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The water-retaining polymer improves the seedlings quality of native forest species in a nursery

El polímero hidroretenedor mejora la calidad de las seedlings de especies forestales nativas en vivero

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SUMMARY

Forest nurseries that produce native species seedlings need to make heigh forestry diversity available to supply the demands of forest ecosystem recovery projects. In this way, understanding the behavior of different species and improving production technology improve management and reduce losses. We here studied the effect of the water-retaining polymer addition to the substrate on the performance of three native species grown under nursery conditions. The experimental design was completely randomized with a factorial scheme. Survival, morphological and physiological attributes of seedlings were investigated. In general, it was observed that studied species proved to be suitable for the subculturing technique, with high survival despite of hydrogel negative influence on this variable in seedlings. On the other hand, the use of water-retaining polymer improved growth in height, stem diameter and seedlings quality, demonstrating to be a viable strategy in native seedlings production in a nursery.

Key words: peaking, survival, *Eugenia uniflora*, *Handroathus albus*, *Cabralea canjerana*.

RESUMEN

Los viveros forestales que producen seedlings de especies nativas deben poner a disposición una alta diversidad forestal para satisfacer las demandas de los proyectos de recuperación. De esta forma, comprender el comportamiento de las diferentes especies y mejorar la tecnología de producción califica el manejo y reduce las pérdidas. Por lo tanto, se estudió el efecto de la adición de polímero hidroretenedor al sustrato sobre el desempeño de tres especies nativas cultivadas en condiciones de vivero. El diseño experimental fue completamente al azar con un esquema factorial. Se investigaron rasgos de supervivencia, morfológicos y fisiológicos en plántulas. En general, se observó que las especies estudiadas resultaron aptas para la técnica de trasplante con alta supervivencia a pesar de la influencia negativa del hidrogel sobre esta variable en las plántulas. Por otro lado, el uso de polímero hidroretenedor mejoró el crecimiento en altura, diámetro del tallo y la calidad de estas plántulas, demostrando ser una estrategia viable en la producción de seedlings nativas en un vivero.

Palabras clave: trasplante, supervivencia, *Eugenia uniflora*, *Handroathus albus*, *Cabralea canjerana*.

INTRODUCTION

Seedlings production of native species in Brazil has limitations due to the lack of knowledge about several forest species, which compromises the offer of quality seedlings and diversity for plantations composition for conservation and/or commercial purposes. On the other hand, there is an increase in demand for native forest species, due to the recent global commitments signed by Brazil for forest restoration (Mews *et al.* 2015, Mezzomo *et al.* 2018).

Scientific investigations have been carried out aiming at qualifying the seedlings through genetic material selection, production techniques, inputs and management in the nursery, thus ensuring adequate performance after planting in the field (Araujo *et al.* 2018). In this context, the development of technologies that can reduce and optimize costs and production time of seedlings in the nursery are fundamental for silviculture with native species.

Nurseries that produce seedlings of native species often depend on strategies to reduce the cost and space in the initial production phase. In these cases, the use of sowing is an operational technique that consists of the accommodation of seeds in beds or trays containing substrate, so that it allows seedling emergence and, later on, transplanting to the final container. This technique is currently used in forest nurseries for the propagation of species with high added value, which show uneven or slow germination, when there is low seed availability, or even ignorance of lot germinative power and species to be cultivated (Araujo *et al.* 2018). Similarly, the use of sowing avoids the waste of materials and labor, as it guarantees lot homogeneity, facilitating subsequent operations. On the other hand, tolerance to growth depends on species and environmental conditions, as high relative humidity air. During plant removal from the substrate, root system dryness may occur, making plants vulnerable to environmental stress, which may increase plant mortality.

