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Lignocellulosic biomass production in six cultivars of *Cenchrus purpureus* (Schumach.) Morrone in the tropics

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ABSTRACT

Objective: To determine the cellulose, hemicellulose, and lignin production in six *Cenchrus purpureus* cultivars, with and without fertilization, harvested every 21 days during 168 days.

Design/methodology/approach: The evaluated cultivars were CT-115, elephant grass, king grass, Maralfalfa, Roxo, and Taiwan, with and without fertilization. The experimental design was a randomized complete block with a split-plot arrangement and four replications. The fertilization dose was 141-43-20 of NPK. Forage was harvested every 21 days during six months in the rainy season. We tested the samples for dry matter (DM), ash, NDF, ADF, hemicellulose, cellulose, and lignin. We performed a nonlinear regression analysis. We determined biomass production with the estimated values and the percentage of each component. The goodness-of-fit indicators were: R^2, the coefficient of determination (R), and the model selection criterion (MSC). The parameters and goodness-of-fit coefficients were analyzed in ANOVA with the SAS software using the GLM procedure. Means were compared with Tukey's test.

Results: The Maralfalfa cultivar presented a higher cellulose production (3,786 kg ha^{-1}), similar ($p>0.05$) to the elephant grass and Taiwan cultivars, which presented values of 3,451 kg ha^{-1} and 3,329 kg ha^{-1}, respectively, and different ($p<0.05$) from CT-115 and king grass, which produced more than 1 t ha^{-1} of cellulose.

Limitations/implications: Cellulose production increased by the effect of fertilization.

Findings/conclusions: The fertilized Maralfalfa, elephant grass, and Taiwan cultivars produced more than 4,000 kg ha^{-1} of cellulose, 3,000 kg ha^{-1} of hemicellulose, and 1,000 kg ha^{-1} of lignin.

Keywords: Biomass, Cellulose, Paper, Hemicellulose, Lignin.

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INTRODUCTION

In the last years, there has been an increased interest in lignocellulosic materials (an array of materials of forestry, agricultural or urban origin). This interest has drawn attention to the use of residual materials from forests, crops, and the timber industry (FAO, 2001). Cellulose, lignin, and hemicellulose can be obtained from said materials in an approximate ratio of 4:3:3, with significant variations depending on the species (Mohammad, 2008). Traditionally, wood has been the preferred material in paper pulp manufacture and, therefore, the primary source of lignocellulosic material. The increased demand for this type of raw material, together with economic and environmental problems, requires the research of alternative sources of lignocellulosic materials other than wood. The *Cenchrus purpureus* (Schumach.) Morrone species, called Napier, Uganda, or elephant grass, is native to Africa. It has been introduced in tropical and subtropical areas for livestock feeding purposes. The paper industry is looking for lignocellulosic resources other than trees (because of their scarcity) or sugar cane (because of a shift toward biofuels). It has opted for the tropical species *C. purpureus*, which has the world's highest potential for lignocellulosic biomass production, due to its erect growth habit, vigorous strains, and large height (Reyes-Castro *et al.*, 2018). Elephant grass and king grass have cellulose and hemicellulose contents of 22.6% and 20.9%, and 23.6% and 21.9%, respectively (Cardona *et al.*, 2013). For the CT-115 cultivar, the reported percentages are 32.9% and 28.8% (Valenciaga *et al.*, 2009). For Maralfafa, Mateus *et al.* report 33.8% and 22.5% (2012). The contents of cellulose and lignin vary greatly, depending on harvest age, soil fertility, variety of grass, and its management, among other factors. Percentages vary between 34% and 39% for cellulose and between 3% and 8% for lignin, considering grasses harvested in weeks 4 and 24 of regrowth for different cultivars of *C. purpureus* (Faria *et al.*, 2017). On average, the cellulose concentration increments for these cultivars ranged from 20% to 40% at 28 to 140 days of regrowth. These data help estimate the theoretical production of paper for each cultivar. Madakadze *et al.* (2010) compared the pulp characteristics of elephant grass and switchgrass (*Panicum virgatum* L.) for paper manufacture. They found that elephant grass had α–cellulose contents of 45.6% and lignin contents of 17.7%; the corresponding contents for switchgrass were 41.2% and 23.9%. The yields for paper pulp were 48% for switchgrass and 50% for elephant grass. These and other characteristics, such as Kappa numbers, and fiber length and freshness, prove the feasibility of using these grasses to produce paper pulp. Agroecological conditions for the cultivation of *C. purpureus* with the required characteristics to produce cellulose and obtain paper are adequate in the tropical areas of Mexico. However, due to a rapid growth rate and variation between cultivars of this species, an assessment is necessary to choose the age of regrowth with the greatest biomass and lignocellulose production rate for each cultivar. Therefore, the objective of our research was to determine the biomass production of cellulose, hemicellulose, and lignin in six cultivars of *C. purpureus*, with and without fertilization with nitrogen, phosphorus, and potassium, harvested every 21 days for 168 days during the rainy season in the tropics of Veracruz.

