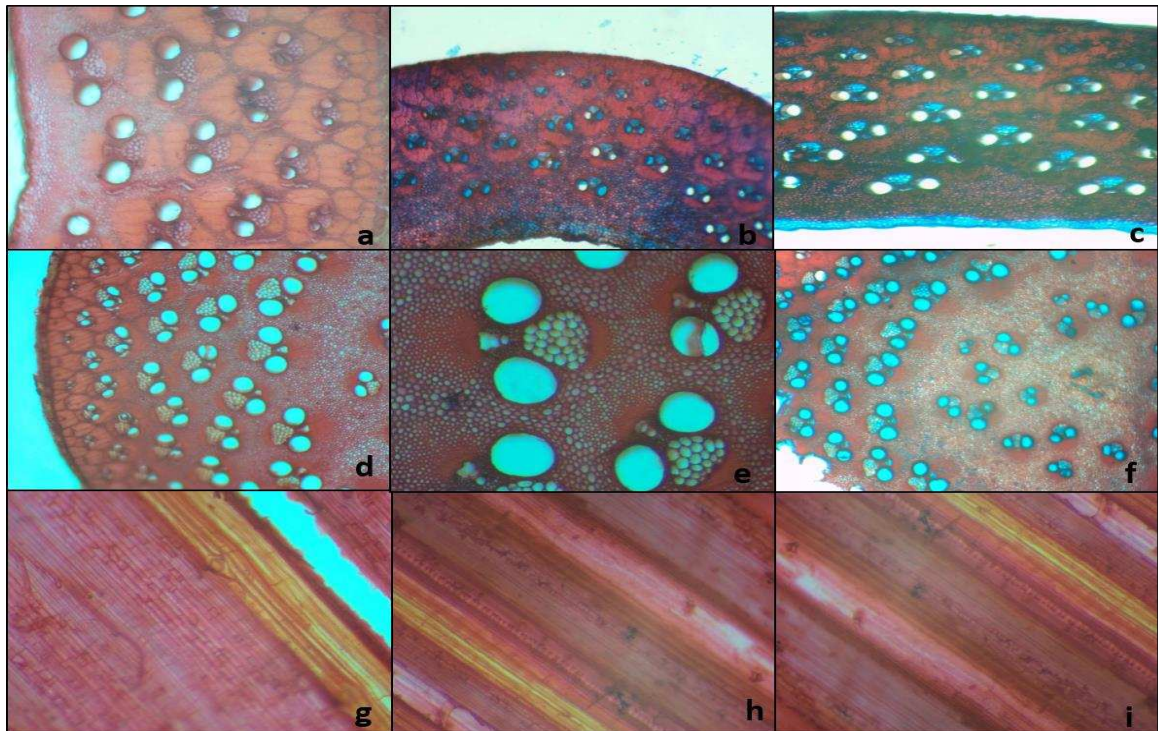
The vascular bundle in culm consists of the xylem with one or two smaller protoxylem elements and two large metaxylem vessels and the phloem with thin walled, unlignified sieve tubes connected to companion cells. Shape, size and distribution of them vary across the internode as well as along culm length (Liese 1998).

The classification, according to Liese (1998), was of type IV. This type consisting of two parts: the central vascular strand with small sclerenchyma sheaths and two isolated fibre bundles outside and inside the central strand (Fig. 5). This type occurs mostly at the basal internodes - which may need the extra support provided by two fibre bundles – and seldom in

the middle part. It occurs combined with type III, which consist of two parts, besides the central vascular strands, a separate fibre stand is present, located at the inner side of the central vascular bundle. The fibre sheath at the protoxylem is usually smaller than the others (Liese 1998).

The fibres constitute the sclerenchymatous tissue and occur in the internodes as caps of vascular bundles and in some species additionally as isolated strands. The fibres are long and tapered at their ends. Across the culm wall the fibre length often increases from the periphery, reaches its maximum at about the middle and decreases towards the inner plan.

The parenchyma cells, forming the basal matrix, are mostly elongated with interspersed shorter cube-like cells. The longer one is characterized by thicker walls with a crossed poly-lammellate structure (Parameswaran & Liese 1975). Apparently, they become lignified at early stages of shoot growth. The proportion of parenchymatous tissue increased from outer to inner part of the culm wall.



**Figure 5:** Culm anatomy of *Merostachys* species. A-F corte transversal, G-I corte longitudinal. A e G: *M. fischeriana*; B-C: *M. ximenaе*; D-F e H-I: *M. tatianaе*.

