Fertilization and substrates influence *Astronium balansae* seedlings in the nursery: one step further for conservation

Fertilización y sustratos influyen en plántulas de *Astronium balansae* en vivero: un paso más para la conservación

Felipe Manzoni-Barbosa ^a*, Maristela Machado-Araujo ^b, Felipe Turchetto ^c, Suelen Carpenedo-Aimi ^b, Adriana Maria Griebeler ^b, Álvaro Luis Pasquetti-Berghetti ^a, Viviane Dal-Souto-Frescura ^d

* Corresponding Author: ^a Universidade Federal do Paraná, Departamento de Ciências Florestais, Curitiba, Brasil, tel.: +55 4133604297, felipe93mb@gmail.com

^b Universidade Federal de Santa Maria, Departamento de Ciências Florestais, Santa Maria, Brasil.

^c Universidade Federal de Santa Maria – campus Frederico Westphalen, Departamento de Engenharia Florestal, Frederico Westphalen, Brasil.

^d Universidade Federal de Santa Maria – campus Cachoeira do Sul, Coordenadoria Acadêmica, Cachoeira do Sul, Brasil.

SUMMARY

Research regarding the conservation of species at risk of extinction is needed worldwide. In this context, the cultivation of seedlings for conservation purposes plays a pivotal role in meeting the demand of projects and the transplantation of these species into natural habitats. There is currently a lack of information regarding the optimal nursery inputs for cultivating high-quality seedlings of *Astronium balansae*, a forest species native to southern Brazil, Argentina and Paraguay. Therefore, this study aimed to investigate the effect of different substrates and fertilizer sources on the production of *A. balansae* seedlings in nurseries. At 130 days after sowing, the morphophysiological attributes of the seedlings were evaluated, revealing a significant influence of the chemical and physical characteristics of substrates on plant quality. Controlled-release fertilizer, particularly when used in specific proportions with carbonized rice husk, proved effective in enhancing production. The species exhibits tolerance to low fertility levels and high-porosity substrates; however, under such conditions, it did not exhibit optimal physiological and morphological features. The use of various fertilizers and different doses of carbonized rice husk impacted the quality and production time of *A. balansae* plants. Morphological and physiological characteristics demonstrated that the application of controlled-release fertilizer at 6 g L⁻¹, in association with 0 %, 20 %, and 40 % carbonized rice husk, enhanced plant quality within 130 days. All the evaluated attributes can be utilized to assess the quality of *A. balansae* plants.

Keywords: carbonized rice husk, endangered species, Pau-ferro-do-sul, quantum yield, fluorescence.

RESUMEN

Estudios que faciliten la conservación de especies en peligro de extinción son importantes a nivel mundial. En este sentido, la producción de plantas para proyectos de conservación es fundamental para que las especies en peligro sean utilizadas en proyectos de plantación en campo. *Astronium balansae* es una especie forestal originaria del Sur de Brasil, Argentina y Paraguay que cuenta con poca información sobre la producción y calidad de plantas en vivero. Este trabajo tuvo como objetivo determinar el efecto de sustratos y fuentes de fertilizantes en la producción y calidad de plantas de *A. balansae* en vivero. A los 130 días después de la siembra se evaluaron los atributos morfofisiológicos de las plantas: se observó que las características químicas y físicas de los sustratos influyeron en la calidad de plantas. El fertilizante de liberación controlada asociado con ciertos niveles de cascarilla de arroz carbonizada facilita la producción de plantas. La especie tolera bajos niveles de fertilidad y sustratos con alta porosidad, sin embargo, en tales condiciones las plantas mostraron valores más bajos de los atributos morfofisiológicos en relación a los mejores tratamientos. El uso de diferentes fertilizantes y dosis de cascarilla de arroz carbonizada afectan la calidad y el tiempo de producción de las plantas de *A. balansae*. Las características morfológicas y fisiológicas demuestran que el fertilizante de liberación controlada 6 g L⁻¹ asociado al 0 %, 20 % y 40 % de cascarilla de arroz carbonizada favorecen la calidad de las plantas producidas en 130 días. Todos los atributos evaluados se pueden utilizar para evaluar la calidad de las plantas de *A. balansae*.

Palabras clave: cascarilla de arroz carbonizada, especie amenazada, Pau-ferro-do-sul, rendimiento cuántico, fluorescencia.

INTRODUCTION

The Brazilian territory is a global biodiversity hotspot, with over 36,400 registered plant species. However, estimates suggest that 2,738 of these species are currently under some degree of threat (Martins et al. 2018). Despite persistent efforts to conserve and assess species at risk of extinction, many still await comprehensive study (Bachman et al. 2018). Furthermore, threats such as habitat loss, overharvesting, and the impacts of climate change have accelerated the processes leading to extinction (Brummitt et al. 2015, Urban 2015). Globally, approximately 20 % of all known species are at risk of extinction (Brummitt et al. 2015). Recognizing the severity of this situation, the United Nations (UN) has declared 2021-2030 as the "decade of restoration", aiming to restore 350 million hectares of degraded areas to reverse the processes of climate change and loss of biodiversity (FAO 2019).

