Biomass and carbon in two planting densities of eucalypt hybrids of energy forests

Biomassa e carbono em duas densidades de plantio em híbridos de eucalipto de florestas energéticas

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Abstract
The search for alternative sources of fuel has modified traditional models of forest production, with new concepts emerging and with new problems for production. Therefore, the biomass and carbon in stands of three Eucalyptus urophylla x E. grandis hybrid clones at two densely planted spacings were quantified. The study was implemented in a completely casualized design, with hybrid clones AEC 1528, I144 and GG100 at spacings of 3.0 m x 0.5 m and 3.0 m x 1.0 m, with 3 replications and 50 plants per plot. After 33 months the trees were harvested for biomass and carbon quantification. The hybrids I144 and GG100 yielded higher productions of biomass and carbon of stemwood and stembark. The smaller spacing increased the production of biomass in all evaluated compartments but did not change the carbon concentration.

Keywords: Short rotation coppice; Bioenergy; Eucalyptus; Dense planting.

INTRODUCTION
Forest for energy or short rotation forest is a forestry system developed for the efficient production of woody biomass, where the woody species used are fast growing (Dillen et al., 2016). Major biomass categories grown specifically for bioenergy include short rotation coppice varieties, such as willow; short rotation forestry cultivated at short intervals, such as eucalypts; and high energy yielding grasses such as Miscanthus (Welfle & Slade, 2018).
This system aims at maximum biomass production per cycle, which is measured by the yield of the crop per area or by the amount of raw material generated (Welfle & Slade, 2018). In Europe such trees are planted in high density, harvested in short cycles of 2 to 5 years and replaced when productivity decreases (Dillen et al., 2016). In Brazil these forests have a variable cycle of 1 to 2 years and spacing of 3.0 m x 1.0 m and 3.0 m x 0.5 m, with population densities of approximately 3300 and 6700 plants per hectare, respectively (Guerra et al., 2012).

Changes in the amount of biomass allocated to the different plant compartments occur as a function of spacing (Bernardo et al., 1998), so the correct choice of spacing will influence individual plant growth, also interfering with stand growth (Stape, 1995). Biomass production and carbon allocation is increased with increasing population density and decreasing spacing (Leles et al., 2001; Oliveira Neto et al., 2003; Reiner et al., 2011; Rocha et al., 2015) thus producing higher amount of biomass in smaller physical and temporal space.

In order to accurately determine all carbon stock in forest stands, there are often constraints and difficulties, both operational and financial, which use hypothetical values to determine carbon concentration and therefore carbon stock. These estimates consider that the elemental carbon present in the biomass constitution represents approximately 50% of the total mass, but Sanquetta & Balbinot (2003) point out that the indiscriminate use of this percentage has been providing totally unrealistic estimates; thus, they recommend the use of appropriate methods for biomass determination as an assessment of carbon sequestration by forests. Sanquetta et al. (2014) and Behling et al. (2014) indicate that carbon stock quantifications should seek to determine the carbon concentration for each species as well as for each biomass component.

The results obtained imply many positive environmental benefits due to the implementation of short rotation crops (Weih & Dimitriou, 2012), but there is still little information about dense eucalypt plantations. The objective of this study was to evaluate biomass production, carbon concentration and content in stands of Eucalyptus urophylla x E. grandis hybrids in energy forests.

**MATERIAL AND METHODS**

The experiment was installed at the Experimental Area of the Federal University of Jataí in Central-West Brazil, at 662 meters altitude and at coordinates 17º 56' 10" S 51º 43' 40" W. The climate is Aw according to the Köppen classification, with a dry season ranging from April to September and a rainy season from October to March. The average annual temperature is 23.7 °C and the average annual rainfall is approximately 1,645 mm (Instituto Nacional de Meteorologia, 2015).

The soil of the experimental area is classified as Oxisol (Santos et al., 2018), which had its fertility evaluated at the beginning of the experiment (Table 1). Subsequently, the fertilization recommendation was made according to Andrade (2004) and the soil preparation occurred with scarification at 25 cm depth and manual planting of the plants, after having the plant root system immersed in a termite controlling solution to prevent injuries of such species' attacks.

| pH  | Ca   | Mg   | Al  | H+Al | Al+H+Al | CTC | K   | P   | S   | B   | Cu  | Zn  | Na  | V   |
|-----|------|------|-----|------|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 4.7 | 1.36 | 0.92 | 0.18| 5.5  | 8       | 74  | 6.3 | 21.1| 0.28| 9.5 | 2.5 | 3.8 | 31  | 6.8 |

Notes: pH in CaCl₂, P in Melhich I, clay 515g dm⁻³, silt 75 g dm⁻³ and sand 410 g dm⁻³.

