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Water regime and substrate influence the growth of *Eugenia uniflora* seedlings

El régimen hídrico y el sustrato influyen en el crecimiento de las plantas de *Eugenia uniflora*

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SUMMARY

Eugenia uniflora is a fruit tree of the Myrtaceae family, native to Brazil, Argentina and Uruguay that has high potential for use in landscape and environmental projects, food and pharmaceutical industry. However, information on the production of quality seedlings is still scarce. The objective was to identify the appropriate substrate and its characteristics, in relation to the water regime for the production of *E. uniflora* seedlings. The experiment was conducted in a randomized block design, in a factorial scheme, with four types of substrates being tested (S1: 100 % peat - commercial substrate (CS), S2: 80 % CS + 20 % Carbonized Rice Husk (CRH), S3: 60 % CS + 40 % CRH and S4: 40 % CS + 60 % CRH) and five water regimes (WR) (4, 8, 12, 16 and 20 mm day^{-1}). Height, stem diameter, dry matter and Dickson's quality index were evaluated. Factors influenced the measured attributes in isolation. Seedlings grown in S1 showed the highest growth and accumulation of dry matter. Results are 72 % and 41 % higher than those shown by S3. *Eugenia uniflora* showed superior growth and quality of seedlings under smaller WR. The use of peat-based substrate, combined with the use of 4 to 8 mm WR day^{-1}, provides rapid development of *E. uniflora* seedlings in a nursery.

Key words: Brazilian cherry, Myrtaceae, seedling production, irrigation, nursey.

RESUMEN

Eugenia uniflora es un árbol frutal de la familia Myrtaceae, nativo de Brasil, Argentina y Uruguay que tiene un alto potencial de uso en proyectos paisajísticos y ambientales, la industria alimentaria y farmacéutica. Sin embargo, todavía falta información sobre la producción de plantas de calidad. El objetivo de este trabajo fue identificar el sustrato adecuado y sus características, en relación al régimen hídrico para la producción de plántulas de *E. uniflora*. El experimento se realizó en un diseño de bloques al azar, en un esquema factorial, con cuatro tipos de sustratos probados (S1: 100 % turba - sustrato comercial (SC), S2: 80 % SC + 20 % cáscara de arroz carbonizada (CAC), S3: 60 % SC + 40 % CAC, y S4: 40 % SC + 60 % CAC) y cinco regímenes hídricos (RH) (4, 8, 12, 16 y 20 mm dia^{-1}). Se evaluó la altura, el diámetro del cuello, la materia seca y el índice de calidad de Dickson. Los factores influyeron en los atributos medidos de forma aislada. Las plantas cultivadas en S1 presentaron el mayor crecimiento y acumulación de materia seca, siendo los resultados 72 % y 41 % superiores a S3. *Eugenia uniflora* mostró un crecimiento y una calidad de plántulas superiores en plantas de semillero más pequeñas. El uso de sustrato a base de turba, combinado con el uso de 4 a 8 mm RH dia^{-1}, proporciona un rápido desarrollo de plántulas de *E. uniflora* en vivero.

Palabras clave: Pitanga, Myrtaceae, producción de plantas, irrigación, vivero.

INTRODUCTION

Brazil is the world's largest producer of fruits of *Eugenia uniflora* L. (Brazilian cherry), a native fruit tree belonging to the Myrtaceae family (Santos *et al.* 2015). In addition to its wide distribution in its places of origin, this species has been widely cultivated in other countries in South America (Paraguay, Argentina and Bolivia), Central America (Panama, Mexico, Honduras, Guatemala, Costa Rica and El Salvador) and Africa (Madagascar, Gabon and South Africa) owing to its easy adaptability to different climatic and edaphic conditions (Migues *et al*. 2018).

Commercial plantations of *E. uniflora* have important potential for economic exploitation and income diversification, especially in niche markets eager for novelties (Franzon *et al*. 2008). In addition to the possibility of exploitation for fresh consumption, the fruit can be used by agribusiness to manufacture juices, ice creams, jellies, sweets, liqueurs and other products (Coradin *et al*. 2011).

