Growth dynamics of container seedlings of *Eucalyptus grandis x Eucalyptus urophylla* and *Hymenaea courbaril* L.[1]

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ABSTRACT

Growth analysis (GA) is used to quantify plant development based on morphophysiological changes. GA is a practical tool to evaluate nursery techniques to make them more efficient. The objective of this essay was to quantity measure morphophysiological growth variables of *Eucalyptus grandis x Eucalyptus urophylla* and *Hymenaea courbaril* (*jatoba*) container seedlings to characterize growth stages. The essay was conducted in a shade house located in the western state of Paraná. GA analyses were performed at 10-day intervals on seedlings of both species. When evaluating *Hymenaea courbaril* seedlings results indicated increased values of height, diameter, root and shoot dry biomass up to 130 days after emergence (DAE). Seedling growth stage-based GA were 70, 100 and 130 DAE for the *Eucalyptus* hybrid and 50, 80, and 110 DAE for *jatoba* which presented accelerated, intermediate and reduced seedling growth.

Keywords: morphological attributes; forest species; photosynthetic pigments; forest nurseries.

INTRODUCTION

In Brazil, *Eucalyptus* spp. farming has adapted to the environmental conditions with an average productivity higher than in the centers of origin of the species (Souza *et al.*, 2012). *Eucalyptus urograndis* or “superclone” when under ideal water, nutritional and climatic conditions show excellent primary and secondary growth (Fernandes *et al.*, 2012). The hybrid is widely used in the forestry industry because of its great timber potential, in addition to faster cutting cycles (six and seven years), high basic density (0.50 to 0.52 g cm^{-3}), high lignin content (up to 29.94%), mechanical strength close to 80.82% and cellulose content around 68.41% (Gonçalves *et al.*, 2009).

Despite the great economic potential and superior wood quality, native wood species are little considered in commercial plantations when compared to exotic species. Therefore, it is important not only to know the prospective use of those species as well as factors related to the conservation and protection of native wood species that are at risk of extinction (Bobato *et al.*, 2008; Dias *et al.*, 2012).

Hymenaea courbaril L. (*jatoba*) has a wide distribution throughout Brazilian states, mainly due to its tolerance to a wide variation in edapho-climatic conditions found in degraded areas (Matheus *et al.*, 2011). *Jatoba* belongs to the Fabaceae family being considered either helophytic or selective xerophyte depending on its occurrence (Costa *et al.*, 2011). Moreover, *jatoba* wood has great value in domestic and foreign markets, and can be used in the furniture industry, flooring, medicines, ingredients, in human and animal food, distilled beverages, as varnish and fuel, but illegal logging is so widespread that laws and regulations have been created in order to reduce the export of wood from illegal logging in countries of the European Union, United States and Australia (Silva *et al.*, 2014; Lowe *et al.*, 2016).

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The morpho-physiological characteristics of seedlings can be modulated by nursery practices that confer greater tolerance or hardness to post-planting conditions. The use of seedlings with low quality quite often results in replanting and uneven stands (Eloy *et al.*, 2014) in spite of other factors (Grossnickle & Macdonald, 2018).

Stress tolerance can be explained as acclimation characterized by the process of transition from the nursery to the field. Therefore, by imposing stressful practices on seedlings, the nurseryman will aim to acclimate them. However, such practices are stressful to a greater or lesser degree (Landis *et al.*, 2010) which makes hardening beneficial or detrimental depending on plant ontogeny (Barton & Boege, 2017).

Growth analysis (GA) appears as a tool that quantifies physio-morphological changes according to species, evaluation period, photosynthetic efficiency and growth strategy. GA lacks the need for sophisticated or expensive equipment, which are replaced by simple and periodic measurements (Benincasa, 2004; Falqueto *et al.*, 2009).

Based on the above, the essay aimed to characterize growth stages in seedlings of *Eucalyptus grandis x Eucalyptus urophylla* and *Hymenaea courbaril* L. propagated in containers to subsidize future hardening practice essays research.

MATERIAL AND METHODS

The essay was conducted in a shade house covered with 150-micron thick anti-UV and low-density polyethylene film located in Universidade Estadual do Oeste do Paraná, Marechal Cândido Rondon, Paraná, with coordinates of 24° 33' 24'' S, 54° 05' 67 ‘’ W and altitude of 420 m. The climatological classification according to Koppen is the Cfa type, subtropical humid mesothermal (Alvares *et al.*, 2013).

Eucalyptus spp. and *jatoba* seeds were purchased from the Instituto de Pesquisas e Estudos Florestais (IPEF) and from the Rede de Sementes (Portal Amazônia), respectively.

Jatoba seeds were scarified in direction against the hypocotyl-radicle axis with a sandpaper (n° 36) to reduce tegument impermeability. Afterwards, seeds were disinfected in 10% sodium hypochlorite for 30 minutes and washed out followed by soaking in tap water for 48 hours and later sown in a 290 cm^3 container. *Jatoba* sowing took place at on December 12, 2018 and the emergency started on December 20. The Growth analysis started on February 9, 2019 and ended on May 30, 2019 with 10-day intervals.