The incorporation of water-retaining polymers to the substrate is carried out to optimize seedlings production of native species in regions of seasonal climate (Mews *et al.* 2015). These polymers are organic substances capable of absorbing and storing significant amounts of water in regard to their weight. When mixed with the substrate, polymers improve its physical and chemical attributes, mainly in the aeration, retention and water availability, reducing water deficit effects and nutrient losses by leaching, and allowing superior nutrients absorption incorporated into a culture medium and consequently larger plant growth (Navroski *et al.* 2016, Azevedo *et al.* 2019).

The benefits caused by hydrogel addition to the substrate in seedlings production of native and exotic forest species have been reported by several authors, as observed by Navroski *et al.* (2016) (*Eucalyptus dunnii*), Felippe *et al.* (2016) (*Eucalyptus benthamii*), Kelling *et al.* (2017) (*Cordia trichotoma*), Turchetto *et al.* (2020) (*Enterolobium contortisiliquum*), Menegatti *et al.* (2017) (*Aspidosperma parvifolium*) and Mews *et al.* (2015) (*Handroanthus ochraceus*). However, there are no studies available in literature addressing the effects of hydrogel incorporation into substrate on seedling survival and initial growth when using the pecking technique, nor on other species of high environmental value such as *Cabralea canjerana*, *Eugenia uniflora* and *Handroathus albus.*

Thus, the hypothesis is that hydrogel use in substrate is an efficient technique to increase seedlings survival and growth of native forest species, when adopting the seedlings technique. Therefore, the aim of the present study is to evaluate the effect of water-retaining polymer incorporation into the substrate on the functioning of seedlings of three native species under nursery conditions.

METHODS

The study was carried out in a greenhouse at the Viveiro Florestal of the Federal University of Santa Maria, Central Depression of Rio Grande do Sul, Brazil (29° 43 'S and 53° 43' W), whose altitude is approximately 90 meters. The local climate, according to the Köppen classification, is subtropical of the fundamental Cfa type, with well-distributed rainfall throughout the year and average 1,700 mm annual rainfall (Alvares *et al.* 2013).

The survey was conducted in the season of maximum growth in the region (November to March), corresponding to the hottest months of the year. During this period, the monthly sunshine hours, maximum and minimum temperatures recorded were 252.9 hours, 29.9 ºC and 18 ºC, respectively (BDMET-INMET 2020).

The species studied were *Cabralea canjerana* (Vell.) Mart. (canjerana), *Eugenia uniflora* L. (pitangueira) and *Handroanthus albus* (Chamiso) Sandwith (ipê-amarelo-ouro), whose selection was based on their ecophysiological and silvicultural characteristics, which suggest the need to use seedbed due to recalcitrance to storage. The seeds were obtained from the Bolsa de Sementes Project, at the Federal University of Santa Maria. afterwards, they were sown in perforated plastic trays (seedlings), containing commercial substrate (80 %) composed of *Sphagnum* peat and expanded vermiculite mixed with carbonized rice husks (20 %). These were placed in a greenhouse, with average photosynthetically active radiation of 1,882 µmol m^{-2} s^{-1}, for approximately three months, until seedlings with a height between 5 and 7 cm, and at least one pair of fully expanded leaves, were able to be grown.

The experimental design used was entirely randomized in a 2 x 3 factorial scheme. The levels of factor A were composed by the absence (P0 – 0 g L^{-1}) or presence (P4 – 4 g L^{-1}) of Hydroplan® water-retaining polymer and factor B, by the different forest species tested (*Cabralea canjerana*, *Eugenia uniflora* and *Handroanthus albus*). The study was carried out using four replicates per treatment, totaling 24 experimental units with 24 containers each.

For seedlings production, cylindrical-conical polypropylene tubes with a volume of 180 cm³ were used, containing the same substrate used in the sowing, plus controlled-release fertilizer, in the NPK 18-05-09 formulation, in 6 g L^{-1} dose. Substrate preparation was carried out in a concrete mixer, with fertilizer addition and water-retaining polymer for the treatment provided for its presence.