Moreover, it has potential in the pharmaceutical industry, as the fruit is rich in vitamins, antioxidants and anti-inflammatory substances (Costa *et al*. 2020). The species is recommended to integrate projects for the recovery of degraded areas, landscape and bee use (Bourscheid *et al*. 2011).

Among the factors that influence the development and quality of seedlings in nurseries, the type of substrate and the water regime, which are closely related, stand out. The substrate is a cultivation medium used to replace soil that supports and acts as a mediator of hydration and nutrition of plants in containers (Fermino *et al*. 2018). In the formulation of the suitable substrate for a given crop, two or more components are used. The management of the proportions of these components aims at improving the physical and chemical attributes, according to the individual needs of each species, type of container, irrigation, local availability and cost. According to Dutra *et al*. (2018), the composition of the ideal substrate should also reconcile reduced water consumption with maximum efficiency for growth.

Thus, water requirement is another factor that must be considered in the production of seedlings of native forest species. According to Andriolo (1999), the water available to the roots and the water required by the aerial part control the water status of the plant. In this case, inadequate changes in water status can negatively affect the development of plants, with responses such as foliage reduction and leaf abscission, loss of turgor pressure and high root growth being expressed (Taiz *et al*. 2017).

In the daily practice in forest nurseries that cultivate multiple species, the irrigation of the seedlings is done in an empirical and generalized way. However, studies on forest species of *Eucalyptus* (Navroski *et al*. 2015) and native species, such as *Parapiptadenia rigida* Benth (Dutra *et al*. 2016), *Cordia trichotoma* (Vell.) Arráb. Ex Steud (Kelling *et al*. 2017), *Luehea divaricata* Mart. et Zucc (Dutra *et al*. 2018) and *Enterolobium contortisiliquum* (Vell.) Morong (Turchetto *et al.* 2020), have shown the importance of aligning daily irrigation levels with the characteristics of the species and stage of nursery growth. According to Dutra *et al*. (2018) identifying regimens suitable for different species will aid in reducing seedling production costs as well as providing superior physiological and sanitary control.

The hypothesis of the present study was that the offer of smaller water regimes, combined with the use of substrates with intermediate proportions of rice husks, favors the growth of seedlings of *E. uniflora* in nursery. Thus, given the importance of defining protocols for the rational use of water resources in nurseries, coupled with the lack of information in the literature on irrigation and the use of suitable substrates in the production of *E. uniflora* seedlings, the present study aimed at identifying the appropriate substrate and its characteristics in relation to the water regime for the production of *E. uniflora* seedlings.

METHODS

The experiment was conducted in the forest nursery of Universidade Federal de Santa Maria (29° 43′ S and 53° 43′ W), located in the central region of Rio Grande do Sul State, Brazil. The region climate is classified as Subtropical of the Cfa type, characterized by an average temperature of the coldest month between -3 and 18 °C, and of the warmest month above 22 °C, and average annual precipitation of 1,769 mm, with heavy rains distributed throughout the year (Alvares *et al*. 2013), the altitude is approximately 90 m. The research was conducted between January and May, corresponding to the summer and autumn seasons.

Mature fruit of *E. uniflora* were collected in December from 14 parent trees located in forest remnants in Santa Maria Region. After collection, the seeds were processed and sorted manually, forming a single batch. They were stored in polyethylene bags in a cold chamber at ± 8 °C and relative humidity of approximately 80 % until the experiment commenced (approximately 30 days).

The experimental design was randomized blocks in a 4 x 5 factorial scheme (substrates x water regimes), using four replicates per treatment, totaling 80 experimental units. As substrates, the commercial product –based on sphagnum peat and containing expanded vermiculite, agricultural plaster, NPK + micronutrients and dolomitic limestone– was mixed with carbonized rice husk (CRH) in different proportions. Four substrate compositions were tested (S1 – 100 % CS, S2 – 80 % CS + 20 % CRH, S3 – 60 % CS + 40 % CRH and S4 – 40 % CS + 60 % CRH) associated with five water regimes (table 1).