At planting fertilization of NPK 04-14-08 (with 12% of Ca and 10% S) was applied with 150 grams per plant. In addition 60 kg N ha⁻¹ and 53 kg K ha⁻¹ both divided at 60 and 90 days after planting were applied. A year after plantig the same doses of N e K were applied again plus B at 3 kg ha⁻¹.
The experiment was implemented in a completely randomized design, in a 3 x 2 factorial arrangement (three hybrids and two spacings) with three replications, resulting in 18 plots, planting 50 plants per plot. The central trees were measured, leaving a simple border line per plot. As hybrid clones of *Eucalyptus urophylla* x *E. grandis* (i.e. *E. urograndis*) the most planted clones in the region were used – AEC1528; I144; and GG100 – and the spacings were 3.0 m x 0.5 m and 3.0 m x 1.0 m.

Data were obtained at 33 months of age, determined through periodic inventories and based on the optimal harvest time (Rodriguez et al., 1997). To evaluate the biomass of the tree components, one tree was felled at ground level per diameter class in each plot, using five diameter classes and based on the central value of DBH of each class. A total of 96 trees were sampled and for the wet biomass measurement two components were separated, one composed of leaves and branches and the other composed of stemwood with stembark.

Compartment sampling was performed according to Soares et al. (2011). A homogeneous sample of leaves and branches was removed from the crown and this sample was weighed to obtain the wet mass. In the determination of the biomass, stemwood discs with stembark of 2.5 cm thickness were removed from the center of each third of the tree trunk. After field collection, the samples from the compartments were sent to the laboratory and dried in a circulation and forced air renewal oven at a temperature of 70 ± 5 ºC, until reaching a constant mass.

Biomass per area was determined by the sum of the product between the biomass of each component and the number of trees at each diameter class. Total biomass resulted from the sum of the two measured compartments and was quantified by the equation cited by Sanquetta et al. (2014) and Soares et al. (2011) (Equation 1):

\[
DB_{(Total)} = \frac{WB(c) \times DB(a)}{WB(a)}
\]

Where: DB (Total) = dried biomass, in kg; WB (c) = wet biomass of the compartment, in kg; and WB (a) = wet biomass of the sample taken to the laboratory, in kg; and DB (a) = dry biomass of the sample, in kg.

The samples of discs with stembark and leaves plus branches were used to determine the carbon concentration using the “loss of ignition” calcination method, according Davies (1974) adapted by Silva et al. (1999) (Equation 2):

\[
\%C = \left\{ \frac{(M - (T + CM)) \times 100}{M} \right\}/2
\]

Where: % C = carbon concentration of the sample; M = mass of the sample after heating to 105 ºC; T = mass of ash plus melting pot after heating to 500 ºC; and CM = mass of melting pot.

It was observed that some plots had trees with diameter at breast height (DBH) of less than four centimeters, suppressed by intraspecific competition. In order to quantify the biomass and carbon in the most significant way in all plots, aiming at mechanized harvesting, besides representing the reality of a standard commercial planting, these suppressed trees were quantified and added to Total Biomass - TB, while the Partial Biomass - PB - did not include the suppressed trees. After obtaining the carbon content of the samples in each compartment, the total carbon amount was estimated in the same way as when determining the total biomass.

Statistical analysis was performed by analysis of variance (ANOVA), according to factorial analysis. When there was a statistically significant difference between the means of the main treatments (hybrids) and between the means of the secondary treatments (spacings), as well
as interactions, the Tukey test was performed. All statistical tests were performed at 5% significance.

RESULTS AND DISCUSSION

BIOMASS PRODUCTION

For all variables analyzed there was no interaction between the studied factors, hybrids and spacing. At cutting, the average DBH (diameter at breast height – 1.30 meters) measured with a caliper was 9.61 cm for AEC1528, 10.25 cm for I144 and 10.02 cm for GG100. Differences between biomass productions occurred in BWB, PB and TB (Table 2). The evaluated spacings showed significant responses for all biomass variables but did not affect the survival rate of the trees (Table 3). This study was the first silvicultural planting on a site which was previously occupied by degraded pastures for livestock, and it is localized close to environmental protection areas. On this site factors like weeds, ants and termites were more important than the planting densities for the survival rate. Resquin et al. (2018) also linked survival rate to silvicultural factors instead of high-density plantations of eucalypt species.