MATERIALS AND METHODS

The experiment was implemented from July 2010 to January 2011 at the La Posta experimental station in Paso del Toro, Veracruz (19° 00' 49" N and 96° 08' 19" W), at 12 masl (INEGI 2009). The prevailing climate in the area is sub-humid tropical Aw_2 (Vidal 2005); with a rainfall of 1,461 mm, a relative humidity of 77.4%, and an average temperature of 25 °C, with a maximum of 35 °C and a minimum of 15 °C. The soil type is vertisol and has an acidic pH (4.9 to 5.4), a clay sandy loam texture, medium content of organic matter (1.6%-2.6%), and inorganic nitrogen (7-21 ppm), phosphorus (17.6-78.5 ppm), potassium (174 ppm), calcium (1,590-1,936 ppm), and magnesium (288-418 ppm). We used cultivars of CT-115, elephant grass, king grass, Maralfalfa, Roxo, and Taiwan. Each plot was subdivided into two areas: one with and the other one without fertilization. The experimental design was a randomized complete block with a split-plot arrangement and four replications per plot, representing each grass cultivar. The land was prepared with subsoil, fallow, double harrow, and 80 cm furrows. We used three-node cuttings planted every 80 cm on the furrow slope, making sure two of the three nodes remained buried at a 45° angle from the surface. The sowing took place in June 2009, while the assessments were conducted from July 2010 to January 2011, after cutting to level. The sampling area on each cut date was 5.76 m^2 (or three furrows), 2.4 m wide per 2.4 m long. We made a total of eight cuts. At the beginning of the experiment, we cut to level at 25 cm. The fertilization dose was 141-43-20 with the following formula: 200 kg of urea, 50 kg of diammonium phosphate, and 200 kg of the 20-10-10 mixture, rationed in two applications. The first was made eight days after cutting to level, and the second 60 days afterwards. Aerial biomass (green matter) was harvested at 25 cm above the ground every 21 days, for six months, during the rainy season. When sampling, we recorded the production of dry matter (DM) of the whole plant in each parcel, took a subsample of 300 g, minced it, dried it at 55 °C in a forced air furnace until reaching constant weight, and finally ground it with a Willey mill using a 1 mm mesh. The chemical composition of DM and ash of these dehydrated and ground subsamples (A.O.A.C., 2000) was determined using an Ankom Fiber Analyzer. In duplicate, neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin were determined using the 72% sulfuric acid method; hemicellulose and cellulose were calculated by difference (Van Soest *et al.*, 1991). With the results of biomass yield at each sampling moment in kg DM ha^{-1}, we proceeded to perform a nonlinear regression analysis using the Gompertz model and applying the Powell algorithm as a minimization method. We used the Micromath Scientist® software with the Gompertz function:

$$Y = A\exp^{-\exp(-\mu(x-B))}$$

Where Y=biomass yield (kg DM ha^{-1}); x=time (days); A=maximum biomass production (kg DM ha^{-1}); B=inflection point, indicating the end of growth and the beginning of decrease (days); and μ=growth rate (kg DM ha^{-1} day^{-1}). To determine the production of lignocellulosic biomass (lignin, hemicellulose, and cellulose) for each period, we multiplied the estimated values of biomass in kg DM ha^{-1} (obtained using the Gompertz model) by the percentage of cellulose, hemicellulose, and lignin. We did so every 21 days during the

168 days of the experiment. Using the nonlinear Gompertz model, we adjusted the data and obtained the curve parameters of cellulose, hemicellulose, and lignin production for each cultivar.

Experimental design and statistical analysis

The obtained parameters and the goodness-of-fit coefficients were analyzed with ANOVA in a randomized complete block design in a split-plot arrangement —where large plots were cultivars and small plots were split into areas with and without fertilization— and four replications. The data were analyzed with the SAS software (2007) using the GLM procedure. Means were compared using Tukey's test ($p<0.05$).