## 4. DISCUSSION

### 4.1 Culm density and stand biomass

Plant density and yield are important criteria for bamboo cultivation. The productivity of biomass depends on the presence of required amounts of sunlight, water supply and nutrients availability, as well as other environmental conditions including temperature and humidity (Demirbas 2009).

The biomass obtained for *Merostachys* species were comparable with biomass productivity of other bamboo species, despite the fact that our study was performed in a forest area in which the productivity is lower compared to a commercial plantation (Table 6).

Thus, to get a high performance in terms of mechanical strength, culms should be cut when they achieve greater productivity in 40 to 45 months old plants (Ghavami & Marine 2005). Furthermore, it has been shown that young culms have large amount of cellulose and starch, and low lignin content, factors that decrease the durability and strength of bamboo culms, respectively (Li 2007).

The leaves and branches of *Merostachys* presented a greater representativeness to biomass compared to other species of bamboo, wherein majority of species present the culm with a large representation of the biomass (>75%) (Veblen et al., 1980; Christanty et al., 1996; Shanmughavel & Francis 1996; Isagi et al., 1997; Mognon et al., 2014, 2015). However, it is noteworthy that those species are more robust compared to this study, reaching a greater height and diameter. In addition, all species in Table 6, except those belonging to the genus *Merostachys*, occurs in cultivation area and in that way they are exposed to different types of resources and competition, for example.

Except to *M. fischeriana* (Table A5), the results showed that the aboveground biomass and both Dbh and Ht has a high correlation (>75%) (Table A6, A7). However, a low correlation was found between aboveground biomass and age (<50%). This correlation implies that age was not a significant variable for biomass estimation and was not selected for further model analysis. Many studies have pointed out that above ground biomass accumulation of bamboo increases with increasing age (Shanmughavel & Francis 1996; Scurlock et al., 2000). In our study as well as that one conducted by Yen et al. (2010), a relationship between aboveground biomass and age was not prevalent.

Table 6: Comparison amongst bamboo biomass studies

Species	Biomass ton h <sup>-1</sup>	Reference
<i>Phyllostachys nigra</i> (Lodd. ex Lindl.) Munro	37.2-95.4	Suzuki & Uchimura (1980)
<i>Chusquea culeou</i> E. Desv.	155.9-161.7	Veblen et al., (1980)
<i>Chusquea tenuiflora</i> Phil.	13	Veblen et al., (1980)
<i>Fargesia spathaceae</i> Franch.	23.7	Taylor & Zisheng (1987)
<i>Phyllostachys bambusoides</i> Catillonis	90.2-135.8	Isagi et al., (1993)
<i>Dendrocalamus strictus</i> Nees	4-22	Tripathi & Singh (1994)
<i>Bambusa bamboos</i> (L.) Voss	122-287	Shanmughavel & Francis (1996)
<i>Gingantochloa ater</i> (Hasskari) Kurz ex. Munro,	45	Christanty et al., (1996)
<i>G.veerticillata</i> (Willdenow) Munro		
<i>Dendrocalamus oldhami</i> Munro	134.49	Lin (1998)
<i>Bambusa oldhami</i> Munro	28.5	Yiming et al., (1998)
<i>Dendrocalamus strictus</i> Nees	30-36	Singh & Singh (1999)
<i>Dendrocalamus latiflorus</i> Munro	28.49	Lin (2000)
<i>Phyllostachys pubescens</i> (Pradelle) Mazel ex. J.	10-15	Hsiung (2000)
Horiz		
<i>Phyllostachys pubescens</i> (Pradelle) Mazel ex. J.	30	Chun (2003)
Horiz		
<i>Phyllostachys pubescens</i> (Pradelle) Mazel ex. J.	15-22	Xingcui (2004)
Horiz		
<i>Yashania alpinia</i>	110	Embaye et al., (2005)
<i>Bambusa cacharensis</i> R.B. Majumdar, <i>B.</i>	121.51	Nath et al., (2009)
<i>vulgaris</i> Schrad. Ex J.C.Wendl. and <i>B. balcooa</i>		
Roxb.		
<i>Bambusa beecheyana</i> Munro	10-120	Darabant et al., (2014)
<i>Dendrocalamus asper</i> (Schult.) Backer	377.78	Mognon et. al., (2015)
<i>Merostachys fischeriana</i> Ruprecht ex Doell	9.8-38	Present study
<i>Merostachys tatiana</i> e Santos-Gonçalves,	8.6-33.7	Present study
Carvalho-Okano & Filgueiras		
<i>Merostachys ximena</i> e D.F. Parma, R. Vinícius-	3.8-14.8	Present study
Silva & A.P. Santos-Gonçalves		
<i>Eucalyptus</i> L'Her.	58.4-212.1	Santana (2008)
<i>Eucalyptus</i> L'Her.	115.83-168.26	Gatto et al., (2011)
<i>Pinus</i> L.	25-35	Dossa et al., (2002)