Harvesting was carried out on a rainy day, with high relative humidity and temperature close to 22 ºC. Substrate was moved between the sowing line in the sowing, to facilitate seedlings removal with minimal damage to the roots. Subsequently, seedlings were inserted into a hole in the substrate, made with the aid of a small wooden stake 1.5 cm wide. After introducing the root, perpendicular to the substrate surface, the substrate was accommodated with the same stake around the roots, thus avoiding air pockets presence. After the trays with the seedlings were irrigated and placed in a greenhouse, being covered by a low tunnel with shading mesh (50 %) so that there was no excessive loss of moisture. The structure was removed after 15 days. The irrigation used was by micro-sprinkler (8 mm day^{-1}). Prior to the experiment installation, physical and chemical analyzes of the tested substrates were carried out at the Laboratory of Substrate Analysis for Plants (LASPP), belonging to the Department of Agricultural Research and Diagnosis - SEAPI/RS.

Seedling survival was evaluated 30 days after growing, by counting plants with green shoots and/or containing leaves. At 120 days after harvesting, the height (H) of eight seedlings was measured, from the substrate level to the apical bud, using a graduated ruler (cm), and the stem diameter (SD) with the aid of a precision digital caliper (mm). Through these data, the relationship between the aerial part height and the diameter of the collection (H/SD) of seedlings was obtained.

To determine the shoot part dry mass (SDM), root dry mass (RDM) and total dry mass (TDM), four sectioned seedlings were used at the height of the collection. Next, the roots were washed through sieves to remove the substrate. Subsequently, shoot and root parts were placed in paper envelopes (*kraft* type) and taken to the forced air circulation oven for drying under 65 ± 5 °C in temperature, until constant weight. Finally, samples were weighed on an analytical balance (precision 0.01 g). From these data, Dickson's quality index (DQI) was calculated, according to the equation [1] (Dickson *et al.* 1960).

$$DQI = TDM/[(H/SD) + (SDM/RDM)] \quad [1]$$

The Chlorophyll Falker Index for seedlings chlorophyll *a* (FCI*a*) and chlorophyll *b* (FCI*b*) was also evaluated. A non-destructive reading with a portable optical chlorophyll ClorofiLOG (Falker®, model: CFL 3010) was performed on selected fully expanded leaves from the middle third of four plants. Measurement was performed on both sides of the central rib.

For data analysis, the assumptions of residues normality and homogeneity of variances were verified by the Shapiro-Wilk and Bartlett tests, respectively. When necessary, the data were transformed by Box-Cox. Subsequently, the means were subjected to an analysis of variance and when a significant difference was found, they were compared using the Tukey test at 5 % probability of error, using the statistical software R (R Team 2020).

RESULTS

Seedling survival evaluated 30 days after harvesting was high, with an isolated effect from the study factors (figure 1). The three species evaluated showed high survival (above 86 %) demonstrating suitability for the subcultu-

Table 1. Physical and chemical attributes of the substrates used in the production of *C. canjerana*, *E. uniflora* and *H. albus* seedlings.

Atributos físicos y químicos de los sustratos utilizados en la producción de seedlings de *C. canjerana, E. uniflora* y *H. albus*.

Substrate	DU (kg/m³)	EA %*	AFD %	CE (dS/m)	Condition CE	pH	Condition pH*
P0	413	0.33	0.22	0.51	Normal	4.43	Adequate
P4	329	0.30	0.25	0.47	Normal	4.53	Adequate

Where: P0 - Absence of water-retaining polymer, P4 - presence of water-retaining polymer, DU - Wet density, EA - Aeration Space, AFD - Easily Available Water, CE - Electrical Conductivity. * (Regan 2014).