RESULTS AND DISCUSSION

Maximum production values of cellulose, hemicellulose, and lignin biomass from the whole plant in the different cultivars of *C. purpureus*, obtained with the Gompertz model, can be observed in Table 1. The variance analysis results show that cultivars presented differences ($p<0.05$) in lignocellulosic biomass. The Maralfalfa cultivar presented the highest cellulose production (3,786 kg ha^{-1}), statistically similar ($p>0.05$) to the elephant grass and Taiwan cultivars, whose values were 3,451 kg ha^{-1} and 3,329 kg ha^{-1} respectively, and statistically different ($p<0.05$) from the CT-115 and king grass cultivars, with variations of more than 1 t ha^{-1} of cellulose. Maralfalfa, elephant grass, and Taiwan cultivars produce more biomass DM ha^{-1}, which accounts for the differences and turns them into a renewable option for the Mexican paper industry. Hemicellulose and lignin are part of the elimination process that the paper industry contemplates in its protocols for paper production. It is therefore important to have materials with low concentrations of these components in dry matter and aerial biomass production per hectare (Segura *et al.*, 2008). In this study, the CT-115, king grass, and Roxo cultivars produced 2,133 kg ha^{-1}, 1,519 kg ha^{-1}, and 2,105 kg ha^{-1} of hemicellulose, respectively, and 550 kg ha^{-1}, 530 kg ha^{-1}, and 688 kg ha^{-1} of lignin. The king grass cultivar excelled in both components (hemicellulose and lignin), being statistically different ($p<0.05$) from elephant grass, Maralfalfa, and Taiwan cultivars. It is worth noting that this maximum in production,

Table 1. Maximum production (A) of cellulose, hemicellulose, and lignin kg ha^{-1} from different *C. purpureus* cultivars at a regrowth age of 168 days, obtained using the Gompertz model.

Cultivars	Cellulose		Hemicellulose		Lignin	
	Mean	SEM	Mean	SEM	Mean	SEM
CT-115 (6)	2701^{c}	258	2133^{ab}	199	550^{ab}	150
Elephant (5)	3431^{ab}	328	2623^{a}	239	702^{ab}	190
King grass (5)	2079^{c}	328	1519^{b}	239	530^{b}	190
Maralfalfa (6)	3786^{a}	258	2983^{a}	199	932^{ab}	150
Roxo (6)	2633^{bc}	258	2105^{ab}	199	688^{ab}	150
Taiwan (5)	3329^{ab}	328	2645^{a}	239	1017^{a}	190

[ab] Different literals within the column, of the different response variables are, statistically significant ($p<0.05$). Tukey means test=0.05. ( )= repetitions.

obtained using the parameter (A) of the Gompertz model, was estimated with a regrowth age of 168 days or more. This coincides with what Martínez *et al.* (2010), and Rodríguez *et al.* (2013) concluded: the Gompertz model is the one that best adjusts the data.

The results of fertilization with or without NPK in *C. purpureus* regarding cellulose, hemicellulose, and lignin production in kg ha^{-1} are shown in Table 2. Cellulose production increased ($p<0.05$) as an effect of fertilization, and biomass production reached 4,007 kg ha^{-1}—two times the amount compared to other cultivars without fertilization. We observed a similar trend in the production of hemicellulose and lignin per hectare, with 3,031 kg ha^{-1} and 963 kg ha^{-1} for fertilized treatments. In non-fertilized plants, hemicellulose reached 1,513 kg ha^{-1} and lignin 462 kg ha^{-1}. The fertilization effect in tropical soils has always been beneficial for increasing biomass production. In this regard, Oliveira *et al.* (2015) found a linear effect when applying nitrogen to the Cameroon Piracicaba variety, which yielded 58 t DM ha^{-1} $year^{-1}$, using a dosage of 1,600 kg ha^{-1} of N. In another study, Ramos *et al.* (2013) show an increment in biomass production due to fertilization in OM-22 and king grass cultivars. The yields, in this case, reached about 150 t DM ha^{-1} $year^{-1}$. However, the response of cultivars is not always similar, since CT-115 had a moderate response to nitrogen application.