## 4.2 Biomass structure and allometric equations

Thirteen types of allometric models were used to estimate biomass according to Yen et al. (2010), Nath et al. (2009), Darabant et al. (2014), Mognon et al. (2014; 2015), Sanquetta et al. (2015) and Sohel et al. (2015) (Table A1). The equation " $\ln Bs = \beta_0 + \beta_1 \ln(Dcolo) + \beta_2 \ln(dap^2) + \beta_3 \ln(dap \cdot h \cdot t^2)$ ", as well as Mognon et al. (2014), showed the highest coefficient of determination for the species *M. tatariana* and *M. ximenesae*. On the other hand, to *M. fischeriana* the equation " $Bs = \beta_0 + \beta_1 \cdot (Dbh^2 \cdot h)$ " presented the best fit, as well as showed by the studies of Sohel et al. (2015).

Some studies used satisfactorily only Dbh to estimate biomass (Shanmugavel & Francis, 1996; Nath et al., 2009; Yen et al., 2010; Darabant et al., 2014). However, to obtain a good estimate of the biomass in *Merostachys* shall be made necessary the additional use of variable height. Shanmughavel & Francis (1996) emphasize the difficulties of using variables such as when certain equations, in view of the difficulties of its measurement, hence the preference for only the Dbh, is more practical and less prone to error.

## 4.3 Growth curve and accumulation of dry weight

In general, bamboos have a rapid growth rate. Some species of paleotropical bamboos have a higher growth rate compared to *Merostachys* ( $0.7 \text{ cm day}^{-1}$ ), however *Merostachys* reaches its maximum height within 45 to 90 days, as observed for *Phyllostachys pubescens* (Lee 1983; Yu 1995). Amongst the paleotropical ones *Dendrocalamus giganteus* Wall. ex Munro presented a maximum increment of  $22 \text{ cm day}^{-1}$  (Azzini et al., 1981) up to  $39 \text{ cm day}^{-1}$  (Ghavami 1995). On the other hand, to *Phyllostachys reticulata* (Rupr.) K.Koch was recorded record growth  $1.20 \text{ cm day}^{-1}$  (Ueda 1960).

## 4.4 Moisture content of bamboo culms

The mechanical characteristics of bamboo can vary according the species, age, cutting time, climate, and soil moisture content (Ghavami 1989). Age and culm region are the most important variables for determining properties of different species (Murad 2007). The moisture content decreases towards the apex (Ghavami & Toledo-Filho 1992), as well as the diameter and the thickness of the culm wall (Table A4). The natural humidity ranges from 13-20%, however there are a number of factors that influence its characteristics and properties (Murad 2011). When the moisture content increases, modulus of elasticity, compressive strength, tensile, shear and bending parallel to the fiber decrease (Chun 2003). Bamboo, like wood, changes in size when it loses or gains moisture (Ahmad 2000). Bamboo is a hygroscopic

material, the moisture content changes when there is a variation of relative humidity and room temperature.

Differently from that observed for the species of *Merostachys*, *Dendrocalamus giganteus* presented 13-15% moisture content (Lopes et al., 2000; Ghavami, 2005). Ghavami & Toledo-Filho (1992) found for the same species the 19.5% at the base, 18.9% in the middle and 13.9% in the apex of moisture content in the region of Rio de Janeiro. However, for the Paraíba, the observed values were 15.6%, 15.3% and 14.5% for base, middle and apex, respectively (Ghavami & Toledo-Filho 1992). *Guadua angustifolia* Kunth had 14.1-16.9% (Lopes et al., 2000) and 13-14% (Ghavami 2005) moisture content. In contrast, *G. verticillata* Munro showed 9.6-12.3% (Lopes et al., 2000) and *G. weberbaueri* Pilg. 14.1% moisture content (Murad, 2007).