Studies regarding endangered species seek ways to rescue and develop techniques for the implantation and reestablishment of these plants in their natural habitats (Volis 2016). However, endemic species are still poorly studied, limiting their utilization. Astronium balansae Engl., (Anacardiaceae) is native to southern Brazil, Argentina, and Paraguay, presenting specimens that can reach 25.0 m in height and 130 cm in diameter (DAP) in adulthood, and its wood has a density of 1.25 g cm⁻³, with rural constructions being significant consumers (Carvalho 2008). Due to these factors, the species was explored during the last century, leading to the sharp reduction of native forest fragments and contributing to its current categorization as at risk of extinction (EN) (Luz et al. 2013). Given this scenario, studies are necessary to qualify the seedling production process as a strategy to meet future conservation projects.

Two basic alternatives for species conservation include *in-situ* and *ex-situ* planting, both techniques aiming to maintain the greatest genetic variability and the production of vegetative material to future plantings (Volis 2016). However, only mastering the production protocols expressed by the morphophysiological quality of the seedlings can guarantee the viability of these plantations (Grossnickle and Macdonald 2018*b*). Additionally, many nurseries in Brazil do not meet the demand for conservation plantations due to their low diversity of available tree species, which limits such projects (da Silva *et al.* 2017).

In order to meet this demand, substrates and fertilizers are essential in seedling production (Aimi *et al.* 2019). However, it is ideal for forest species to characterize the combined effect of these factors (Barrett *et al.* 2016) to optimize morphological and physiological responses, thus ensuring higher quality seedlings (Grossnickle and Macdonald 2018*a*, Aimi *et al.* 2019).

In this context, an ideal substrate must possess favorable biological, physical, and chemical conditions, maintaining a balance between water retention and oxygen diffusion to the roots (Barrett *et al.* 2016, Kern *et al.* 2017). For example, due to its availability and physical characteristics, *Sphagnum peat* (Peat moss) is widely used as a substrate component for seedling production. However, optimizing production with a significant cost reduction can be achieved by adding other organic products to peat. In this sense, the partial replacement of this material is possible using locally available organic materials, such as carbonized rice husks, which are available in southern Brazil (Mieth *et al.* 2019).

Substrate fertility is a critical factor in seedling production in nurseries, as it is responsible for providing essential nutrients for plant growth during the production period. Producers can choose from ready-release, controlled-release, or compost fertilizers, with the choice depending on the type of production and the requirements of the target species (Jacobs and Landis 2009). In recent years, several studies have aimed to characterize the suitable substrate and the most appropriate fertilizer for native forest species seedling production (Aimi *et al.* 2019), highlighting the distinct demands of these species.

Based on the information presented above, it is evident that there is a limited number of studies investigating the impact of substrate and fertilizer formations on the morphological and physiological characteristics and production time of A. balansae seedlings. Therefore, this study aims to evaluate the influence of substrates and fertilizers on the morpho-physiological attributes of A. balansae seedlings in the nursery. The study seeks to answer the following questions: (i) How do the morpho-physiological attributes of A. balansae respond to different substrates and fertilizers? (ii) Which attributes best express the quality of the species' seedlings? The results of this study contribute to discussions on aspects related to the quality standards of seedlings, the production potential of this species for restoring degraded areas, and the implementation of replanting projects for *in-situ* and *ex-situ* conservation.

METHODS

The experiment was conducted from September 2016 to January 2017 at the Laboratório de Silvicultura e Viveiro Florestal at the Department of Forest Sciences, Federal University of Santa Maria, Santa Maria, Rio Grande do Sul, Brazil.

The *A. balansae* diaspores collection took place in March 2016 from seven mother trees located in the species' natural distribution region, at (S 29° 00' 39.98", W 55° 14' 54.43"). After collection, the diaspores were placed in paper packaging and stored in kraft paper drums in a humid cold chamber (10 °C and about 80 % relative humidity).

The experimental design was a completely randomized 4 x 4 factorial scheme (substrates x fertilizers), with four replications of eight seedlings each, totaling 16 treatments. The substrates consisted of a commercial mixture of *Sphagnum* peat and expanded vermiculite, with increasing proportions (20 to 60 %) of carbonized rice husk (CRH).

The fertilizers used were: a) controlled-release fertilizer (CRF), with a formulation of 05-18-09 (N- P_2O_5 - K_2O); b) ready-release fertilizer (NPK1) composed of urea, P2O5, and KCl (18-05-09); c) (NPK2) composed of urea, P2O5, and KCl (36-10-18); and d) control, without fertilizer application (table 1). To determine the volume of fertilizers for base fertilization, the dosage recommended by the manufacturer of the controlled-release fertilizer was adopted, with the same dosage applied for ready-release NPK1 and double that for NPK2.