Table 2. Means of biomass values produced by *Eucalyptus grandis* W. Hill. × *E. urophylla* hybrids in an energy forest, Central-West Brazil.

| Hybrid  | Survival (%) | BLB (Mg ha⁻¹) | BWB (Mg ha⁻¹) | PB (Mg ha⁻¹) | TB (Mg ha⁻¹) |
|---------|--------------|---------------|---------------|--------------|--------------|
| AEC1528 | 87.5±3.7a    | 12.2±2.3a     | 90.1±17.2b    | 102.3±18.8b  | 102.4±18.8b  |
| I144    | 88.2±7.2a    | 13.2±1.9a     | 109.9±18.1a   | 123.1±19.5a  | 123.5±19.5a  |
| GG100   | 84.0±4.9a    | 12.9±2.1a     | 104.5±21.3ab  | 117.4±23.0ab | 117.9±23.6ab |
| MSD     | 8.53         | 2.60          | 15.53         | 17.08        | 17.25        |

MSD - minimum significant difference, BLB - biomass of leaves and branches, BWB - biomass of stemwood with stembark, PB - partial biomass (without suppressed trees, DBH < 4 cm), and TB - total biomass. The means ± standard deviation followed by different letters in the column differ statistically from each other at the 5% level of significance.

Table 3. Means of biomass values in *Eucalyptus grandis* W. Hill. × *E. urophylla* hybrids in an energy forest, Jataí-GO.

| Spacing | Survival (%) | BLB (Mg ha⁻¹) | BWB (Mg ha⁻¹) | PB (Mg ha⁻¹) | TB (Mg ha⁻¹) |
|---------|--------------|---------------|---------------|--------------|--------------|
| 3.0 m x 0.5 m | 86.1±6.5a   | 13.9±1.8a     | 116.2±15.0a   | 130.1±16.4a  | 130.6±16.5a  |
| 3.0 m x 1.0 m | 84.7±4.6a   | 11.6±1.5b     | 86.8±10.8b    | 98.4±11.3b   | 98.6±11.6b   |
| MSD     | 5.70         | 1.76          | 10.12         | 11.42        | 11.54        |

MSD - minimum significant difference, BLB - biomass of leaves and branches, BWB - biomass of stemwood with stembark, PB - partial biomass (without suppressed trees, DBH < 4 cm), and TB - total biomass. The averages ± standard deviation followed by different letters in the column differ statistically from each other at the level of 5% significance.

Several authors have observed a significant interaction between clones and planting sites, indicating that some clones may have better adaptation to specific sites, as reported by Sevel et al. (2012) when evaluating hybrids of *Salix* sp.; Reis et al. (2014) when evaluating various seedling and clonal hybrid materials of *Eucalyptus* sp.; Tenorio et al. (2016) for *Gmelina arborea*; and Niemczyk et al. (2018) when evaluating cultivars of *Populus* sp.

Reis et al. (2014) highlight the differences in the responses of the evaluated hybrids to different locations, even when installed in geographically close areas. Clone responses may vary from site to site by up to 40%, from 5.2 to 8.67 Mg ha⁻¹ year⁻¹ (Sevel et al., 2012). The higher biomass yields of hybrids I144 and GG100 may be related to their genetic origin and
their efficiency in macronutrient absorption, translocation and utilization, as evidenced in cuttings by Pinto et al. (2011) who observed higher yields of shoot dry matter and total dry mass for I144 when compared to six other hybrids. Pinto et al. (2011) also observed that the nutrient use efficiency for macronutrients was higher for I144 than for GG100, especially regarding N, P, K, Mg and S.

Caron et al. (2015) observed decreasing trends in biomass production in different plant compartments due to the increase in useful area. Higher biomass yields at 3.0 m x 0.5 m spacing indicate the influence of planting spacing, where increasing the number of trees per hectare increased the biomass produced per unit area. This situation was also observed by Bernardo et al. (1998), Muller (2005) and Caron et al. (2015), where the increase in planting density was directly related to volume and biomass production per unit area.

The PB and TB produced in the 3.0 m x 0.5 m spacing presented a value 30% higher than the 3.0 m x 1.0 m spacing. This result resembles the reports of Tenorio et al. (2016) for Gmelina arborea, at 12 months of age, in different spacings and locations.