#### **4.5 Carbon content determination**

Bamboo covers an area of approximately 37 million ha in the world (Kant, 2010). Many bamboo species studied have high carbon storage capacity (Sohel et al., 2015). According to this study, the carbon content (57%) observed for *Merostachys* species was homogeneous amongst the different compartments and species. Differently from *Merostachys*, *Phyllostachys makinoi* presented culm with 47.49-47.82%, foliage with 38.12-44.78% and branches with 45.66-46.23% of carbon content (Yen et al., 2010).

Based on this study, the total carbon sequestration for *M. fischeriana*, *M. tatariana* and *M. ximenesii* were 5.58-21.66 t ha<sup>-1</sup>, 4.90-19.20 t ha<sup>-1</sup> and 2.16-8.43 t ha<sup>-1</sup>, respectively. Such carbon stock is nearly similar or higher than many fast growing timber species, for example, *Acacia auriculiformis* A. Cum. ex Benth., *Dipterocarpus turbinatus* Gaerth. and *Swietenia mahagoni* (L.) Jacq. 19.38 t ha<sup>-1</sup>, 8.98 t ha<sup>-1</sup> and 28.81 t ha<sup>-1</sup>, respectively (Shin et al., 2007). Previous studies on bamboo from tropical and subtropical countries have shown that various bamboo species have close or higher carbon amount than many valuable fast growing timber species or tropical forest ecosystem (Sohel et al., 2015).

#### **4.6 Chemical composition**

This low amplitude of variation may be due to the species belonging to the same genera, corroborating with the results found for *Phyllostachys* (Felgel & Wegener, 1984; Li et al., 2007) and *Gigantochloa* (Wahab et al., 2013). In addition, *M. fischeriana*, *M. tatariana* and *M. ximenesii* were located in the same environment and the soil slightly differed amongst species. The

environmental factors have great influence in the properties of the wood (Ferreira et al., 1978; Barcellos et al., 2005).

Cellulose is the main component of the cell wall of the fiber and determinant of mechanical properties of bamboo and wood, with a linear structure constituted by a single type of sugar unit (Janssen 1981; Penedo 1980). However, the composition varies according to the species, growth conditions, age of the bamboo and the part of the culm (Liese 1985). The *Merostachys* species studied presented around 75% de cellulose. In contrast, Paleotropical bamboos such as *Bambusa*, *Gigantochloa* and *Phyllostachys*, which are more robust than *Merostachys*, the cellulose content ranged from 25.5-53% (Higuchi 1957; Youdi et al., 1985; Li et al., 2007, Wahab et al., 2013).

The combination of cellulose and hemicellulose is called holocellulose and its content of plant material is important to industries like pulp paper, wood hydrolysis and charcoal because it is a key factor affecting the quality of these products. Its contents in bamboo culms are generally higher than 70%, which can be compared with that of reed (75.4%), cotton shaft (75.1%) and bagasse (75.6%) (Youdi et al., 1985). For the species of bamboo *Phyllostachys* (Fengel & Shao, 1984; Youdi et al., 1985; Li et al., 2007), *Bambusa* (Youdi et al., 1985) e *Gigantochloa* (Wahab et al., 2013), the observed holocellulose content is 60-85% different from that found for *Merostachys* species (98%).

The high concentration of this component in *Merostachys* species makes them not suitable for the production of coal, since almost all holocellulose has thermal degradation between 200 and 350 ° C. Therefore, its high concentration is not suitable for the production of coal, because the charcoal is produced at temperatures above 400 °C.

The basic density is related to the chemical composition of the cell wall, which is defined by cooking conditions to achieve adequate pulp (IPT 1998). Thus, for the investigated species, the basic density is related to the holocellulose content. Therefore, *Merostachys* requires milder cooking conditions compared to the species of conifers and eucalyptus that contains lower concentration of holocellulose (Araújo 2000).

The lignin present in cell walls of plants is always associated with hemicellulose not only through physical interaction but as covalent bonds as well (Philipp 1988). Their structure may be different, depending on its location in the plant, with the contribution of topochemical factors that influence their formation (Rowell et al., 2005). In addition, the lignin content stabilizes at maturity, around one year of age depending on the species (Pereira & Beraldo 2007).

Lignin is obtained on a large scale around the world, as a by-product of the pulping industry, whose main advantage is still as an energy source by burning in the recovery boiler. The lignin is the most resistant compound to thermal decomposition, since its structure is quite complex (Brito & Barrichelo 1977). When in lower concentration, as observed for the species of *Merostachys* (< 2%), in turn, results in a lower yield of coal, because wood with higher lignin content results in coal with higher calorific value (Pastore et al., 1989).