The seedlings were produced in conical polypropylene tube containers with a volume of 180 cm³, arranged in trays of 54 cells, with each sample unit occupying half a tray. Substrate preparation and fertilizer addition were carried out using a concrete mixer, followed by filling the containers. Substrate samples were collected and sent for physical and chemical analysis (table 2). Each tube was initially sown with three diaspores. Subsequently, the trays were placed in a greenhouse where they received micro-sprinkler irrigation at a rate of 8 mm day⁻¹. Thinning was performed thirty days after sowing (DAS), eliminating excess seedlings and leaving only one per container.

At 130 DAS, when the seedlings were in a satisfactory condition for dispatch, the seedlings' morphological and physiological attributes were evaluated. Height (H) was measured using a ruler (cm), and stem diameter (SD) was measured with a digital caliper (mm). Additionally, aerial dry matter (ADM), root dry matter (RDM), and leaf area (LA) were quantified. To achieve this, the seedlings were sectioned into shoots and roots, and the root system was washed using running water over a 0.84 mm mesh sieve. Subsequently, the aerial and root samples were placed in kraft paper packages and dried in an oven with forced air

Table 1. Proportions of substrate and fertilizer tested for A. balansae seedling production.

Proporciones de sustrato y fertilizantes utilizadas para la producción de plantas de A. balansae.

Substrates	Fertilizers			
0 % CRH – 100 % commercial substrate (CS)	Control – No Fertilizing			
20%CRH-80%CS and $20%$ of carbonated rice husk (CRH)	CRF – Controlled release fertilizer $Osmocote^{\circledast},\ 18\text{-}05\text{-}09$ $(N\text{-}P_2O_5\text{-}K_2O)\ 6\ g\ L^{-1}$			
40 % CRH – 60 % CS and 40 % CRH	NPK1 – 187 g of ureia, 133 g of simple superphosphate (P_2O_5) e 72 g of potassium chloride (K_2O) per m ³ of substrate*			
60 % CRH – 40 % CS and 60 % CRH	NPK2 – 373 g of ureia, 267 g of simple superphosphate (P_2O_5) e 145 g of potassium chloride (K_2O) per m ³ of substrate*			

* Based on the controlled release fertilizer nutrient dosage. Where: CS: commercial substrate, CRH: carbonated rice husk, CRF: controlled release fertilizer. Basado en la dosis de nutrientes del fertilizante de liberación controlada. Donde: CS: sustrato comercial, CRH: cascarilla de arroz carbonizada, CRF: fertilizante de liberación controlada.

Table 2. Means of dry density (DD), total porosity (TP), aeration space (AS), readily available water (RAW), buffer water (BW), remaining water (RW), electrical conductivity (EC) and pH of substrate in combination with carbonated rice husk used in the production of *A. balansae* seedlings.

Medias de densidad seca (DD), porosidad total (TP), espacio de aireación (AS), agua fácilmente disponible (RAW), agua tampón (BW), agua remanente (RW), conductividad eléctrica (EC) y pH, del sustrato en combinaciones con cascarilla de arroz carbonizada utilizadas en la producción de plantas de *A. balansae*.

Substrates	DD	ТР	AS	RAW	BW	RW	EC	pН
	g L-1			m ³ m ⁻³			dS cm ⁻¹	H ₂ 0
0 % CRH	144	0.73	0.10	0.13	0.13	0.63	0.19	5.10
20 % CRH	141	0.89	0.23	0.22	0.11	0.67	0.21	5.10
40 % CRH	131	0.86	0.33	0.19	0.07	0.52	0.09	5.70
60 % CRH	134	0.94	0.46	0.19	0.07	0.49	0.17	5.45

Where: CRH - carbonated rice husk, 0 % CRH + 100 % commercial substrate (CS); 20 % CRH + 80 % CS; 40 % CRH + 60 % CS; 60 % CRH + 40 % CS. Donde: CRH - cascarilla de arroz carbonizada, 0 % <math>CRH + 100 % sustrato comercial (CS); 20 % CRH + 80 % CS; 40 % CRH + 60 % CS; 60 % CRH + 40 % CS. circulation at 65 °C until reaching a constant weight. They were then weighed on an analytical balance to obtain the dry matter in grams. To determine leaf area, the leaves were arranged on A4 white paper, pressed with glass, and photographed with a digital camera with a 1.4x zoom, supported by a structure with a fixed height of 0.40 m. The images were processed in the Image J[®] program to obtain the leaf area in mm².