The propagation of Eucalyptus spp. used 120 cm^3 containers with approximately five seeds each. Two weeks after emergence, germinates were selected (based on height and well-developed leaflets). According to Gomes *et al.* (2003) the volume of tubes of 50 and 110 cm^3 were the most suitable for *Eucalyptus grandis* seedlings aged close to 90 days. In addition, from this period onwards, the volumes used of the tubes were restricting the growth of the seedlings, however, on the other hand, there was greater growth in diameter, increase in dry biomass and greater rusticity for these seedlings. Therefore, the research used, based on the recommendation, the objective of the work and availability of containers provided by the University, tubes with volume equal to 120 cm^3.

The sowing of eucalyptus spp. took place on August 8, 2018 with the beginning of the emergency as of August 6 of the same year. The growth analysis covered the period from November 9, 2018 to August 2, 2019 with intervals of 10 days.

Jatoba seedlings were evaluated for a period of 140 days, while for eucalyptus spp. morphometric measurements occurred up to 180 days. The analysis were carried out separately, since the objective is not to compare the species and therefore there is a discrepancy between the evaluation periods.

The containers used for the two species needed to be different due to the characteristics of each species, since *Jatoba* seeds are larger than eucalyptus spp. seeds, moreover, the first species forms much larger seedlings, and in this case, to soften the effects of physical limitation, different containers were chosen.

For propagation of both species, we used Humusfertil® vermicompost based on pine bark, sand as substrate, and vermiculite. During evaluation periods, a nutrient solution composed of 2 mL of KH_2PO_4 L^{-1}; 2 mL of $MgSO_4$ L^{-1}; 5 mL of KNO_3 L^{-1}; 5 mL L^{-1} of $Ca(NO_3)_2$ $4H_2O$; complete micronutrient equal to 1 mL L^{-1}; and 1 ml L^{-1} of Fe-EDTA in 1.000 mL of distilled water was delivered (Hoagland & Arnon, 1950). The nutrient solution delivered per seedling weekly was 5 mL up to 60 days after emergence followed by 10 mL until the end of evaluations.

Irrigation during propagation was carried out from a micro-sprinkler in five daily 10-minute intervals in the summer (at 06:45am, 08:45am, 12:45pm, 03:45pm and 05:45pm) and in three daily intervals during the winter (at 08:45 am, 01:45 pm and 05: 45 pm) for both species.

The experimental design used was a completely randomized one with sixteen evaluation periods, 20 repetitions per period totaling 260 Eucalyptus spp. seedlings, while with *jatoba* there were twelve evaluation periods, 15 repetitions per period totaling 180 seedlings.

The GA variables included height, stem diameter, above ground dry biomass (AGDB), below ground dry biomass (BGDB), leaf area (LI-3000A, Li- Cor®, USA) and

Spad index (Minolta ®, chlorophyll meter RS-232). The Spad index was quantified in four Eucalyptus spp. leaves and in first and second pair of *jatoba* leaves during the essay.

Additionally, we calculated the slenderness index according to Ritchie *et al.* (2010), the Dickson quality index according to Dickson *et al.* (1960), and the leaf area ratio (LAR), the absolute growth rate (AGR), the relative growth rate (RGR) and the net assimilation rate (NAR) according to Benincasa (2004).

The results were tested for normality of the data with Kolmogorov-Smirnov, Cramér-von Mises, Anderson-Darling, Kuiper, Watson, Lilliefors and Shapiro-Wilk tests, while the Bartllet test was used for homogeneity. Due to the significance of the data, they were segregated and adjusted by the sigmoidal model. Graphs were constructed with Sigma Plot 12.0 and the curves were constructed according to the means and their respective standard deviations.

RESULTS AND DISCUSSION

Seedling height of *Eucalyptus urophylla x Eucalyptus grandis* showed accelerated growth until 110 days after emergence (DAE). After that period, seedlings reduced growth rate with a tendency to stabilize up to 180 DAE (Figure 1A).

In Eucalyptus spp. seedlings, stem diameter increased up to 140 DAE showing that the hybrid invests initially in height maintaining stem development for a longer time (Figure 1B). When evaluating seedling quality, a large stem diameter results in higher survivorship (Gomes & Paiva, 2011).

The slenderness index (SI) expresses the ratio between seedling height and diameter. Therefore, the higher the value the lower is seedling stability (Klein *et al.*, 2017). The results of SI as a function of the DAE from *eucalipto*. seedlings (Figure 1C) tend to evolve to stability, after the maximum point observed at 90 DAE (8.76 cm mm^{-1}). Studies such as Gonçalves *et al.* (2005) described that height should vary between 20 and 35 cm and stem diameter from 5 to 10 mm, resulting in a SI from 2 to 7 cm mm^{-1} in seedlings of native wood species. Wendling and Dutra (2010) with seedlings of *eucalipto* concluded that the ideal height for shipping purposes is between 15 and 25 cm and stem diameter greater than 2 mm, with a SI ranging from 7.1 to 11.9 cm mm^{-1}.