These results show that *Merostachys* can be to be interesting for the production of biofuels, for example, in which one of the limiting factors for the conversion of cellulosic materials is the high concentration of lignin. The opposite is observed for other bamboo species, *Bambusa*, *Dendrocalamus*, *Gigantochloa* and *Phyllostachys*, in which the observed lignin content was 22-27% (Higuchi, 1957; Fengel & Shao, 1984; Youdi et al., 1985; Li et al., 2007; Prota 2010; Marinho et al., 2012; Wahab et al., 2013) similar to that of softwoods and hardwoods. *Pinus* and *Eucalyptus*, for example, presents a lignin content, which is relatively high, so, the wood is not indicated for bleached pulp but for unbleached pulp. The opposite is observed for the investigated *Merostachys* species that is indicated for bleached pulp.

Ash is a term generally used to refer to inorganic substances such as silicates, sulfates, carbonates, or metal ions (Rydholm 1965) that do not enter into combustion being in solid form and are undesirable for energy use (Chaves et al., 2013). Higher ash content in some species can adversely affect the processing machinery, because silica is generally the main constituent in ash and ultimately represents a problem for the pulp and paper making process. The silica content of the bamboo culm is generally higher than that of wood (0.5-4.0%) and mostly deposited in the epidermis (Lwin et al., 2006).

*Merostachys* presented ash content between 0.52-0.68%, lower than that found for other species of bamboo (1-2.9%), *Bambusa*, *Dendrocalamus* and *Phyllostachys* (Higuchi, 1957; Youdi et al., 1985; Prota, 2010; Marinho et al., 2012). The ash content is positively correlated to the volatile content. The lower volatile material contents have higher fixed carbon contents (Chaves et al., 2013). In this way, the calorific value has a direct relation with the fixed carbon content. Therefore, what is expected is that high levels of fixed carbon imply in higher calorific (Chaves et al., 2013).

Ashes concentration is low for 1-3% bamboo species (Table 6), but for *Merostachys* the observed values were even lower 0.52-0.68% (Figure 4) and similar to that observed for some species of *Eucalyptus* (Araújo 2000; Trugilho et al., 2003; Chaves et al., 2013).

#### 4.7 Anatomy

The main constituents of the fibrovascular bundles are fibers and conducting vessels (xylem and phloem). The size and density of the bundles vary according to the position, thickness and species of bamboo. However, the number of fibrovascular bundles is equal to the length of the culm, but since the wall thickness at the base is larger than at the top, this causes the bundle concentration to be lower in the base (Chun 2003).

The fibers are characterized by their thin shape. Its length influences the mechanical strength of the stems and contribute to 40-50% of the total culm tissue and 60-70% by weight. The parenchymal tissue, with the function of storing water and nutrients, occupies most of the stem of the bamboo, about 40 to 60%. It surrounds the fibrovascular bundles, but a small part passes through the bundles (Chun 2003). The shorter cells have a denser cytoplasm and thinner walls; they retain their cytoplasmatic and strong peroxidase activities for a long time (Grosser & Liese 1971).

The rigidity of the thickenings and the flexibility of the intervening wall areas free of lignin may be involved in the control of water flow through the wall. The protoxylem structure can be considered as a special feature of the elongation process of the bamboo culm, with both functional and structural implications (Liese 1998).

#### 5. CONCLUSION

Studies of characterization of the chemical compounds of bamboo cell wall are rare. Comprehensive knowledge of the chemical compounds in the bamboo species will facilitate the use of materials in the forestry industrial sector and help to enhance their utilization in the chemical and biochemical related industry. In addition, it is interesting to quantify the chemical components, mainly lignin, of herbaceous bamboos in order to compare with *Merostachys*, which is considered a true woody bamboo.

Our results indicate that *Merostachys* shown a good carbon credit and viable option for the biofuel industry, pulp and paper. However, further research work is necessary for development of agronomic and biotechnological strategies for cultivation and production in large scale. Furthermore, the features of the species covered will be useful in studies of taxonomic and ecological approaches.