The chlorophyll a fluorescence was determined using a portable fluorometer (Junior-Pam Chlorophyll Fluorometer Walz). The evaluation took place on a sunny day from 8:00 am to 11:00 am, focusing on fully expanded leaves in the upper third of the plant, with four seedlings per treatment. The leaves were previously dark-adapted for 30 minutes using aluminum foil, and were then exposed to saturated light pulses with a wavelength induced by red light, measuring approximately 3,000 µmol m⁻² s⁻¹. This process provided the initial fluorescence (F_o) and maximum fluorescence (F_m) signals, which were used to determine the maximum photochemical efficiency of photosystem II (F_v / F_m), obtained through the variable fluorescence ratio ($F_v = F_m - F_o$).

The data underwent analysis of variance (ANOVA) (P < 0.05), and the residuals were subjected Shapiro-Wilk and Bartlett tests to assess assumptions of normality in error distribution and homogeneity of variances, respectively. Variables that did not meet these assumptions were transformed using the Box-Cox method. When an interaction between the substrate (quantitative) and fertilizer (qualitative) factors was detected, means were adjusted using linear regression models. Furthermore, morphological and physiological variables were subjected to Pearson's correlation. The Scott-Knott test at 95 % confidence level was applied for post hoc analysis. All analysis and graphics were conducted in R ver. 4.0.2 (R Core Team 2020).

RESULTS

The physical characteristics of the substrates exhibited variations with different proportions of carbonized rice husk (CRH) (table 2). The dry density decreased, leading to an increase in total porosity and aeration space. However, the readily available water indicated that the highest volume could be retained in the SC80 (0.22 m³ m⁻³). Buffering and remaining water decreased from 0 % CRH to 60 % CRH. Furthermore, the results of the chemical analysis indicated higher electrical conductivity at 20 % CRH (0.21 dS cm⁻¹), despite the lower pH (5.10).

There was a significant interaction between substrate and fertilizer levels (P < 0.05) for plant morphological attributes, including height (H), stem diameter (ST), aerial dry matter (ADM), and root dry matter (RDM) (figure 1).

Seedling production time was 130 days for the best treatments. The attributes of height, stem diameter, and aerial dry matter showed a quadratic behavior when controlled-release fertilizer (CRF) was used, without employing carbonized rice husk (0 % CRH), remaining constant in the 20 % CRH and 40 % CRH treatments associated with FLC. The models exhibited a negative linear behavior with NPK1, NPK2, and control fertilizers. The utilization of 60 % carbonized rice husk (60 % CRH) without fertilizer application (control) yielded the lowest averages for the morphological attributes (figure 1). Root dry matter displayed a negative linear behavior in all fertilizers, with the highest values observed in 0 % CRH and FLC, differing from the other treatments (figure 1).

There was no significant interaction between substrate and fertilizer levels (P < 0.05) when analyzing the leaf area (LA), only the effect of the factors in isolation (figure 2). The highest average for LA was obtained using FLC (302.0 mm²), while in the control sample, it was 15.6 mm² (figure 2A). When considering the effect of the substrate, the LA values decreased as the carbonized rice husk increased, with 158.0 mm² at 0 % and the smallest area (64.0 mm²) at the 60 % dose (figure 2B).

The physiological attributes initial fluorescence (F_o), maximum fluorescence (F_m), and maximum PSII quantum yield (F_v / F_m) showed no significant interaction between substrate and fertilizer levels (P < 0.05). The doses of carbonized rice husk tested, in turn, had a significant effect on these attributes (figure 3).

The control treatment for the fertilizing factor had the highest F_o values (215.72), not differing from NPK1 and NPK2, while CRF had the lowest mean (figure 3A). On the other hand, seedlings cultivated without the use of fertilizer (control) (553.79) showed the highest Fm value, not differing from NPK1 and NPK2, while the CRF showed the lowest mean (figure 3B). Thus, seedlings produced with the presence of CRF evidenced the highest quantum yield (0.735) (figure 3C).

We observed a significant correlation for the morphological and physiological variables observed at the end of the seedling production period (table 3).

DISCUSSION

The physiology and growth of *A. balansae* seedlings were notably influenced by the substrate and fertilizer formulations, demonstrating the species' responsiveness to input management in a nursery setting. Seedling quality, as indicated by suitable physiological and morphological characteristics post-production, is pivotal for ensuring robust survival and growth rates following field planting (Grossnickle and Macdonald 2018*b*).

The seedling production period in this study spanned 130 days for the optimal treatments, resulting in the production of quality seedlings in a shorter timeframe than described in studies involving other native tree species, where production time ranged from 150 to 250 days (Rossa *et al.* 2013, Faria *et al.* 2017, Aimi *et al.* 2019). This efficiency can be attributed to the enhanced physical and fertilizer characteristics of the substrate (table 1), together with the species' inherent robustness. Despite the quadratic behavior observed in the models adjusted for height, stem diameter, and aerial dry matter attributes, the values obtained were considered suitable for forest seedlings when utilizing 0 % CRH, 20 % CRH, and 40 % CRH, in conjunction with controlled-release fertilizer (figure 1).