The above ground dry biomass from *eucalipto* seedlings (Figure 1D) followed the trend observed for height, with increasing averages up to 160 DAE. BGDB values increased up to 140 DAE in the BGDB (Figure 1E) with posterior stabilization.

Mafia *et al.* (2005) reported stabilization in root development at the end of the evaluation period (*i.e.*, approximately after 72 days) as a consequence of the mechanical limitation imposed by the container (50 cm^3) used with two *eucalipto* clones. The above authors concluded from the root biomass that the ideal period for planting good quality seedlings of *Eucalyptus urophylla* Blake was at 100 DAE.

Studies have reinforced that Dickson's quality index (DQI) has been considered one of the best indicator of seedling quality, as it relates slenderness and dry biomass distribution. High DQI results in planting success since it is directly related to seedling quality (Gomes & Paiva, 2011). The DQI values calculated from ours *eucalipto* seedlings increased up to 140 DAE (Figure 1F). Therefore, it would be recommended to send those seedlings for planting at that age.

Corroborating the results obtained with the same hybrid, propagated by cuttings, Silva *et al.* (2012) reported a DQI value less than 0.2 regardless of the treatments.

Leaf area (LA) values in *eucalipto* seedlings were increasing and linear up to 90 DAE (Figure 2A), coinciding with the phases of plant development mentioned by Peixoto and Peixoto (2004). The Spad index decreased with increased evaluation periods (Figure 2B). One of the reasons for such a reduction is that *eucalipto* leaves may present a change in color as a form of protection against the oxidation of photosynthetic pigments with anthocyanin accumulation. Araújo *et al.* (2018) associated leaf color change to hardening of *eucalipto* seedlings.

Plants invest in increasing leaf area to improve photosynthetic production. However, during the vegetative development, leaves ripen and, consequently, senescence occurs. So, these photoassimilates found and stored in those structures will be redistributed. In addition, the leaf area ratio (LAR) will decrease due to the increase in the number of leaves and consequently self-shading (Benincasa, 2004; Peixoto & Peixoto, 2004).

The leaf area ratio in *eucalipto* seedlings decreased up to 170 DAE with values ranging from 7.95 to 14.75 as a result of the progressive shading of the leaves in the lower third of the crown. Thus, the synthesis of photoassimilates was also reduced (Figure 2C).

The absolute growth rate (AGR) will predict the average growth rate over the entire observation period and express the relationship between the total dry biomass and the interval between evaluations (Benincasa, 2004). Thus, as the morphometric variables shape the rate, it will also reduce the AGR value, demonstrating that extrinsic factors can be the main limiting factors in seedling development. In *eucalipto* seedlings, growth speed was accelerated up to 90 DAE (Figure 2D). The decrease of those values coincided with the reduction in leaf area, that is, for an absolute growth rate, self-shading will also be decisive in defining the way in which the growth will occur. Similar results were obtained by Lima *et al.* (2008) who associated the reduction in AGR with the increase in shade levels in *Caesalpinia ferrea* M.

Eucalipto seedlings showed first an accumulation in the relative growth rate (RGR) followed by a reduction after 90 DAE which also expressed reduction of height, diameter and leaf area (Figure 2E). Povhl and Onoll (2008) described that RGR of *Salvia officinalis* L. treated with plant regulators as well as control seedlings behaved in a similar way, first with an accelerated accumulation and later a rapid decrease.

Initial v*alues* of net assimilation rate (NAR) may be related to the increase in dry biomass, such as leaf area, since the liquid assimilation is directly linked to photosynthesis and as a consequence its better use in the production of photoassimilates. Up to 90 DAE, the hybrid *Eucalyptus grandis x Eucalyptus urophylla* showed an upward trend. From that and following the