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## APPENDIX

Table 1: Mathematical models tested for estimation of the total dry biomass (Bs) of species

Number	Model
1	$B_s = \beta_0 + \beta_1 ht$
2	$B_s = \beta_0 + \beta_1 Dbh$
3	$B_s = \beta_0 + \beta_1 Dbh^2$
4	$B_s = \beta_0 + \beta_1 Dbh * ht^2$
5	$B_s = \beta_0 + \beta_1 Dbh + \beta_2 ht$
6	$B_s = \beta_0 + \beta_1 dcolo + \beta_2 Dbh^2 + \beta_3 (Dbh * ht^2)$
7	$\ln B_s = \beta_0 + \beta_1 \ln ht^2$
8	$\ln B_s = \beta_0 + \beta_1 \ln dap$
9	$\ln B_s = \beta_0 + \beta_1 \ln Dcolo + \beta_2 \ln dap^2 + \beta_3 \ln (dap * ht^2)$
10	$B_s = \beta_0 + \beta_1 (Dbh^{0.5} * \ln Dbh)$
11	$\log B_s = \beta_0 + \beta_1 \log Dbh$
12	$B_s = \beta_0 + \beta_1 (Dbh^2 h)$
13	$B_s = \beta_0 + Dbh^{\beta_1}$

$\beta_0 \dots \beta_n$  = model coefficients; ps = dry weight (kg); Dbh = diameter at breast height (cm); ht = height (m); dc = diameter neck (cm).

Table 2: Soil chemical properties at *Merostachys* species

Species	pH H <sub>2</sub> O	P	K	S	Cu	Mn	Fe	Zn	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Al <sup>3+</sup>	H + Al	SB	t	T	V	m	Mo	N	P-Rem
		-----mg/dm <sup>3</sup> -----								-----cmol/dm <sup>3</sup> -----					%	dag/kg	g/L			
<i>M. fischeriana</i>	5.3 ±	4.2±	96.2±	2.8±	1.4±	120.3±	48.1±	3.5±	3.7±	1.7±	0.3±	5.8±	5.6±	5.9±	11.4±	49.4±	5.0±	3.8±	0.56±	29.2±
	0.3	0.8	28.5	0.7	0.1	6.1	15.9	0.9	0.3	0.3	0.1	0.6	0.6	0.5	0.2	5.3	2.9	0.3	0.01	2.7
<i>M. tatarica</i>	4.8 ±	4.4±	44.1±	2.4±	1.6±	142.6±	75.0±	1.1±	2.0±	0.9±	0.6±	8.0±	3.0±	3.6±	11.1	25.7±	23.9±	4.1±	0.52±	24.5±
	0.2	0.6	3.2	0.3	0.1	21.6	10.3	0.1	0.5	0.1	0.1	0.4	0.6	0.5	±0.7	4.7	6.3	0.4	0.04	1.1
<i>M. ximenes</i>	5.3 ±	1.8±	35.0±	2.1±	0.9±	113.9±	91.8±	2.15±	3.2±	1.4±	0.4±	6.0 ±	4.7±	5.1±	10.7	45.3±	16.6±	3.4±	0.55±	28.6±
	0.3	0.3	3.4	0.9	0.1	8.4	76.8	0.6	1.0	0.4	0.44	1.8	1.5	1.1	±1.3	12.8	16.6	0.9	0.04	6.1

pH in H<sub>2</sub>O – Relation 1:2.5; Ca<sup>2+</sup> - Mg<sup>2+</sup> - Al<sup>3+</sup> - Extractor: KCl – 1mol/L; SB= sum of exchangeable bases; T - cation exchange capacity in pH 7.0; m= aluminum saturation index; MO (Organic matter)= C. org. x 1.724 –Walkley-Black; S – Extractor - acetic acid monocalcium phosphate; N – N<sub>total</sub>- sulfuric acid digestion - Kjeldahl distillation P-Na-K-Fe-Zn-Mn-Cu-Cd-Pb-Ni-Cr- Mehlich-1 Extractor; H + Al - Extractor calcium acetate 0.5mol/L – pH 7.0; t - Cation exchange capacity; V= cation exchange capacity; P-rem= remaining phosphorus.