A. balansae is typically associated with rocky and welldrained soils (Carvalho 2008). However, the increased total porosity resulting from a high volume of macropores in the highest doses of carbonized rice husks proved detrimental to plant development. Conversely, carbonized rice husk demonstrates its benefits when used in appropriate proportions (Faria *et al.* 2017), serving as an alternative material for substrate conditioning. This allows for cost reduction in the production of forest seedlings, particularly in regions where rice cultivation is prevalent (Fermino *et al.* 2018).

Conversely, high doses of CRH reduce the waterholding capacity of the substrate, necessitating more frequent irrigation (Aklibasinda *et al.* 2011). As a result,

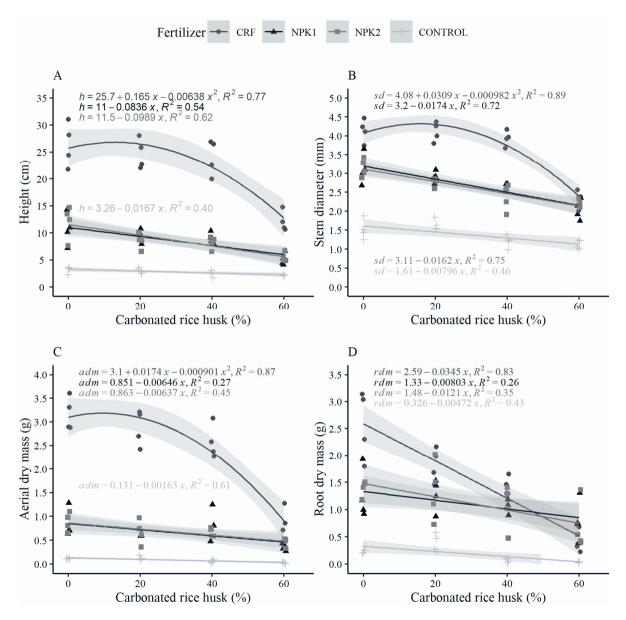


Figure 1. (A) Height, (B) stem diameter, (C) aerial dry mass and (D) root dry mass of *A. balansae* seedlings. Where: CRF - Controlled release fertilizer, 18-05-09 (N-P2O5-K2O): NPK1 - Ready Release Fertilizer, P_2O_5 and KCl (18-05-09): NPK2 - P_2O_5 and KCl (36-10-18). Lighter areas indicate confidence band (P < 0.05).

(A) Altura, (B) diámetro del tallo, (C) masa seca aérea y (D) masa seca de raíces de plantas de *A. balansae*. Donde: CRF - fertilizante de liberación controlada, 18-05-09 (N-P2O5-K2O): NPK1 - Fertilizante de Liberación Rápida, P2O5 y KCl (18-05-09): NPK2 - P2O5 y KCl (36-10-18). Las áreas más claras son la franja de confianza (P < 0.05).

A. balansae seedlings produced in a substrate containing 60 % CRH were significantly smaller than the others. This outcome may have been influenced by the leaching of nutrients provided by the CRF and the ready-release fertilizer, compromising seedling growth. Additionally, Aklibasinda *et al.* (2011) highlighted that an excess of CRH reduces the substrate's water retention capacity and increases pH, which can cause micronutrient deficiencies and plant stress.

The use of CRF for native forest species seedling production has demonstrated significant benefits for plant growth compared to ready-release fertilizer (Aimi *et al.* 2019). Furthermore, due to the characteristics of CRF, the gradual release of nutrients contributes to the reduction of leaching, maintaining nutrient in the substrate for an extended period (Jacobs and Landis 2009). The advantages of this fertilizer were evident in both physiological attributes, with higher values of F_v / F_m , and morphological

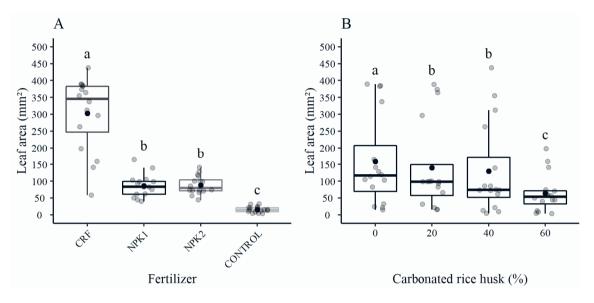


Figure 2. (A) Leaf area (mm²) vs fertilizers, (B) Leaf area (mm²) vs carbonated rice husk of *A. balansae* seedlings. Where: black dots are the mean value. * mean values with different letter are compared using Scott-Knott test (P < 0.05).