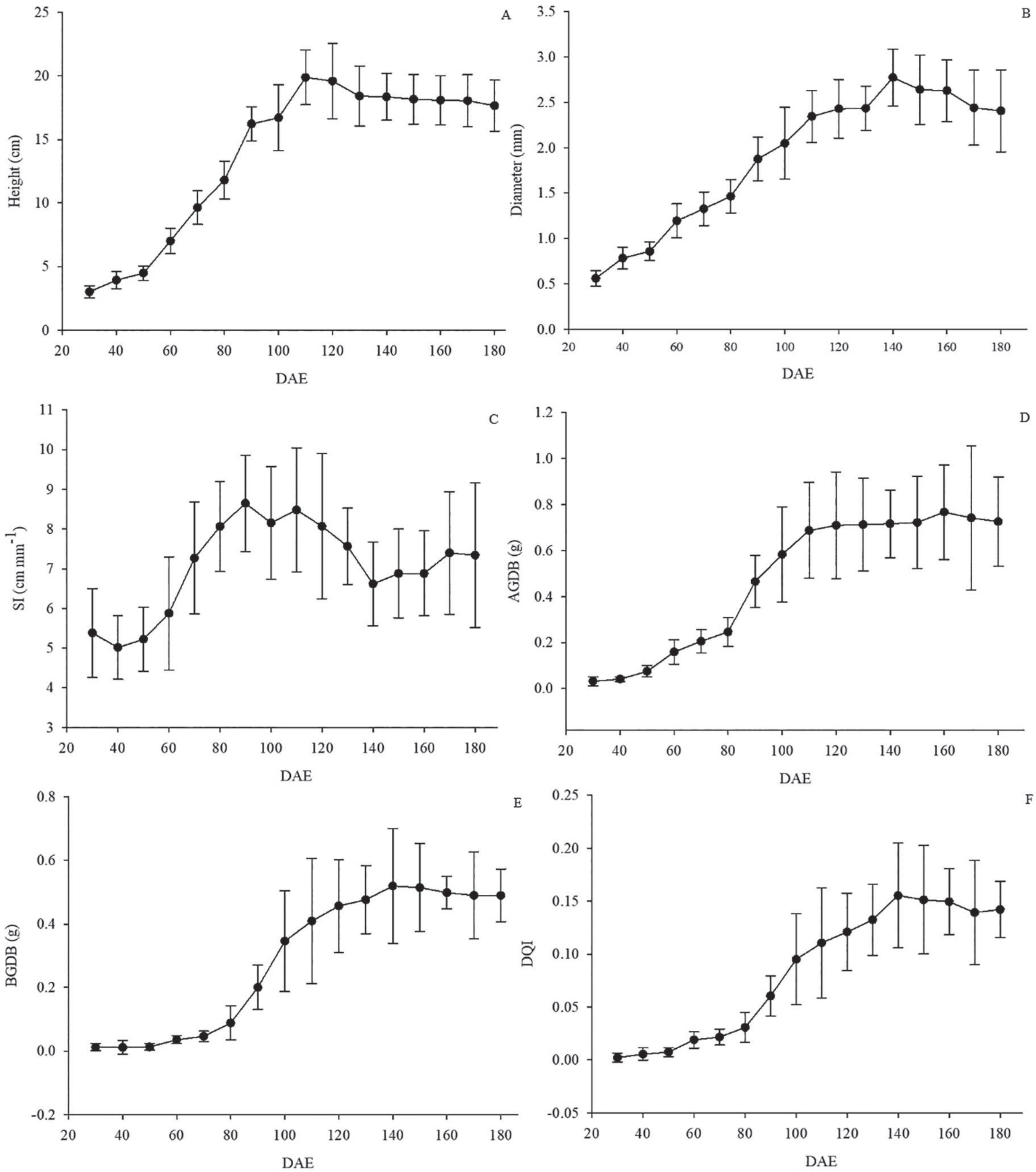

Figure 1: Average growth in height (A), stem diameter (B), slenderness index (C), above ground dry biomass (AGDB) (D), below ground dry biomass (BGDB) (E) and Dickson quality index (F) in *Eucalyptus grandis x Eucalyptus urophylla* seedlings.

results of AGR and RGR, mean NAR was reduced due to the reduction in the speed of growth (Figure 2F).

Another factor that alters NAR is respiration, as it represents the relationship between what is produced and consumed by plant metabolism (*i.e.*. efficiency in conversion of light energy into dry biomass) (Peixoto & Peixoto, 2004).

After 130 DAE, *jatoba* seedlings started to reduce height growth rate. Regarding nursery management, plant growth limitation may result in dispatch seedlings for planting in advance (Figure 3A). Gonzaga *et al.* (2016) evaluated containers with volumes of 110 cm^3 and plastic bags (15 cm in diameter and 20 cm in height) for propagation of *jatoba* seedlings. The authors reported that at 210 DAE the highest averages were calculated in seedlings produced in plastic bags. This is a consequence of the greater volume of substrate to be explored, greater nutritional availability and, consequently, favoring the