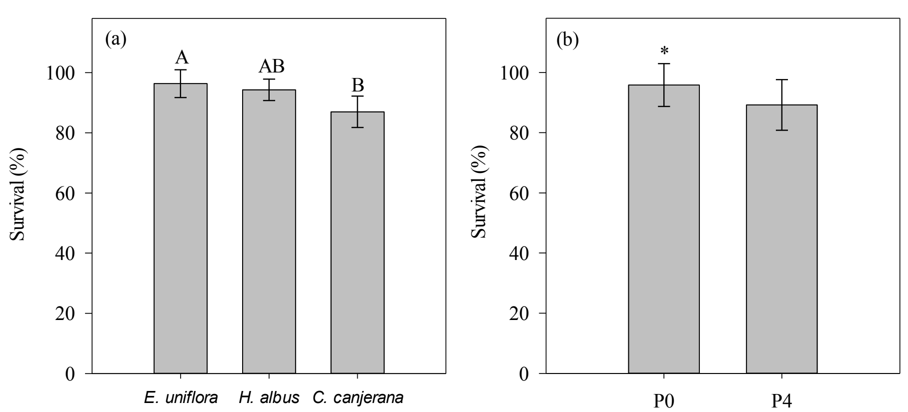

Figure 1. Seedlings survival of different native forest species (A) in water-retaining polymer absence (P0) or presence (P4) in the substrate (B), 30 days after growing in a nursery. Means followed by the same letter do not differ by Tukey's test at 5 % probability of error. Vertical bars indicate standard deviation. Asterisk (*) indicate significant difference according to Anova.

Supervivencia de plántulas de diferentes especies forestales nativas (a) en ausencia (P0) o presencia (P4) de polímero hidroretenedor en el sustrato (b), 30 días después del trasplante en vivero. Las medias seguidas de la misma letra no difieren según la prueba de Tukey. Las barras verticales indican la desviación estándar. El asterisco (*) indica una diferencia significativa según Anova.

ring technique, with emphasis on *E. uniflora*, which obtained 96.4 %. When analyzing seedlings behavior when using the hydrogel, seedlings survival was lower when they were seeded into the substrate with the water-retaining polymer (P4) presence with an average of 89.23 %, while in its absence the survival was 95.83 % (figure 1).

The analysis of variance showed that there was a significant interaction between the use of water-retaining polymer incorporated into the substrate and evaluated species ($P < 0.05$) for the variables H, SD, H / SD, FCI*a* and FCI*b* (table 2). At 120 days after harvesting, the water-retaining polymer use provided the highest H values for all analyzed species (table 2). In hydrogel presence, *E. uniflora* was the species showing the highest growth in H (27.93 cm), followed by *H. albus* and *C. canjerana* with 18.59 cm and 14.09 cm, respectively.

Capralea canjerana presented the highest SD regardless of polymer use, while *H. alba*, which also had a high diameter, was favored by its use (table 2). Despite the high height, *E. uniflora* had a smaller diameter compared to the other species, mainly in polymer absence, which resulted in the highest H/SD, although within accepted standards (HSD < 10) for seedlings suitable for planting (table 2). It can be seen that *E. uniflora* showed higher chlorophyll levels with the polymer presence. *C. canjerana* had the opposite behavior, with higher chlorophyll values in its absence and *H. albus* was indifferent to the hydrogel for this attribute (table 2).

Table 2. Height (cm), stem diameter (cm), H/SD ratio, and Falker chlorophyll index (FCI*a* and FCI*b*) of seedlings of different native forest species according to the use of water-retaining polymer in the substrate, evaluated 120 days after peaking.

Altura (cm), diámetro del cuello (cm), relación H / DC e índice de clorofila Falker (ICFa y ICFb) de seedlings de diferentes especies forestales nativas según el uso de polímero hidroretenedor en el sustrato, evaluado en 120 días después del trasplante.