Bernardo et al. (1998), Leles et al. (2001), Caron et al. (2015) and Rocha et al. (2015) observed that planting spacing affects the growth and accumulation of total biomass: in larger spacings there is an increase in diameter and individual biomass (per tree), but there is a reduction in total biomass production per unit area. In larger spacings there is an increase in diameter, probably due to the greater availability of resources such as soil, humidity, and light (Bernardo et al., 1998; Benin et al., 2014), providing more space for the growth of each tree, thus resulting in better plant growth and development by promoting larger diameters, greater DBH, good root and crown development. This set of characteristics directly contributes to the quality and quantity of individual production (Lima et al., 2013).

Planting density and harvesting age are closely related; thus, denser spacing usually requires thinning or shorter harvesting cycle, as competition between plants anticipates the stagnation of stand growth (Caron et al. 2015). Rocha et al. (2015), in the evaluation of the average values of biomass (Mg ha\(^{-1}\) year\(^{-1}\)) in five plant densities from 1.5 to 9.0 m\(^2\) per plant with ages ranging from 4 to 7 years, observed that at 4 years the largest increases in biomass were obtained in the spacing with the largest number of plants per hectare.

The average annual increment (AAI) observed for biomass production in the 3.0 m x 0.5 m and 3.0 m x 1.0 m spacing was 47.47 and 35.85 Mg ha\(^{-1}\) year\(^{-1}\), respectively. The denser spacing gave higher values than those reported by Guerra et al. (2012); while in Brazil energy forests present a productivity of the order of 120 m\(^3\) hectare\(^{-1}\) (approximately 45 Mg ha\(^{-1}\) in annual cycles).

The values of average biomass production per hectare per year demonstrate the Brazilian advantage in biomass production for energy purposes. Lamere et al. (2015) observed a production ranging from 8.0 to 16 Mg ha\(^{-1}\) year\(^{-1}\) in clones of the hybrid Populus nigra L. x P. maximowiczii Henry and P. tremula L., with 10,000 plants per hectare, planted in northwestern Germany. In northern Poland, Niemczyk et al. (2018) obtained incremental values proportional to planting density at seven years for six different hybrids of Populus sp. The mean annual increment under these conditions ranged from 8.0 Mg ha\(^{-1}\) year\(^{-1}\) to 2.5 Mg ha\(^{-1}\) year\(^{-1}\), with 1,333 plants per hectare. In Gmelina arborea at 12 months, Tenorio et al. (2016) reported values of 4.2, 18.8 and 22.6 Mg ha\(^{-1}\) year\(^{-1}\) for spacings of 2.0 m x 2.0 m, 1.0 m x 1.0 m and 0.5 m x 0.5 m, respectively, at different sites in Costa Rica.

**CARBON FIXING**

A mean carbon concentration of 48.40% was found, reaching 47.53% to 49.76% for stemwood with stembark (CCWB) and 45.78% to 49.57% for leaves and branches (CCLB). The results showed no interaction between the hybrid factors and spacing. For CCWB, a statistical difference was observed for hybrids (Table 4). The values found for CCWB were higher for the GG100 hybrid.
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Table 4. Averages of carbon concentration and content for *Eucalyptus grandis* W. Hill. × *E. urophylla* hybrids in an energy forest, Jataí-GO.

| Hybrid | CCLB (%) | CCWB (%) | CLB (Mg ha⁻¹) | CWB (Mg ha⁻¹) | PC (Mg ha⁻¹) | TC (Mg ha⁻¹) |
|--------|----------|----------|----------------|---------------|--------------|--------------|
| AEC1528 | 47.2±0.2a | 49.4±0.2b | 5.8±1.1a       | 44.5±8.2b     | 50.2±9.0b    | 50.3±9.0b    |
| I144   | 47.5±0.4a | 49.5±0.0ab| 6.3±0.9a       | 54.3±8.9a     | 60.6±9.6a    | 60.9±9.6a    |
| GG100  | 47.4±0.2a | 49.6±0.0a | 6.1±1.0a       | 51.8±10.5ab   | 58.0±11.4ab  | 58.2±11.6ab  |
| MSD    | 0.48      | 0.15      | 1.22           | 7.60          | 8.33         | 8.42         |

MSD - Minimum Significant Difference; CCLB - carbon concentration of leaves and branches; CCWB - carbon concentration of stemwood with stembark; CLB - carbon content of leaves and branches; CWB - carbon content of stemwood with stembark; PC - partial carbon content; and TC - total carbon content. The averages followed by different letters in the column differ statistically from each other at the level of 5% significance.

The carbon concentrations observed in this study were no more than 50%, as in Silva et al. (2015). Sanquetta & Balbinot (2003) warn against the indiscriminate use of the factor of 0.5 or 50% to convert the biomass stock into carbon stock related to carbon credit generation projects, as it causes under or overestimation, and may introduce a bias in carbon stock estimates (Amaro et al., 2013).