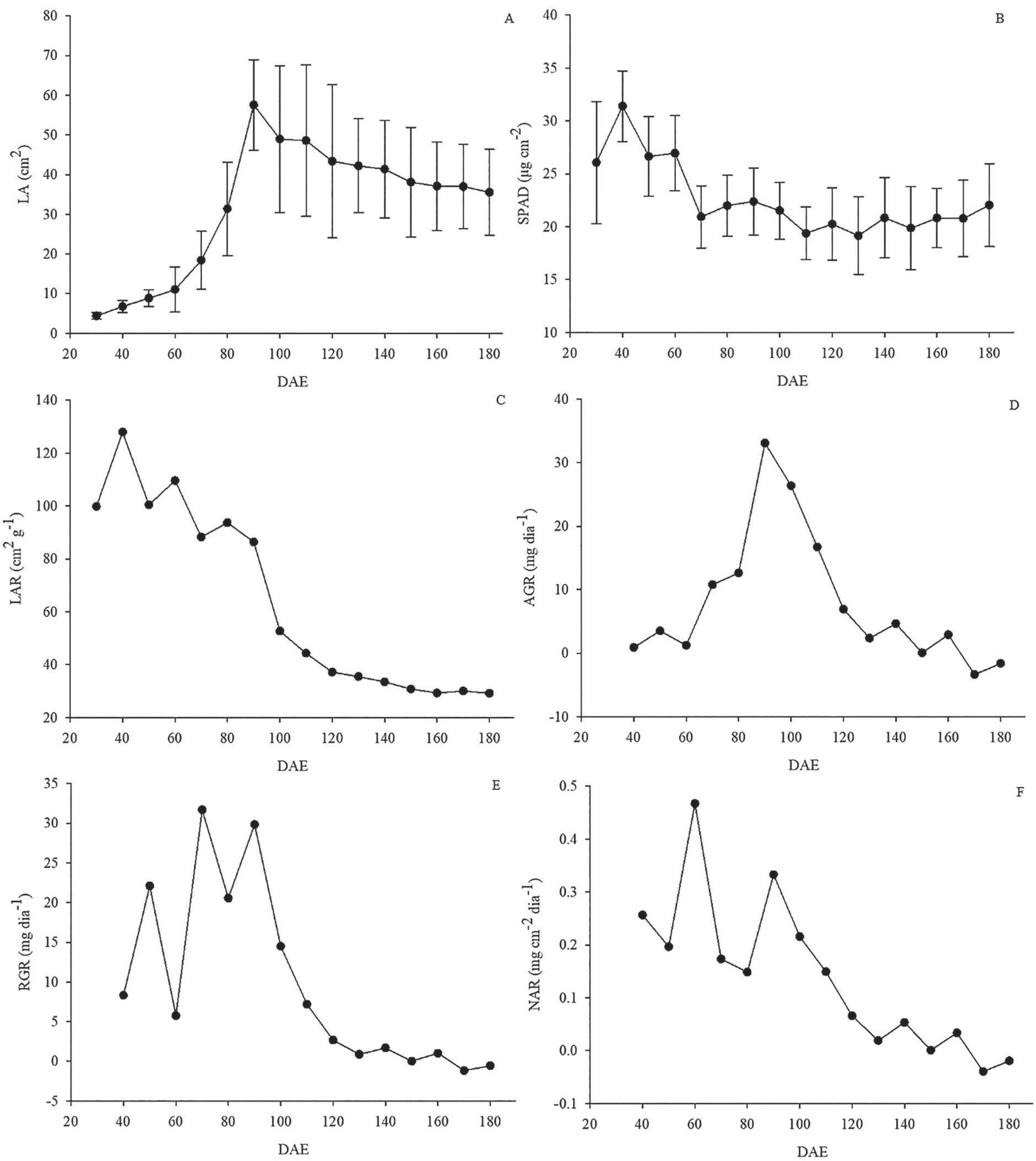

Figure 2: Leaf area (A), Spad index (B), leaf area ratio (C), absolute growth rate (D), relative growth rate (E) and net assimilation rate (F) in *Eucalyptus grandis x Eucalyptus urophylla* seedlings.

formation of new roots. Ferraz and Engel (2011) recommended that the use of containers with a volume equal to or greater than 300 cm^3 is not very restrictive and favors growth in seedlings height and stem diameter as well as anticipation of the production cycle.

Stem diameter from *jatoba* seedlings showed a similar trend to height (Figure 3B) with a reduction at 140 DAE. The reduction may result from the growth natural growth cycle of the species. Alternately, aboveground growth decreases to direct energy reserves to other plant structures, mainly the root, as a way of protection against environmental adversities (Mencuccini, 2014).

In *jatoba* seedlings, values of SI ranged from 5.95 to 7.61 cm mm^{-1} from 30 to 140 DAE. The tendency is that terrestrial plants seek a balance (aerial/root systems) to stabilize their development (Figure 3C). Gonzaga *et al.* (2016) reported that the SI from *jatoba* seedlings varied from 5.95 to 7.16 cm mm^{-1} close to those calculated in this essay.