Characteristics	Substrate	Species		
		E. uniflora	*H. albus*	*C. canjerana*
Height (cm)	P0	23.67 Ab	16.06 Bb	9.43 Cb
	P4	27.93 Aa	18.59 Ba	14.09 Ca
	CV (%) = 3.86		F(*P*) substrate = 175.24 (< 0.0001)	
	F(*P*) substrate*species= 5.14 (0.017)		F(*P*) species = 801.79 (< 0.0001)	
Stem Diameter (mm)	P0	3.67 Cb	4.56 Bb	5.41 Aa
	P4	4.21 Ba	5.40 Aa	5.19 Aa
	CV (%) = 5.03		F(*P*) substrate = 15.562 (0.00009)	
	F(*P*) substrate*species= 10.461 (0.047)		F(*P*) species = 70.991 (< 0.0001)	
H/SD	P0	6.44 Ab	3.53 Bb	1.74 Cb
	P4	6.63 Aa	3.44 Ba	2.71 Ca
	CV (%) = 6.45		F(*P*) substrate =11.05 (0.0037)	
	F(*P*) substrate*species= 8.63 (0.0023)		F(*P*) species = 594.23 (< 0.0001)	
FCI*a*	P0	33.71 Ab	25.31 Ba	36.40 Aa
	P4	37.88 Aa	23.85 Ca	31.20 Bb
	CV (%) = 6.87		F(*P*) substrate = 0.893 (0.357)	
	F(*P*) substrate*species= 9.550 (0.0015)		F(*P*) species = 61.535 (< 0.0001)	
FCI*b*	P0	14.30 Ab	6.35 Ba	15.15 Aa
	P4	19.53 Aa	4.93 Ca	8.64 Bb
	CV (%) = 14.23		F(*P*) substrate = 1.810 (0.195)	
	F(*P*) substrate*species= 26.001 (< 0.0001)		F(*P*) species = 95.573 (< 0.0001)	

Regarding biomass accumulation, it was found that dry mass of *H. albus* aerial part (3.61 g changes^{-1}) and *E. uniflora* (3.49 g changes^{-1}) exceeded *C. canjerana* (1.38 g changes^{-1}) (figure 2A), while for RDM this superiority was maintained only by *H. albus* (2.86 g changes^{-1}), being on average 2 to 3 times higher than in the other species (figure 2B). Consequently, this trend was maintained for TDM (6.49 g changes^{-1}), followed by *E. uniflora* and *C. canjerana*, with 4.66 g changes^{-1} and 2.06 g changes^{-1}, respectively (figure 2C).

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The Dickson Quality Index, which expresses the quality of produced seedlings, demonstrated an isolated effect of the study factors, which is convenient, as the attribute is not indicative for comparing species, but for treatments. In this sense, the water-retaining polymer addition to the substrate provided the highest DQI values (4.12), being 32 % higher when compared to seedlings conducted without the presence of the polymer (3.12) (figure 3).

DISCUSSION

High survival (> 86 %) for *Eugenia uniflora*, *Handroathus albus* and *Cabralea canjerana* seedlings was evidenced in the study, however, values were approximately 8 % lower when using the water-retaining polymer mixed with the substrate (figure 1). Sousa *et al.* (2013), evaluating the survival of *Anadenanthera peregrina* (L.) SPEG seedlings grown in substrates with different hydrogel doses, also observed higher survival rates in the control treatment, in which there was no presence of water-retaining polymer.