The formulated substrates were sent to the Substrate Laboratory of the Department of Horticulture and Silviculture of Federal University of Rio Grande do Sul, where physical and chemical characteristics were determined (table 2), following the methodology described in Normative Instruction no. 17 of the Ministry of Education. Agriculture, Livestock and Supply (Brasil 2007). The pH was evaluated with a pH meter and the electrical conductivity with a conductivity meter. To determine the water holding capacity, water columns of 10, 50 and 100 cm of suction were used.

Prior to the installation of the experiment, to define the frequency and intensity of irrigation to adjust the daily irrigation depth of each treatment, a sprinkler uniformity test was carried out according to the methodology described by Salassier *et al*. (2006). We obtained 85.6 % uniformity, which is considered a good value, according to the above study.

For the production of seedlings, polypropylene tubes with a capacity of 110 cm^3 were used, packed in 96 cell trays. Before sowing, a controlled release fertilizer (NPK 18-05-09) was added to the substrates at 6.0 g L^{-1}. Sowing was carried out directly on the substrates, using two seeds per container. After sowing, the trays were placed in a greenhouse and 4 mm daily irrigation was applied for 30 days. At the end of this period, thinning was carried out, leaving only a vigorous and central seedling in each container. In addition, the containers in the trays were alternated, leaving 50 % of the cells occupied, whose experimental units were configured with 24 seedlings. The trays were then placed in an open and sunny environment, and the seedlings were subjected to different water regimes (table 1). Transparent plastic tunnels were used to cover the plants during rain events, being monitored with rain gauges, so that the seedlings received only the amount of water defined in the irrigation regimes.

Table 1. Water regimes used, with their respective irrigation depths, used in the production of *Eugenia uniflora* seedling.

Descripción de los regímenes hídricos (RH) utilizados, con sus respectivas láminas de irrigación, en la producción de plantas de *Eugenia uniflora*.

Water regime	Irrigation schedule	Water blade (mm)	Total (mm day^{-1})
WR4	08:00 a.m	2	4
	01:00 p.m	2	
WR8	08:00 a.m	2	8
	01:00 p.m	2	
	03:00 p.m	4	
WR16	07:45 a.m	4	16
	11:00 a.m	4	
	02:00 p.m	4	
	04:00 p.m	4	
WR20	09:00 a.m	4	20
	12:30 p.m	4	
	03:00 p.m	4	
	05:00 p.m	8	

Table 2. Physical and chemical characteristics of the substrates used in the production of *Eugenia uniflora* seedlings.

Características físicas y químicas de los sustratos utilizados en la producción de plantas de *Eugenia uniflora*.

Characteristic	Substrates			
	S1	S2	S3	S4
pH (H_2O)	5.31	5.65	5.76	6.04
EC mS cm^{-1}	0.51	0.41	0.34	0.33
WD kg m^{-3}	172.06	218.23	154.29	220.17
DD Kg m^{-3}	136.95	135.98	141.91	143.96
TP (%)	84.37	83.25	83.03	84.61
AE (%)	27.75	32.15	36.78	48.16
EAW (%)	22.88	18.71	14.42	14.39
CRA10 (%)	56.62	51.09	46.26	36.45
CRA50 (%)	35.74	32.39	31.83	22.06
CRA100 (%)	31.80	28.39	29.09	19.18

Where: EC: electrical conductivity, WD: wet density, DD: dry density, TP: total porosity, AE: aeration space, EAW: easily available water, CRA10, 50 and 100: water retention capacity under suction 10, 50 and 100 cm water column. S1: commercial substrate (100 % CS), S2: 80 % CS + 20 % carbonized rice husk (CRH), S3: 60 % CS + 40 % CRH and S4: 40 % CS + 60 % CRH.

At 120 days after sowing, the morphological attributes height (H), stem diameter (SD), ratio of height to stem diameter (H/SD), shoot dry mass (SDM), root dry mass (RDM), total dry mass (TDM) and the Dickson Quality Index (DQI) were evaluated. The analyses of non-destructive attributes were performed on eight plants per sampling unit, and the destructive ones (dry mass) on three plants.