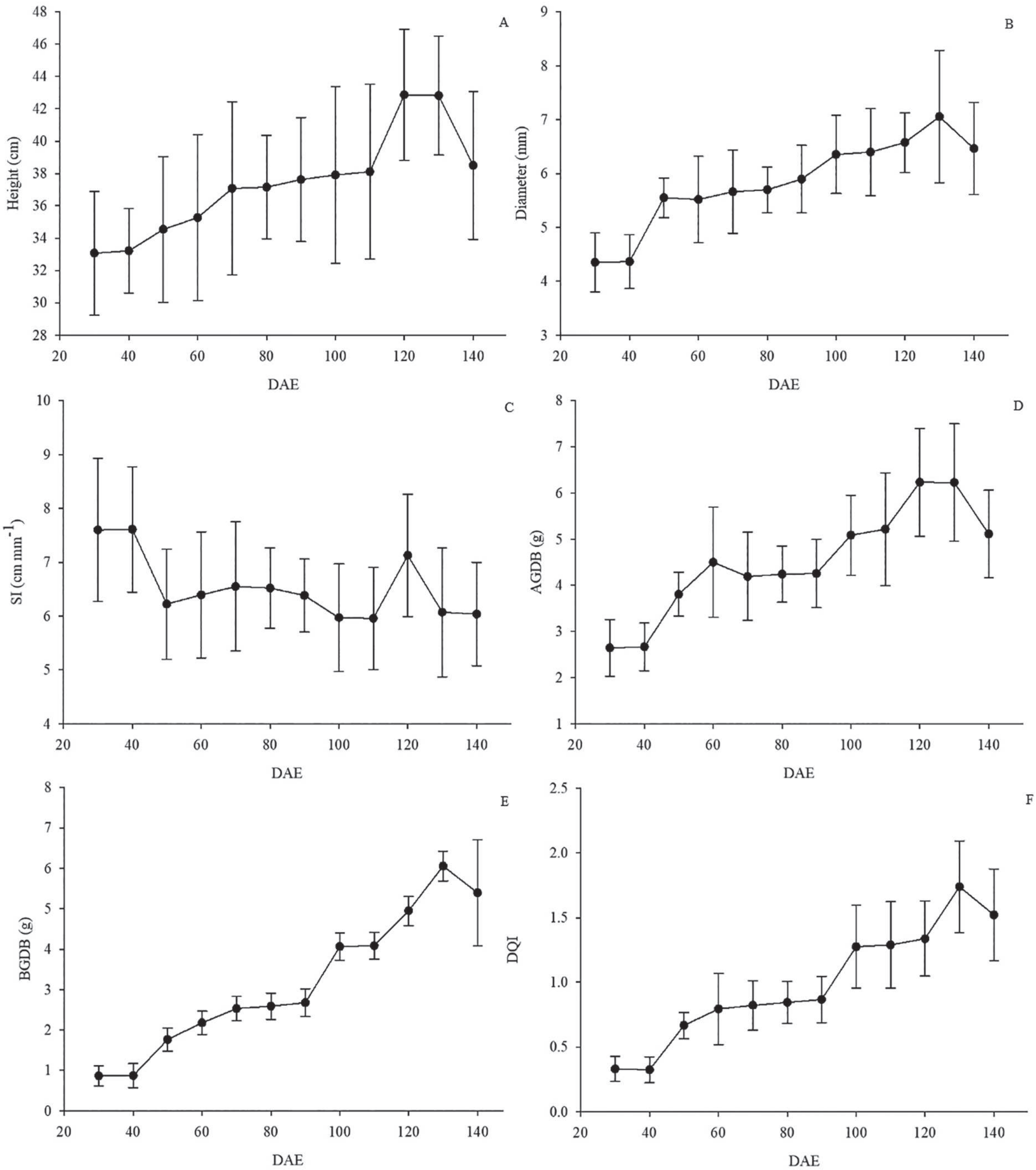

Figure 3: Average growth in height (A), stem diameter (B), slenderness index (C), above ground dry biomass (AGDB) (D), below ground dry biomass (BGDB) (E) and Dickson quality index (F) in *Hymenaea courbaril* seedlings.

Above ground dry biomass of *jatoba* seedlings (Figure 3D) indicated that the highest average was obtained at 130 DAE, with reduced values after that period. In fact, the relationship between above and belowground tissues in terrestrial wood plants are complex and still little explored for some species. Therefore, understanding the dynamics, as well as the relationship between above and belowground tissues is extremely important regarding the metabolism of assimilation, absorption and transformation of the main substrates used by plants (Laclau *et al.*, 2013).

Jatoba may lose leaves under certain circumstances (semi-deciduous) investing, primarily in root growth and as compensation for climatic adversities (Costa *et al.*, 2011) seen in the present essay. From 100 DAE on, there was a reduction in leaf area coinciding with leaf loss, as well as a reduction in the Spad index. However, root biomass increased until the final assessment period (130 DAE) and remained close to that value at 140 DAE (Figure 3E). Comparing the increase in above ground biomass (Figure 3D) and root biomass (Figure 3E) and the difference between the lowest and highest averages, it was observed that the increase in dry biomass was 2.37 times in aboveground tissues, while it was 7.01 times for belowground ones, approximately.

Dickson's quality index of *jatoba* seedlings increased up to 130 DAE, coinciding with an increase in above and belowground biomasses with values greater than 0.30 (Figure 3F). However, this increase depends on factors such as crop treatment, management, type of substrate, container and stage of seedling development (Gomes *et al.*, 2013).

Leaf area (LA) of *jatoba* seedlings showed a quadratic trend up to 90 DAE (Figure 4A). The LA of *Handroanthus albus* (Cham.) Mattos, guarucaia and *jatoba* seedlings quantified by Ferraz and Engel (2011) showed the highest averages with 300 cm^3 containers compared to the 50 and 110 cm^3 ones. We detected leaf spots with increased DAE, which contributed to the reduction of Spad index. The spots appeared on older leaves which evolved to necrosis and irregular spots in the leaf blade. With the increase of leaf spots there was a reduction in the pigmented area and, consequently, a reduction in the Spad index (Figure 4B).