(A) Área foliar (mm²) vs fertilizantes, (B) Área foliar (mm²) vs cascarilla de arroz carbonizada, de plantas de *A. balansae*. Donde: los puntos negros son el valor medio. * Los valores medios con letras diferentes se comparan mediante la prueba de Scott-Knott (P < 0.05).

Table 3. Correlation coefficients among morphological variables height (H), stem diameter (SD), aerial dry mass (ADM), root dry mass (RDM), leaf area (LA), and the physiological variables initial fluorescence (F_0), medium fluorescence (F_m) and maximum photochemical efficiency of PSII (F_v / F_m) of *A. balansae*.

Coeficientes de correlación entre las variables morfológicas: altura (H), diámetro del tallo (SD), masa seca aérea (ADM), masa seca de la raíz (RDM), área foliar (LA), y las variables fisiológicas fluorescencia inicial (F_o), fluorescencia media (F_m) y máxima eficiencia fotoquímica del PSII (F_v / F_m) de *A. balansae*.

	Н	SD	ADM	RDM	LA	F _o	F _m	F_v / F_m
Н	1	-	-	-	-	-	-	-
SD	0.97**	1	-	-	-	-	-	-
ADM	0.95**	0.94**	1	-	-	-	-	-
RDM	0.98**	0.96**	0.94**	1	-	-	-	-
LA	0.95**	0.94**	0.96**	0.94**	1	-	-	-
F _o	-0.61**	-0.57**	-0.61**	-0.62**	-0.64**	1	-	-
F _m	-0.53**	-0.50**	-0.54**	-0.51**	-0.55**	0.89**	1	-
F_v / F_m	0.49**	0.42**	0.45**	0.51**	0.52**	-0.79**	-0.46**	1

** Significant correlation P < 0.01

attributes, showcasing increased biomass when combined with an appropriate proportion of carbonized rice husk.

The increase in the values of F_o and F_m , along with the reduction in the average value of F_v / F_m indicates that a higher amount of carbonized rice husk and low nutrient availability impede the growth of *A. balansae*. The decline in the F_v / F_m ratio is considered an indicator of photoin-hibitory damage in stressed plants (Su *et al.* 2015). This

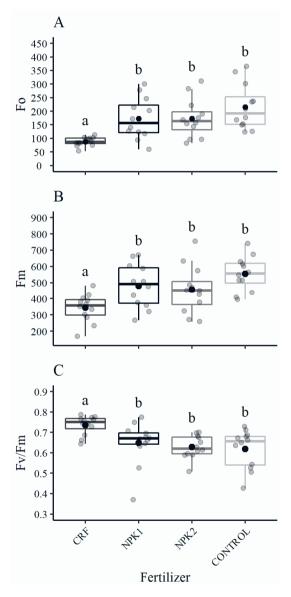


Figure 3. (A) Maximum quantum yield of photosystem II F_v / F_m , (B) Maximum fluorescence, (C) Initial fluorescence of *A. balansae* seedlings. Where: black dots are the mean value. * Mean values with different letter are compared using Scott-Knott test (P < 0.05).

(A) Rendimiento cuántico máximo del fotosistema II F_v / F_m , (B) Fluorescencia máxima, (C) Fluorescencia inicial de plantas de *A. balansae*. Donde: los puntos negros son el valor medio. * Los valores medios con letras diferentes se comparan mediante la prueba de Scott-Knott (P < 0.05). lower mean of F_v / F_m may be attributed to a reduction in chlorophyll content in plant leaves due to nutrient deficiency (Cambrollé *et al.* 2015). Chlorophyll a fluorescence is an efficient non-destructive method for assessing plant stress (Dąbrowski *et al.* 2015). The superior results obtained in plants grown in substrate containing CRF combined with 0 % CRH or 20 % CRH demonstrate that the species is responsive to nutrient availability.

The correlation observed between leaf area and F_v/F_m serves as a reliable predictor of seedling quality, suggesting that seedlings with larger leaf area and enhanced photosystem II efficiency exhibit better growth capacity and biomass assimilation. Additionally, mature green leaves tend to have higher F_v/F_m values, indicating greater photosynthetic capacity and an increase in biomass (Catoni *et al.* 2019). These factors, according to Grossnickle and Macdonald (2018b), can improve the performance of these seedlings in conservation and restoration plantations.

In the studied conditions, the production of *A. balansae* seedlings within approximately 130 days is deemed viable, especially when compared to other species that may require up to 250 days for dispatch (Rossa *et al.* 2013). This highlights the potential of this species to contribute to the diversity of seedlings produced in nurseries in southern Brazil. Moreover, the pursuit of cost reduction in production leads nurseries to opt for locally available substrate components (Kern *et al.* 2017), such as rice husks. Therefore, substrates composed of up to 40 % carbonized rice husk, combined with 6 g L⁻¹ of controlled-release fertilizer, are recommended for the production of high-quality *A. balansae* seedlings.