The height of the plants to the apical bud was measured with a millimeter ruler, and the diameter of the collection with a digital caliper (precision of 0.01 mm). For the determination of shoot dry mass (SDM), root dry mass (RDM) and total dry mass (TDM), the shoot and root parts were separated; the latter being washed in running water with sieves. The shoot and root parts were then placed separately in Kraft paper bags and taken to the air circulation oven (70 °C) until they reached constant weight. After weighing on a digital scale (accuracy of 0.001 g), the values were recorded in grams. With these data, the Dickson Quality Index (Dickson *et al*. 1960) was calculated using the following formula:

$$QI = \frac{TDW}{[(H\ /\ SD)\ + (SDW/RDW)]} \qquad [1]$$

Where:
DQI = Dickson Quality Index.
TDW = total dry weight (g).
H = height (cm).
SD = stem diameter (mm).
SDW = shoot dry weight (g).
RDW = root dry weight (g).

Data were initially analyzed on the assumptions of normality of residues and homogeneity of variances, by means of the Shapiro-Wilk and Bartlett tests, respectively. Afterwards, the data were subjected to analysis of variance (factorial ANOVA) and, when a significant difference was found ($P < 0.05$), the means were compared using the Tukey test at 5 % probability of error. For the statistical analysis, the statistical software Sisvar (Ferreira 2014) was used.

RESULTS

From the analysis of variance, it was found that the interaction between substrates and water regimes was not significant ($P > 0.05$) for the evaluated attributes. However, when the factors were analyzed separately, there was a significant effect of the substrate for the attributes height ($P < 0.0000$), stem diameter ($P = 0.00002$), H/SD ratio ($P = 0.042$), shoot dry mass ($P < 0.0000$), root dry mass ($P = 0.0007$), total dry mass ($P = 0.0008$) and Dickson Quality Index ($P = 0.0001$). For water regimes, a significant effect was observed for the variables height ($P < 0.00001$), stem diameter ($P = 0.000015$), Dickson Quality Index ($P = 0.0018$) and shoot dry mass ($P < 0.00001$).

Eugenia uniflora seedlings grown on S1 (100 % CS) also showed the best results for H and DQI, with 22.2 cm and 0.41, respectively (figure 1A and 1D). With regard to SD, the best averages were found in S1 (3.4 mm) and S2 (3.2 mm) (S2: 80 % CS + 20 % CRH), while the substrates with the highest proportion of CRH (S3: 60 % CS + 40 % CRH and S4: 40 % CS + 60 % CRH) gave values below 3.0 mm (figure 1B), which is insufficient for the seedlings to be dispatched. On the other hand, the values of the H/SD ratio varied between 5.7 (S2) and 6.6 (S1) (figure 1C).

The highest accumulation of dry mass in *E. uniflora* seedlings, both shoot (2.11 g plant^{-1}) and root (0.58 g plant^{-1}), was provided by S1, whose results were around 72 % and 41 % higher, respectively, than for substrate S3 (60 % CS + 40 % CRH) (figure 2A).

Considering the irrigation regimes, for H the best results were found in WR4 (4 mm day^{-1}) and WR8 (8 mm day^{-1}), with values of 22 and 20 cm, respectively (figure 3A), SD and DQI presented the highest averages when using the water regime WR4 (4 mm day^{-1}) (figure 3B and 3D). The H/SD ratio, with an average value of 6.13, was not influenced by water regimes (figure 3C).

DISCUSSION

In general, it was shown that *Eugenia uniflora* seedlings grew better in a nursery with the substrate S1 (100 % CS). According to Barrett *et al*. (2016) sphagnum peat has favorable characteristics for seedling production, such as adsorption and release of nutrients, good aeration and high water retention capacity. Thus, these results can be associated with this substrate physical characteristics, whose values of aeration space (AE ≅ 28 %) and easily available water (EAW ≅ 23 %) (table 2) were on average 74 % less EA and 59 % more EAW compared to S4. Regan (2014) described values of approximately 10 to 30 % and 22.5 to 32.5 %, respectively, as appropriate percentages for the AE and EAW of substrates, which corroborates the results obtained for S1 in this study.