The concentration of photosynthetic pigments is strongly influenced by biotic and abiotic factors. With *jatoba* an increase in the number of leaves was observed, varying from 7.86 to 14.26. New leaves act as "drains" and later become productive (Taiz *et al.*, 2017). During leaf senescence or under stressful conditions that affect older leaves, some cellular constituents will be relocated. Amino acids, proteins and components related to pigmentation are degraded and transported from younger leaves to other defense-related organs for structuring new constituents, mainly for roots (Himelblau & Amasino, 2001; Reyes-Arribas *et al.*, 2001). We hypothesize that there is growth limitation caused by container size because all *jatoba* seedlings developed leaf spots. Alternatively, leaf senescence could be influencing such symptoms. Reich (1995) reported that 67% of the species from tropical forests have leaf longevity greater than 10 months and in the case of *jatoba*, seedlings remained only four months in the shade house.

Self-shading is easily observed in *jatoba* seedlings, because of the conformation of its growth and leaf size and to the fact that the species requires more light in early stages of growth (Costa *et al.*, 2011). Thus, leaf area rate (LAR) from *jatoba* seedlings also showed a reduction over DAE (Figure 4C). Variations in the relationship between the total leaf area and the total dry matter mass result from the growth strategy, both in morphological and physiological aspects (Santos Júnior *et al.*, 2006; Ferreira *et al.*, 2009).

Long-lived terrestrial plant species are vulnerable to both temperature variation and water deficit in the early stages of growth. Under stress, growth will be interrupted, and the defense process will be triggered (Hansen & Turner, 2019).

In *jatoba* seedlings, there was greater growth in root biomass until the final evaluation periods, which resulted in a higher rate of growth quantified via absolute growth rate (AGR) (Figure 4D). According to Silva (2014), early growth of *jatoba* can occur under shading conditions. However, in order to reach reproductive maturity, it requires high incidence of light.

The relative growth rate (RGR) trend was similar to the AGR due to leaf area and dry biomasses. It is evident, therefore, that RGR can be associated with photosynthetic rate, in the same way that a high respiratory consumption is associated with a low photosynthetic rate, caused by any disturbance in plant metabolism (Figure 4E).

Net assimilation rate (NAR) varied depending on DAE as a result of the natural growth strategy which alter their development according to external stimuli (Figure 4F). Barbieri Júnior *et al.* (2007) reported that *jatoba* seedlings treated with 8.400 g m^{-3} of phosphorus and without the inoculation of mycorrhiza showed a reduction of NAR between 30 and 90 days DAE and evolved until stabilization at 120 DAE.

In *eucalipto* and *jatoba* seedlings, below ground dry biomass and stem diameter variables showed the highest correlation with DQI (Table 1). Binotto *et al.* (2010) concluded that the root and total dry biomass as well as stem diameter were positively correlated with DQI in *Eucalyptus grandis* seedlings. The larger the root system, the greater the absorption of water and nutrients, resulting in greater aboveground growth and accumulation of biomass.

For *jatoba* seedlings, in addition to DGDG mass, another variables that stood out was the correlation between Dickson's quality index and stem diameter. In this case, the latter contributed positively to the index on most days of evaluation (Table 1).

Seedlings with a larger stem diameter may have a greater field survival because of greater investment in root growth and accumulation of reserves (Gomes & Paiva, 2011). Aimi *et al.* (2016) evaluated seedlings of *Cabralea canjerana* (Vell.) Mart. in the final stage of development in a nursery at 210 DAE and observed a high correlation (0.91) between stem diameter and below ground dry biomass.

Recent research about the concept of target seedling (Landis *et al.*, 2010) criticized the use of generalized values in determining seedling quality of wood species, whether exotic or native. Thus, detailed studies shall aim to determine the proper expedition stage and hardening as a function of the species.

Considering that hardening practices can be beneficial or harmful to seedling development, characterization of