CONCLUSIONS

The use of different fertilization methods and carbonized rice husk influenced both the production time and the quality of *A. balansae* seedlings.

Morphological responses were evident through the increase in variables such as height, stem diameter, leaf area, root mass, and areal dry mass. Higher values of the physiological attribute F_v / F_m were observed, particularly under the application of controlled-release fertilizer at 6 g L⁻¹ in combination with 0 %, 20 %, and 40 % carbonized rice husk, indicating the production of high-quality seedlings.

Morphological attributes, including height, stem diameter, leaf area, root mass, and aerial dry mass, along with the physiological variable F_v / F_m , can serve as reliable indicators to predict the overall quality of *A. balansae* seedlings in a nursery setting.

ACKNOWLEDGMENTS

The authors would like to thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for granting a scholarship to the first author, and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the productivity research scholarship granted to the second author.

AUTHOR CONTRIBUTIONS

FMB; Project idea, funding, database, processing, analysis, wrote the original draft. MMA; Project idea, funding. FT; Project idea, analysis, writing – review & editing. SCA, AMG and VDSF Writing – review & editing. ALPB; Analysis, writing – review & editing. All authors approved the final and submitted version of this manuscript.

FUNDING

Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) – Financing Code 001.

REFERENCES

- Aimi SC, MM Araujo, MH Fermino, LA Tabaldi, TC Zavistanovicz, P Mieth. 2019. Substrate and fertilization in the quality of *Myrocarpus frondosus* seedlings. *Floresta* 49(4): 831-840. DOI: <u>http://dx.doi.org/10.5380/rf.v49i4.59748</u>
- Aklibasinda M, T Tunc, Y Bulut, U Sahin. 2011. Effects of different growing media on scotch pine (*Pinus sylvestris*) production. *Journal of Animal and Plant Sciences* 21(3): 535-541.
- Bachman SP, EM Lughadha, MC Rivers. 2018. Quantifying progress toward a conservation assessment for all plants. *Conservation Biology* 32(3): 516-524. DOI: <u>https://doi.org/10.1111/cobi.13071</u>
- Barrett GE, PD Alexander, JS Robinson, NC Bragg. 2016. Achieving environmentally sustainable growing media for soilless plant cultivation systems – A review. *Scientia horticulturae* 212: 220-234. DOI: <u>https://doi.org/10.1016/j. scienta.2016.09.030</u>
- Brummitt NA, SP Bachman, J Griffiths-Lee, M Lutz, JF Moat, A Farjon, JS Donaldson, C Hilton-Taylor, TR Meagher, S Albuquerque, E Aletrari, AK Andrews, G Atchison, E Baloch, B Barlozzini, A Brunazzi, J Carretero, M Celesti, H Chadburn, E Cianfoni, C Cockel, V Coldwell, B Concetti, S Contu, V Crook, P Dyson, L Gardiner, N Ghanim, H Greene, A Groom, R Harker, D Hopkins, S Khela, P Lakeman-Fraser, H Lindon, H Lockwood, C Loftus, D Lombrici, L Lopez-Poveda, J Lyon, P Malcolm-Tompkins, K Mcgregor, L Moreno, L Murray, K Nazar, E Power, M Quiton Tuijtelaars, R Salter, R Segrott, H Thacker, LJ Thomas, S Tingvoll, G Watkinson, K Wojtaszekova, EM Nic Lughadha. 2015. Green plants in the red: A baseline global assessment for the IUCN sampled red list index for plants. *PLOS ONE* 10(8): e0135152. DOI: https://doi.org/10.1371/journal.pone.0135152
- Cambrollé J, JL García, ME Figueroa, M Cantos. 2015. Evaluating wild grapevine tolerance to copper toxicity. *Chemosphere* 120: 171-178. DOI: <u>https://doi.org/10.1016/j.che-</u> <u>mosphere.2014.06.044</u>
- Carvalho PER. 2008. Espécies arbóreas brasileiras. 3ed. Brasília, Brasil. Embrapa Informações Tecnológicas. 1039 p.