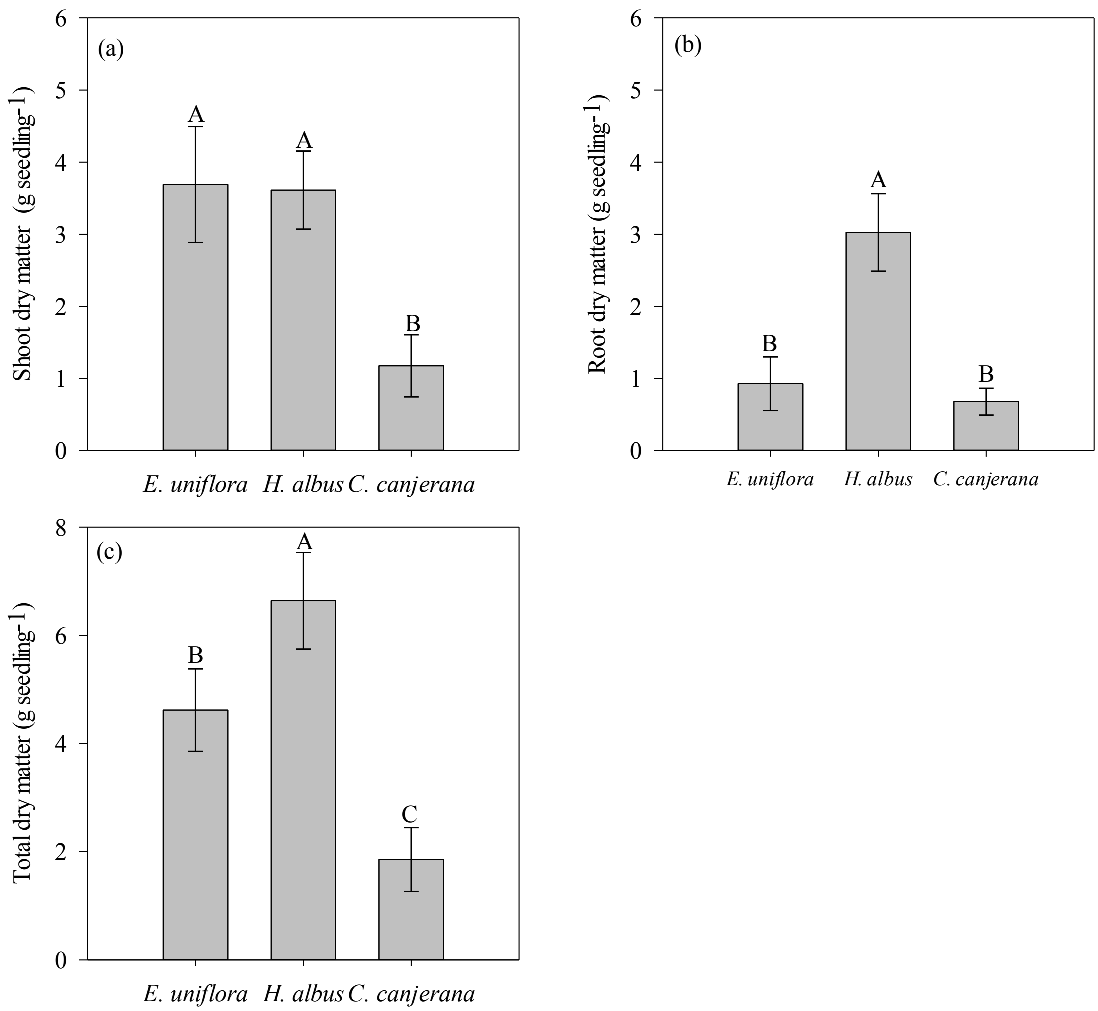

Figure 2. Average values of shoot dry mass (A), root dry mass (B) and total dry mass (C) of seedlings of different native forest species, 120 days after growing in nursery. Means followed by the same letter do not differ by Tukey's test at 5 % probability of error. Vertical bars indicate standard deviation.

Valor medio de materia seca de la parte aérea (a), materia seca radicial (b) y materia seca total (c) de seedlings de diferentes especies forestales nativas, 120 días después del trasplante en vivero. Las medias seguidas de la misma letra no difieren según la prueba de Tukey. Las barras verticales indican la desviación estándar.