Table 3 shows the percentage chemical composition of DM, ash, cellulose, hemicellulose, and lignin of the whole plant, and the yield of the six cultivars of *C. purpureus* with and without fertilization harvested at a regrowth age of 168 days. We observe a slightly higher effect of fertilization with NPK on the increment in cellulose content: 36.3% without fertilization and 38.1% when fertilized. There is also a decrease in hemicellulose, from 30.0% to 28.1%. Regarding lignin content in the whole plant, there were no observed effects of fertilization ($p<0.05$), so the average value remained at 8.3%. Cane pulp is a sub-product used in the paper industry for being a good source of cellulose, but it has a much higher lignin production (16%) (Bhatti and Khan, 1996) as compared to the percentages found in the

Table 2. Maximum production (A) of cellulose, hemicellulose, and lignin kg ha^{-1}, with or without fertilization (NPK), in *C. purpureus* at a regrowth age of 168 days or more.

Treatment	Cellulose		Hemicellulose		Lignin	
	Mean	EEM	Mean	EEM	Mean	EEM
Fertilized (18)	4007^{a}	88	3031^{a}	66	963^{a}	86
Unfertilized (15)	1786^{b}	106	1513^{b}	80	462^{b}	110

^{ab} Different literals within the column, of the different response variables are, statistically significant ($p<0.05$). Tukey means test=0.05. ( )=repetitions.

Table 3. Fertilization with NPK and its effect on percentage chemical composition and yield of *C. purpureus* cultivars harvested at a regrowth age of 168 days.

Treatment	DM	Ashes	Cellulose	Hemicellulose	Lignin	Yield (kg DM ha^{1})
Fertilized	30.8^{a}	7.4^{b}	38.1^{a}	28.1^{a}	8.2^{a}	9991^{a}
Unfertilized	27.9^{a}	9.5^{a}	36.3^{a}	30.0^{a}	8.4^{a}	4438^{b}

^{ab} Different literals within the column of the different response variables are, statistically significant ($p<0.05$). Tukey means test=0.05.

grasses we evaluated. Due to the effect of fertilization, DM production doubled its yield, that went up from 4,438 kg DM ha^{-1} to 9,991 kg DM ha^{-1} ($p<0.05$).

Figure 1a shows the behavior of each cultivar due to the effect of fertilization. Maralfalfa and elephant grass cultivars responded rapidly to fertilization, presenting a cellulose yield of more than 4,000 kg DM ha^{-1} in a short period (115 days), during which the curve became asymptotic. The Taiwan cultivar did not respond so fast, its asymptote beginning at 168 days. Still, its cellulose values are higher than 4,000 kg DM ha^{-1}. Rengsirikul *et al.* (2013) reported a variable dry matter production in eight *C. purpureus* cultivars, which were fertilized with 375 kg ha^{-1} of N and harvested every 90 days. Their yields ranged from 27 t DM ha^{-1} $year^{-1}$ to 58 t DM ha^{-1} $year^{-1}$. Their cellulose content varied between 35% and 47% and their hemicellulose content went from 19% to 26%, values attesting to this species' potential for cellulose production. These results substantiate harvesting biomass to obtain cellulose starting at 115 days of age and up until the maximum production age, established using parameter A of the Gompertz model (168-211 days). Figure 1b shows cultivars that grow or produce more cellulose without fertilization. These were the Maralfalfa, elephant grass, and Roxo cultivars (1600-2400 kg DM ha^{-1}). However, all cultivars, except for king grass (115 days), have an asymptote that starts at 168 days of regrowth, which is very close to their maximum production age as calculated with the Gompertz model.

The hemicellulose biomass curve of fertilized cultivars in Figure 2a presented a behavior similar to the cellulose biomass curves with Maralfalfa, elephant grass, and Taiwan cultivars. These cultivars produced between 3,000 kg DM ha^{-1} and 3,600 kg DM ha^{-1}, with an asymptote starting at a regrowth age of 168 days. Caballero-Gómez *et al.* (2016) obtained a higher hemicellulose production: 4,350 kg DM ha^{-1}. This was due to a higher biomass yield (15 t ha^{-1}) in the various *C. purpureus* cultivars, with 29% hemicellulose contents on a dry base. Figure 2b shows that the Maralfalfa cultivar grew exponentially and reached its maximum hemicellulose production in the first 60 days of regrowth. Meanwhile, king grass grew slower without fertilization with NPK, not reaching 1,000 kg DM ha^{-1} of hemicellulose at 168 days of age.