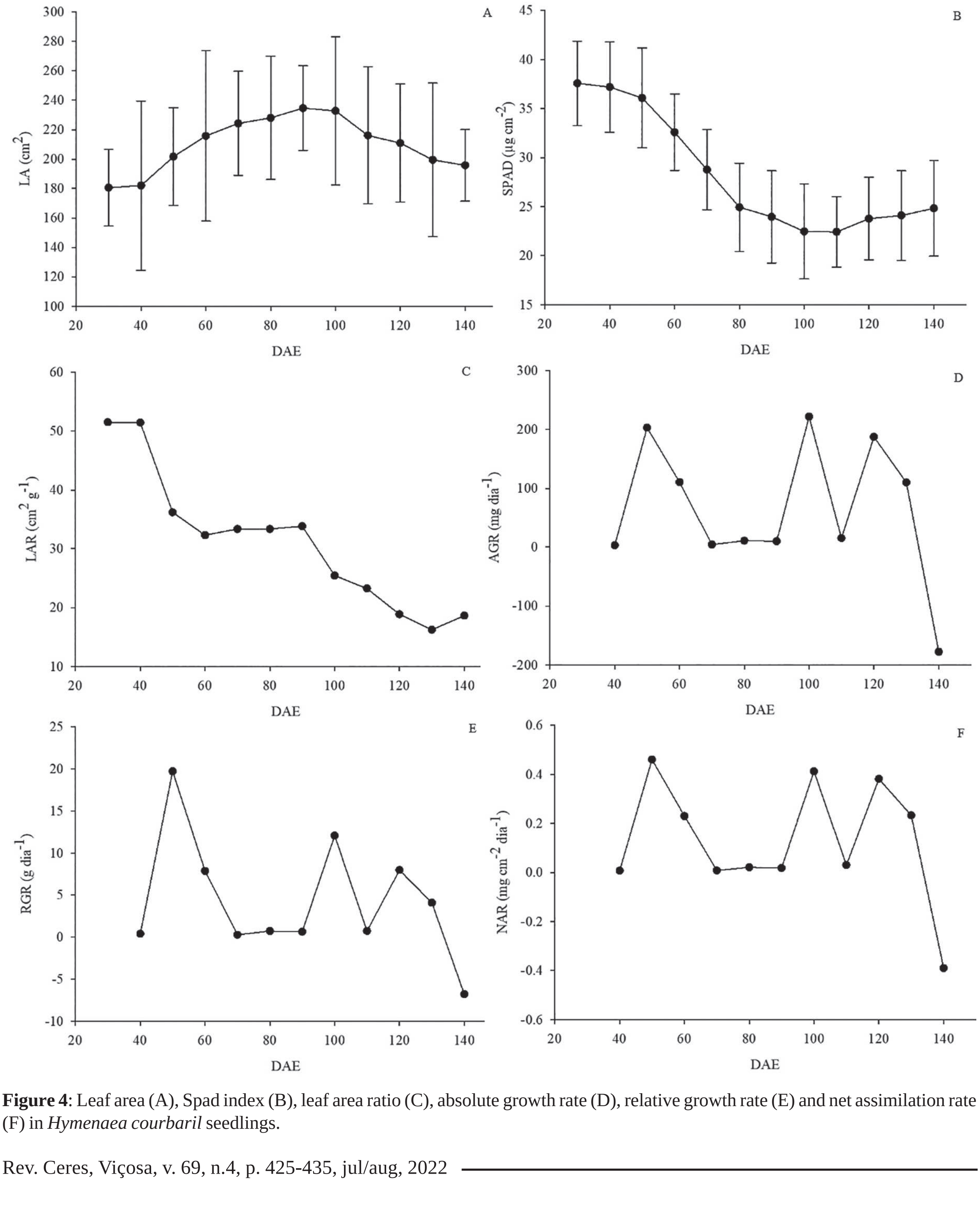

Figure 4: Leaf area (A), Spad index (B), leaf area ratio (C), absolute growth rate (D), relative growth rate (E) and net assimilation rate (F) in *Hymenaea courbaril* seedlings.

Table 1 - Correlation among below ground dry biomass (BGDB) and diameter in *Eucalyptus grandis* x *E. urophylla* and *Hymenaea courbaril* seedlings with Dickson quality index (DQI)

	Eucalyptus urograndis							
	30	**40**	**50**	**60**	**70**	**80**	**90**	**100**
	DAE							
BGDB	0.997*	0.994*	0.997*	0.919*	0.947*	0.902*	0.823*	0.943*
Stem diameter	0.527*	0.544*	0.259*	0.839*	0.830*	0.589*	0.617*	0.712*
	110	**120**	**130**	**140**	**150**	**160**	**170**	**180**
BGDB	0.938*	0.752*	0.844*	0.930*	0.804*	0.568*	0.636*	0.765*
Stem diameter	0.861*	0.399*	0.391*	0.844*	0.900*	0.797*	0.846*	0.765*
	Hymenaea courbaril							
	30	**40**	**50**	**60**	**70**	**80**	**90**	**100**
	DAE							
BGDB	0.890*	0.909*	0.751*	0.911*	0.653*	0.833*	0.775*	0.836*
Stem diameter	0.896*	0.334*	0.431*	0.802*	0.828*	0.737*	0.474*	0.668*
	110	**120**	**130**	**140**	-	-	-	-
BGDB	0.788*	0.836*	0.519*	0.760*				
Stem diameter	0.853*	0.280*	0.897*	0.728*				

(*) Bilateral t-test / Decision-making level = 0.05 (*)

Columns represent evaluation days (every 10 days). And the lines represent the two correlated parameters, to mention the stem diameter and BGDB.

ontogenic or growth stages, are important to compare results of morphophysiological effects.

A major concern when determining the optimal age for dispatching seedlings to the field is that their quality will be directly related to the length of stay in the protected environment and the type of container they are cultivated. Because, these factors alone or together can contribute to the poor quality of plants. After the seedling rotation period is exceeded, the roots begin to curl, as well as the yellowing of the leaves that evolve into necrosis (Araújo *et al.*, 2018). Thus, based on the results obtained, it is clear that the container, as well as other climatic factors, may have limited the development and accumulation of plant biomass in the final evaluation phase, approximately after 140 days for both species.

CONCLUSION

The dynamics of seedling growth indicated three growth stages of hybrid *Eucalyptus grandis x Eucalyptus urophylla* at 70, 100 and 130 DAE and in *Hymenaea courbaril* at 50, 80 and 110 DAE when propagated in containers of 120 and 290 cm^3, respectively. The stages, namely initial, intermediary and final reflect variations of accelerated, constant and decreasing growth rate, respectively.

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