- Catoni R, F Bracco, L Gratani, MU Granata. 2019. Physiological, morphological and anatomical leaf traits variation across leaf development in *Corylus avellana*. *Mediterranean Botany* 40(2): 185-192. DOI: <u>https://dx.doi.org/10.5209/</u> mbot.62325
- Da Silva APM, D Schweizer, HR Marques, AMC Teixeira, TVMN Dos Santos, RHR Sambuichi, CG Badari, U Gaudare, PHS Brancalion. 2017. Can current native tree seedling production and infrastructure meet an increasing forest restoration demand in Brazil?. *Restoration Ecology* 25(4): 509-515. DOI: https://doi.org/10.1111/rec.12470
- Dąbrowski P, B Pawluśkiewicz, AH Baczewska, P Oglęcki, HS Kalaji. 2015. Chlorophyll a fluorescence of perennial ryegrass (*Lolium perenne* L.) varieties under long term exposure to shade. *Zemdirbyste-Agriculture* 102(3): 305-312. DOI: https://dx.doi.org/10.13080/z-a.2015.102.039
- FAO (Food and Agriculture Organization). 2019. New UN decade on ecosystem restoration offers unparalleled opportunity for job creation, food security and addressing climate change. Accessed 15 nov. 2019. Available in <u>http://www. fao.org/news/story/en/item/1182090/icode</u>
- Faria JCT, LA Melo, GE Brondani, WM Delarmelina, DSN Silva, EM Nieri. 2017. Substrates formulated with organic residues in the production of seedlings of *Moquiniastrum polymorphum. Floresta* 47(4): 523-532. DOI: <u>http://dx.doi.org/10.5380/rf.v47i4.50568</u>
- Fermino MH, MM Araujo, SC Aimi, F Turchetto, ALP Berghetti, TC Zavistanovicz, P Mieth, AM Griebeler, JMV Vilella. 2018. Reutilization of residues as components of substrate for the production of *Eucalyptus grandis* seedlings. *Cerne* 24(1): 80-89. DOI: https://doi.org/10.1590/01047760201824022522
- Grossnickle SC, JE Macdonald. 2018a. Seedling quality: History, application, and plant attributes. *Forests* 9(5): 283. DOI: <u>https://doi.org/10.3390/f9050283</u>
- Grossnickle SC, JE Macdonald. 2018b. Why seedlings grow: influence of plant attributes. *New Forests* 49: 1-34. DOI: <u>https://doi.org/10.1007/s11056-017-9606-4</u>
- Jacobs DF, TD Landis. 2009. Fertilization. *In* Dumroese RK, T Luna, TD Landis eds. Nursery manual for native plants: A guide for tribal nurseries. Washington, D.C. Department of Agriculture, Forest Service. p. 200-215.
- Kern J, P Tammeorg, M Shanskiy, R Sakrabani, H Knicker, C Kammann, E Tuhkanen, G Smidt, M Prasad, K Tiilikkala, S Sohi, G Gascó, C Steiner, B Glaser. 2017. Synergistic use of peat and charred material in growing media – an option to reduce the pressure on peatlands?. *Journal of Environmental Engineering and Landscape Management* 25(2): 160-174. DOI: https://doi.org/10.3846/16486897.2017.1284665
- Luz CLS, JR Pirani, ASM Valente, EP Fernandez, TSA Penedo, RAX Borges. 2013. Anacardiaceae. *In* Martinelli G, MA Moraes eds. Livro vermelho da flora do Brasil. Rio de Janeiro, Brasil. CNCFlora. p. 140-143.
- Martins E, G Martinelli, R Loyola. 2018. Brazilian efforts towards achieving a comprehensive extinction risk assessment for its known flora. *Rodriguésia* 69(4): 1529-1537. DOI: <u>https://doi.org/10.1590/2175-7860201869403</u>
- Mieth P, MM Araujo, MH Fermino, SC Aimi, DR Gomes, JM Vilella. 2019. Ground peach pits: alternative substrate component for seedling production. *Journal of Forestry Research* 30: 1779-1791. DOI: <u>https://doi.org/10.1007/ s11676-018-0740-4</u>

- R Core Team. 2020. R: A language and environment for statistical computing. <u>https://www.R-project.org/</u> [version 4.0.2]. R Foundation for Statistical Computing, Vienna, Austria.
- Rossa ÜB, AC Angelo, AC Nogueira, DJ Westphalen, MVM Bassaco, JEF Milani, JE Bianchin. 2013. Fertilizante de liberação lenta no desenvolvimento de mudas de Schinus terebinthifolius e Sebastiania commersoniana. Floresta 43(1): 93-104. DOI: http://dx.doi.org/10.5380/rf.v43i1.25690
- Su L, Z Dai, S Li, H Xin. 2015. A novel system for evaluating drought-cold tolerance of grapevines using chlorophyll

fluorescence. *BMC Plant Biology* 15: 82. DOI: <u>http://</u> dx.doi.org/10.1186/s12870-015-0459-8

- Urban MC. 2015. Accelerating extinction risk from climate change. *Science* 348(6234): 571-573. DOI: <u>http://dx.doi.org/10.1126/science.aaa4984</u>
- Volis S. 2016. Conservation meets restoration rescuing threatened plant species by restoring their environments and restoring environments using threatened plant species. *Israel Journal of Plant Sciences* 63(4): 262-275. DOI: <u>https://doi.org/10.1080/07929978.2016.1255021</u>

Recibido: 01/02/23 Aceptado: 21/09/23