Table 3: Soil physical properties at *Merostachys* species

Species	Coarse sand (kg/kg)	Fine sand (kg/kg)	Silt (kg/kg)	Clay (kg/kg)	Textural classification <sup>1</sup>	Type of soil <sup>2</sup>
<i>M. fischeriana</i>	0.29 ± 0.04	0.13 ± 0.0	0.11 ± 0.01	0.45 ± 0.04	sandy clay	clayey
<i>M. tatarica</i>	0.15 ± 0.02	0.10 ± 0.01	0.14 ± 0.01	0.52 ± 0.06	clay	clayey
<i>M. ximenes</i>	0.27 ± 0.10	0.09 ± 0.02	0.11 ± 0.03	0.50 ± 0.09	clay	clayey

<sup>1</sup>SBCS; <sup>2</sup> IN SPA/MAPA 02/2008

Table 4: Thickness of the culm wall of *Merostachys* species

Species	Base (cm)	Middle (cm)	Apex (cm)
<i>M. fischeriana</i>	1.92	1.5	0.99
<i>M. tatarica</i>	2.57	2.23	2.74
<i>M. ximenesae</i>	1.88	1.21	0.69

Table 5: Correlation analysis between biometric variables of *Merostachys fischeriana*

	Cbh	Dbh	Dc	Ht	Nodes	Culm	Leaves	Branches	Total
Cbh	1								
Dbh	0.99*	1							
Dc	0.99*	0.99*	1						
Ht	0.25	0.25	0.23	1					
Nodes	0.17	0.16	0.16	0.80*	1				
Culm	0.86*	0.86*	0.86*	0.48*	0.40	1			
Leaves	0.29	0.28	0.31	-0.01	0.04	0.44*	1		
Branches	0.43	0.42	0.43	0.19	0.16	0.48*	0.67*	1	
Total	0.79*	0.78*	0.80*	0.39	0.33	0.94*	0.69*	0.71*	1

\*p < 0.05

Table 6: Correlation analysis between biometric variables of *Merostachys tatarica*

	Cbh	Dbh	Dc	Ht	Nodes	Culm	Leaves	Branches	Total
Cbh	1								
Dbh	0.98*	1							
Dc	0.98*	0.99*	1						
Ht	0.46*	0.43	0.46*	1					
Nodes	0.09*	0.04	0.07	0.62*	1				
Culm	0.81*	0.79*	0.78*	0.77*	0.33	1			
Leaves	0.30	0.38	0.34	0.33	0.06	0.31	1		
Branches	0.33	0.40	0.39	0.64*	0.25	0.54*	0.80*	1	
Total	0.67*	0.82*	0.68*	0.75*	0.29	0.85*	0.73*	0.88*	1

\*p < 0.05

Table 7: Correlation analysis between biometric variables of *Merostachys ximenesae*

	Cbh	Dbh	Dc	Ht	Nodes	Culm	Leaves	Branches	Total
Cbh	1								
Dbh	0.99*	1							
Dc	0.99*	0.99*	1						
Ht	0.85*	0.84*	0.85*	1					
Nodes	0.82*	0.80*	0.80*	0.88*	1				
Culm	0.95*	0.94*	0.93*	0.82*	0.76*	1			
Leaves	0.84*	0.84*	0.84*	0.71*	0.66*	0.87*	1		
Branches	0.92*	0.92*	0.92*	0.85*	0.73*	0.92*	0.89*	1	
Total	0.91*	0.92*	0.91*	0.77*	0.70*	0.90*	0.95*	0.95*	1

\*p < 0.05

Table 8: Fitting total dry biomass equations for *Merostachys fischeriana*

Model	$\beta_0$	$\beta_1$	$\beta_2$	$\beta_3$	R <sup>2</sup> ajustated
1	7.269	0.0021			0.1506
2	1.2623	0.0012			0.6175
3	-0.3328	0.0068			0.5918
4	381.67	2.2855			0.5918
5	-614.7980	495.9009	38.1869		0.6552
6	1156.1762	-645.0139	193.1017	0.8318	0.7873
7	1.3962	0.4395			0.0789
8	-2.141	0.4382			0.433
9	4.5706	4.8756	-1.7893	0.1542	0.6243
10	0.3161	0.001			0.5989
11	2.5563	0.9881			0.433
12	396.76	8.7575			0.8103
13	360	0.9881			0.433

Table 9: Fitting total dry biomass equations for *Merostachys tatarica*

Model	$\beta_0$	$\beta_1$	$\beta_2$	$\beta_3$	R <sup>2</sup> ajustated
1	-307.59	161.51			0.5753
2	-202.15	592.93			0.5007
3	560.07	108.12			0.4412
4	745.63	19407			0.6134
5	1.7708	-0.0594	0.0011		0.5239
6	-208.4889	573.5092	-65.4733	1.4934	0.6193
7	54.272	699.45			0.5313
8	54.272	1398.9			0.5313
9	4.2614	-4.4448	2.5183	0.4585	0.8420
10	35016	64732			0.5074
11	24563	15605			0.6841
12	676.38	8.6335			0.6131
13	285.98	1.5605			0.6841