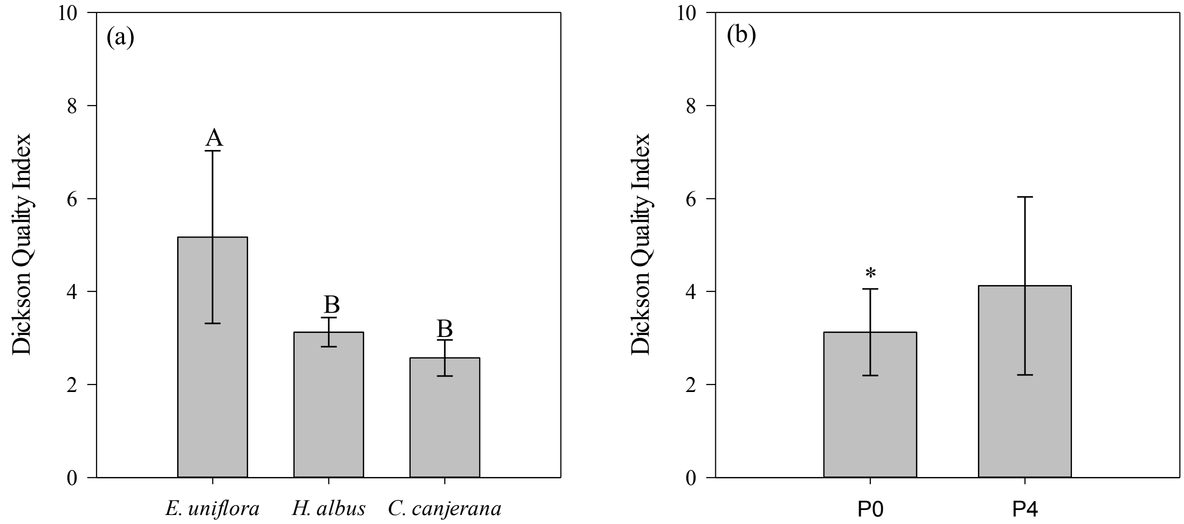

Figure 3. Dickson's quality index of different native forest species (A) in the absence (P0) or presence (P4) of water-retaining polymer in the substrate (B), 120 days after growing in nursery. Means followed by the same letter do not differ by Tukey's test at 5 % probability of error. Vertical bars indicate standard deviation. Asterisks (*) indicate significant difference according to Anova.

Índice de calidad de Dickson de diferentes especies forestales nativas (a) en ausencia (P0) o presencia (P4) de polímero hidroretenedor en el sustrato (b), 120 días después del transplante en vivero. Las medias seguidas de la misma letra no difieren según la prueba de Tukey. Las barras verticales indican la desviación estándar. El asterisco (*) indica una diferencia significativa según Anova.

Such results are possibly explained by the fact that in this phase, despite the root system needing moist substrate, it is essential that the culture medium also provides adequate aeration space for mitochondrial breathing (Marenco and Lopes 2009). In the present study, the substrate P4 aeration space should have a few more points (about 3 %) of aeration space (table 1), to increase oxygen presence to the roots.

When the roots of plants not adapted to flooding are submitted to deficiency (hypoxia) or absence of oxygen (anoxia), ATP synthesis occurs at a very low rate and cellular metabolism is impaired (Marenco and Lopes 2009). According to Kennedy *et al.* (1992), most plant tissues can tolerate oxygen deficiency for short periods before suffering irreversible damage. Thus, given that the small seedlings that were grown only occupied the surface of the substrate, the use of 8 mm day^{-1} of irrigation combined with the water-retaining polymer addition to the substrate may have contributed to the seedling mortality.

Concerning the survival and performance of the species, they were shown to be able and tolerant to the pecking technique, with emphasis on *E. uniflora*. The differentiated behavior of native tree species in nurseries is known in seedlings production of native forest species when propagated by seeds (Gonçalves *et al.* 2000). In addition, differentiated survival rates of native species have also been reported by other authors evaluating seedling transplantation from natural regeneration to nursery (Viani and Rodrigues 2007, Calegari *et al.* 2011, Turchetto *et al.* 2016).