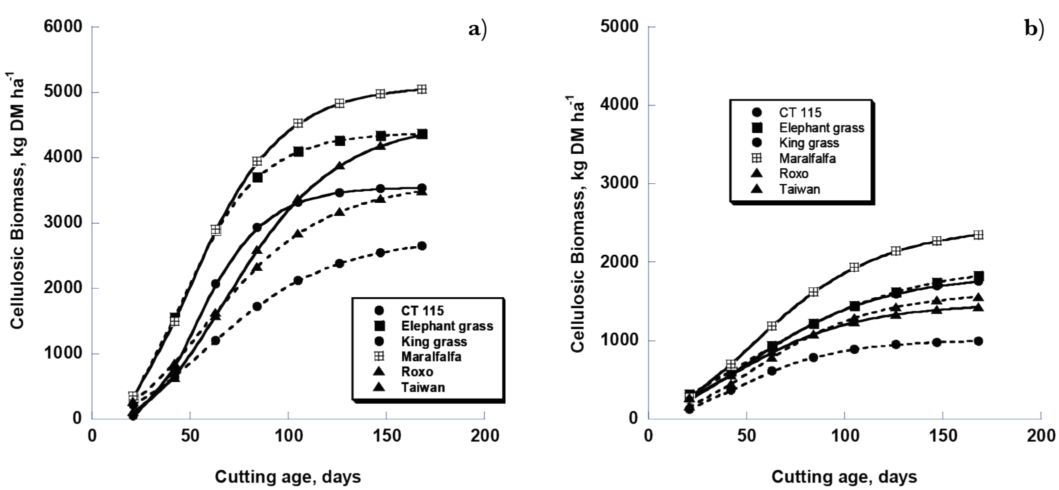

Figure 1. Cellulosic production curves: a) fertilized, b) not fertilized, in different *C. purpureus* cultivars harvested every 21 days in the tropics.

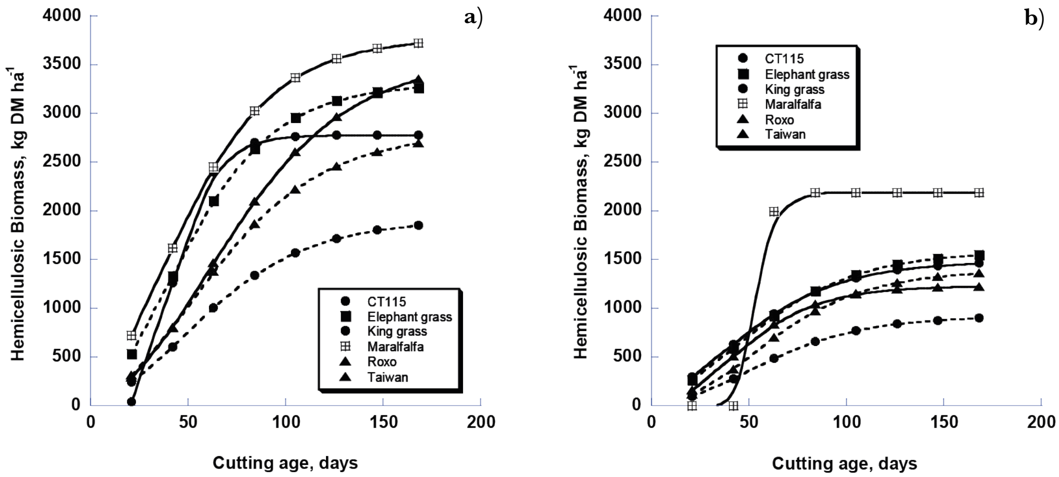

Figure 2. Hemicellulosic production curves: a) fertilized, b) not fertilized, in various *C. purpureus* cultivars, harvested every 21 days in the tropics.

Chemically and structurally, lignin represents a barrier to separating fiber. The association between lignin and polysaccharides determines plant material rigidity and structural resistance. Therefore, the paper industry requires materials with low concentrations of this compound since its elimination is an expensive process (Alvarez-Vasco *et al.*, 2017; Sun *et al.*, 2000). The Maralfalfa cultivar fertilized with NPK produces more than 1,100 kg DM ha^{-1} of lignin. Even though this occurs at 168 days of regrowth age, its asymptote starts at 115 days; it is advisable to harvest the forage starting at this age. We can observe a similar behavior in the elephant grass and CT-115 cultivars, but with lower lignin productions: 600 kg ha^{-1} and 800 kg ha^{-1}, respectively.

CONCLUSIONS

Fertilized Maralfalfa, elephant grass, and Taiwan cultivars are an alternative for the paper industry. They produce more than 4,000 kg ha^{-1} of cellulose, 3,000 kg ha^{-1} of hemicellulose, and just under 1,000 kg ha^{-1} of lignin during the rainy season in the tropics of Veracruz. Biomass cuts for paper production should start at 115 days and continue until a regrowth age of 168 days.

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