Table 10: Fitting total dry biomass equations for *Merostachys ximena*

Model	$\beta_0$	$\beta_1$	$\beta_2$	$\beta_3$	R <sup>2</sup> ajustated
1	-323.5	123.98			0.5875
2	-220.82	441.77			0.8488
3	42.379	148.19			0.849
4	64731	52376			0.7742
5	-207.1801	457.0677	-6.1164		0.8492
6	-53.1030	143.1020	92.8510	0.2572	0.8513
7	-0.2966	0.6551			0.7177
8	-2.5216	0.4887			0.8741
9	4.6789	-3.1014	21693	0.2315	0.9091
10	221.62	452.17			0.848
11	2.2581	1.7886			0.8741
12	90.953	18.243			0.8347
13	181.16	1.7886			0.8741

Table 11: Anova nested factor species (region) effect sliced by species

<b>Lignin</b>					
<b>Source of variation</b>	<b>DF</b>	<b>Type I SS</b>	<b>Mean Square</b>	<b>F Value</b>	<b>Pr &gt; F</b>
Species	2	0.3031	0.1515	0.69	0.5086
<i>M. fischeriana</i>	2	0.7975	0.3987	1.82	0.1807
<i>M. tatiana</i>	2	3.2834	1.6417	7.51	0.0026
<i>M. ximena</i>	2	0.3833	0.1916	0.88	0.4276
Error	27	5.9025	0.2186		
Corrected Total	35	10.6699			

<b>Cellulose</b>					
<b>Source of variation</b>	<b>DF</b>	<b>Type I SS</b>	<b>Mean Square</b>	<b>F Value</b>	<b>Pr &gt; F</b>
Species	2	99.6685	49.8342	0.39	0.6822
<i>M. fischeriana</i>	2	59.7006	29.8503	0.23	0.7943
<i>M. tatiana</i>	2	132.3318	66.1659	0.51	0.6033
<i>M. ximena</i>	2	12.3902	6.1951	0.05	0.953
Error	27	3469.344	128.4942		
Corrected Total	35	3773.435			

<b>Hemicellulose</b>					
<b>Source of variation</b>	<b>DF</b>	<b>Type I SS</b>	<b>Mean Square</b>	<b>F Value</b>	<b>Pr &gt; F</b>
Species	2	226.825	113.412	0.84	0.4492
<i>M. fischeriana</i>	2	48.4587	24.2293	0.18	0.8377
<i>M. tatiana</i>	2	43.0087	21.5043	0.16	0.8544
<i>M. ximena</i>	2	49.2517	24.6258	0.18	0.8353
Error	18	2439.02	135.501		
Corrected Total	26	2806.56			

<b>Ash</b>					
<b>Source of variation</b>	<b>DF</b>	<b>Type I SS</b>	<b>Mean Square</b>	<b>F Value</b>	<b>Pr &gt; F</b>
Species	2	0.000124	0.000062	0.25	0.7837
<i>M. fischeriana</i>	2	0.000072	0.000036	0.14	0.8672
<i>M. tatiana</i>	2	0.000289	0.000144	0.57	0.5749
<i>M. ximena</i>	2	0.000803	0.000401	1.59	0.2320
Error	18	0.004553	0.000252		
Corrected Total	26	0.005842			

## GENERAL CONCLUSIONS

- *Merostachys fischeriana*, *M. tatiana* and *M. ximena* showed a productivity, in cultivation 3m x 3m, highly competitive with crops of other species of bamboo and pine, for example.
- The *Merostachys* species have a higher productivity in the fourth year and the culm shares the highest proportion, followed by foliage and branch.
- Adjusted equations by the method of linear regression to estimate the total biomass of *Merostachys* were satisfactory.
- *Merostachys* present rapid growth, averaging 7 cm daily.
- *Merostachys* in all compartments, showed decreasing moisture content towards the apex. The lowest level was presented by the culm, followed by branches and leaves.
- The carbon content observed for *Merostachys* species was homogeneous for the different species and compartments.
- The content of cellulose, hemicellulose, lignin and ash between *Merostachys* species as well as between the regions, base, middle and apex did not presented significant difference between each other. However, only *M. tatiana* presented significant differences for the lignin concentration between regions.