In general, it was observed that hydrogel addition to the substrate contributed to H, SD, H/SD growth, chlorophyll indexes *a* and *b* improvement and quality of seedlings produced. Mews *et al.* (2015), studying the influence of water-retaining polymer use in *Handroanthus ochraceus* (Cham.) Mattos seedlings production, also found the positive effect of adding 4.0 g L^{-1} hydrogel to the substrate on growth in height and diameter of the collection. Turchetto *et al.* (2020), evaluating the effect of different substrates and irrigation regimes on morphophysiological attributes of *Enterolobium contortisiliquum* seedlings, observed that hydrogel incorporation to the substrate provides higher growth rates and biomass allocation, regardless of the daily water regime adopted.

Such results may be related to the improvement of substrate physical-chemical characteristics before hydrogel incorporation, reducing substrate density, increasing the easily available water and pH (table 1). This effect starts to occur when the seedling has a larger root system and shoot, in a second production phase. Hydrogel addition to the substrate allows the reduction of water deficit and leachate losses effects, contributing to the higher absorption of nutrients incorporated into the culture medium and, consequently, better plant growth (Navroski *et al.* 2016, Azevedo *et al.* 2019).

FCI*a* and FCI*b* indicate that the main photosynthetic pigments levels in plants are specific to each species. The highest chlorophyll indexes *a* and *b* values were observed in plants produced in the water-retaining polymer presence, except for *C. canjerana* species. This result allows us to infer, once again, that hydrogel addition contributes to avoid nutrients leaching from the substrate, especially nitrogen, which is a constituent element of chlorophyll molecules (Taiz *et al.* 2017).

The differences evidenced in the growth attributes of evaluated species were already expected and are directly related to their ecophysiological characteristics. *Eugenia uniflora* and species of *Handroanthus* genus, such as *H. albus,* are categorized as initial secondary (Carvalho 2003), since both show intermediate to fast growth under nursery conditions, while *C. canjerana* has slow to intermediate growth (Araujo *et al.* 2018). In seedlings production, species such as *E. uniflora* tend to increase more in height and aerial part biomass in response to a higher photosynthetic rate, in addition to having a thin and abundant root system that ensures better nutrients absorption, and such behaviors are evidenced in the present study. However, species such as *H. albus* and *C. canjerana* show less growth in aerial parts, though, superior biomass allocation in the root system, since their roots are thicker (figure 2A). Thus, plants that invest in a larger root mass have higher rusticity, which may denote larger survival and initial development in the field, considering the adverse conditions in these environments, such as water and nutrient availability, sometimes limited.

Regarding DQI, it was observed that all seedlings produced were of adequate quality to be taken to the field. The highest values were observed for seedlings produced in hydrogel presence, and as for the species, *E. uniflora* was the one that presented a higher value. DQI is considered a good indicator of seedling quality, since robustness (H/SD ratio) and biomass distribution balance (MSPA/RDM ratio) are used for its calculation (Gonzaga *et al.* 2016), considering the results of several important morphological characteristics used for quality evaluation. The higher the DQI, the better the quality of seedling produced (Gomes *et al.* 2002), however, it is an attribute associated with each species, not deserving comparison among different species.

Information related to native forest species regarding morphological and physiological behavior in the seedling production process in nurseries and the optimization of processes, such as the use of water-retaining polymers, is scarce. Therefore, there is a need for further studies with species *C. canjerana, E. uniflora* and *H. albus*, testing different polymer dosages associated with irrigation regimes in the production of native species with silvicultural potential.

CONCLUSIONS

The hypothesis formulated that hydrogel use in the substrate as an efficient methodology to increase the survival and growth of seedlings of native forest species is partially confirmed, since the survival was negatively influenced by the polymer. The evaluated species proved to be suitable for the subculturing technique, with differences in behavior. *Eugenia uniflora* and *H. albus* expressed the highest rates of survival, growth and biomass allocation compared to *C. canjerana*. The water-retaining polymer use improves growth in height, diameter of the collection, physiological status and quality of *Eugenia uniflora*, *Handroanthus albus* and *Cabralea canjerana* seedlings, representing a viable strategy in seedling production of native species in nursery.

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