The carbon concentration for CCLB was lower than in CCWB, which was different from those reported by Sanquetta et al. (2014) for *Acacia mearnsii* De Wild, with ages ranging from 1 to 7 years. They reported that the values obtained for foliage had the highest carbon content, while the wood part had the lowest.

A similar fact was observed by Silva et al. (2015) for *Eucalyptus* spp. at ages ranging from 2.3 to 8 years. The authors reported that carbon concentrations hardly exceed 50%, except for the leaf compartment, which ranged from 51.06% at the age of 3.3 years to 49.75% at the age of 4.7 years. Lopes & Aranha (2006), studying *Eucalyptus globulus* and *Pinus pinaster*, observed that carbon concentrations varied according to stand ages and differed between biomass compartments, being higher in foliage and lower in wood. The difference observed between the results found in the literature and this study can possibly be explained by the methodology employed. The methodology used in this study quantified the CCLB in a single sample, as well as stemwood with stembark composed a second sample, thus influencing the carbon concentration of each compartment.

Evaluating the carbon content between the spacings, there was no interaction between the hybrid factors and spacings and there was no significant difference between the spacings. For the carbon content of leaves and branches (CLB), stemwood carbon with stembark (CWB), partial carbon (PC) and total carbon (TC) there was a significant difference (Table 5).

Table 5. Carbon concentrations and stocks for *Eucalyptus grandis* × *E. urophylla* hybrids in dense spacing, Jataí-GO.

| Spacing (Meters) | CCLB (%) | CCWB (%) | CLB (Mg ha⁻¹) | CWB (Mg ha⁻¹) | PC (Mg ha⁻¹) | TC (Mg ha⁻¹) |
|-----------------|----------|----------|----------------|---------------|--------------|--------------|
| 3.0 m x 0.5 m   | 47.3±0.2a| 49.4±0.2a| 6.6±0.9a       | 57.5±7.4a     | 64.1±8.1a    | 64.3±8.2a    |
| 3.0 m x 1.0 m   | 47.4±0.4a| 49.5±0.1a| 5.5±0.7b       | 43.0±5.4b     | 48.5±5.6b    | 48.6±5.8b    |
| MSD             | 0.32     | 0.10     | 0.81           | 5.08          | 5.56         | 5.62         |

MSD - Minimum Significant Difference; CCLB - carbon concentration of leaves and branches; CCWB - carbon concentration of stemwood with stembark; CLB - carbon content of leaves and branches; CWB - carbon content of stemwood with stembark; PC - partial carbon; TC - total carbon and Mg ha⁻¹ - Megagram per hectare. The averages followed by different letters in the column differ statistically from each other at the level of 5% significance.
The CWB of the AEC1528, I144 and GG100 hybrids represented 88.5%, 89.4%, and 89.1% of the carbon stock, respectively. For CWB, it was observed that the values are above to those observed by Amaro et al. (2013), where the value of around 80% resulted from 64.42 Mg ha⁻¹ for stemwood with stembark and 15.89 Mg ha⁻¹ for canopy. This difference between the studies shows the efficient stemwood carbon allocation by the hybrids. Furthermore, when comparing these values, it could be noted that eucalypt hybrids produce as much biomass as a native forest of over 70 years.

The variation of C concentration for the hybrids I144 and GG100 maintained the same proportion of the biomass results, where the observed values were higher than for the hybrid AEC1528. When the carbon stock is compared, the 3.0 m x 0.5 m spacing differed statistically for all evaluated components, presenting higher average carbon stock (Table 5), being 32.3% higher than in the spacing 3.0 m x 1.0 m.

Rocha et al. (2015) in hybrid clones of *Eucalyptus grandis* x *E. camaldulensis* at different spacings and ages, observed that biomass, carbon stock, volume, and energy per hectare increased as the spacing between the trees was reduced. The authors report that over time, from the fourth to the seventh year, there was a reduction in the values obtained for the increase in biomass (Mg ha⁻¹ year⁻¹), carbon (Mg ha⁻¹ year⁻¹) and energy power (kW h⁻¹ ha⁻¹ year⁻¹), which can be largely explained by the decrease in the average annual increment values.

**CONCLUSIONS**

For the hybrids it was found that biomass production, carbon concentration and content differ for the stemwood with stembark compartment. Planting spacing influenced biomass production and carbon content in all evaluated compartments but did not influence carbon concentration.

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