Further research on the effect of adding CRH to the substrate has shown better growth and quality of the seedlings produced when they are grown under the lowest proportions. Fermino *et al*. (2018), evaluating the use of agro-industrial residues in the production of seedlings of *Eucalyptus grandis* W. Hill ex Maiden, did not verify a significant effect of the use of CRH on the height and diameter attributes of the collection. Dutra *et al*. (2016), studying the effects of adding CRH on the development of *Parapiptadenia rigida* seedlings, found that doses above 20 % reduced growth in H, SD and dry mass.

Additionally, Fermino *et al*. (2018) highlighted that the use of tube-type containers is associated with the use of substrates with better physical quality, given the limited space for root growth, in addition to rapid drainage with possible nutrient leaching. In this sense, although the use of CRH is recommended as a substrate conditioner (proportion of up to 30 %) for the production of seedlings of other species in regions with high availability of this

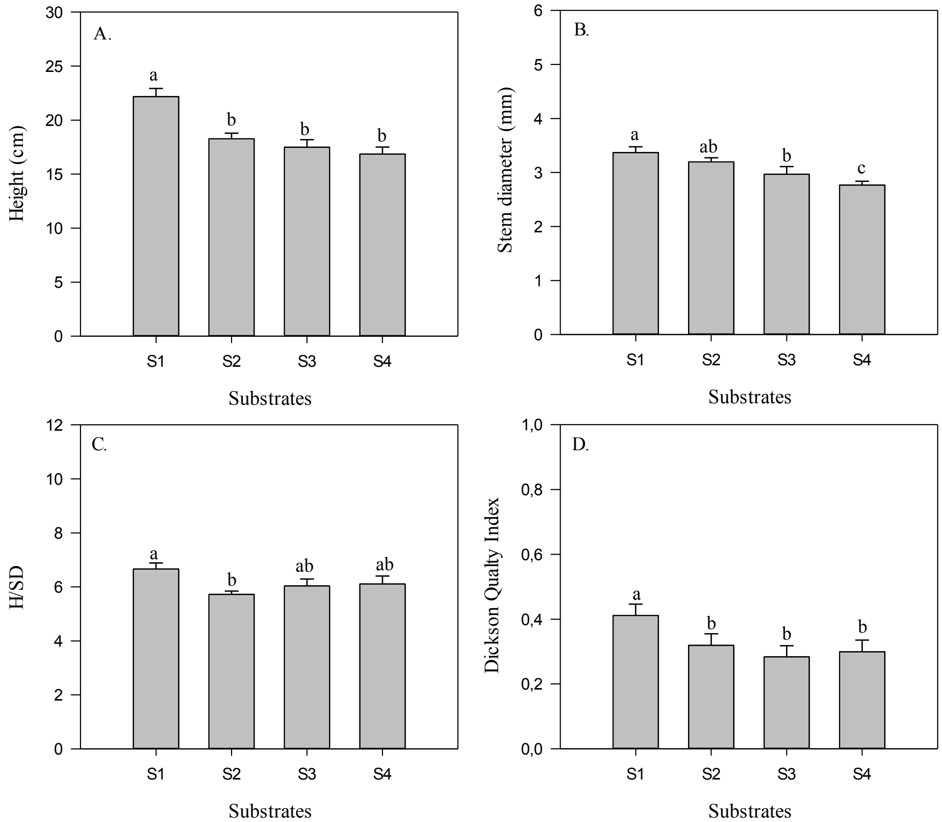

Figure 1. Height (A), stem diameter (B), height/stem diameter ratio (C) and Dickson Quality Index (D) of *Eugenia uniflora* seedlings as a function of different substrates at 120 days after sowing. Averages followed by the same letter do not differ at 5 % probability ($P < 0.05$) of error by the Tukey Test. S1: Commercial substrate (100 % CS), S2: 80 % CS + 20 % carbonized rice husk (CRH), S3: 60 % CS + 40 % CRH and S4: 40 % CS + 60 % CRH. Vertical bars indicate standard deviation.

Altura (A), diámetro del cuello (B), relación altura / diámetro (C) e Índice de Calidad Dickson (D) de plantas de *Eugenia uniflora* en función de diferentes sustratos a los 120 días después de la siembra.

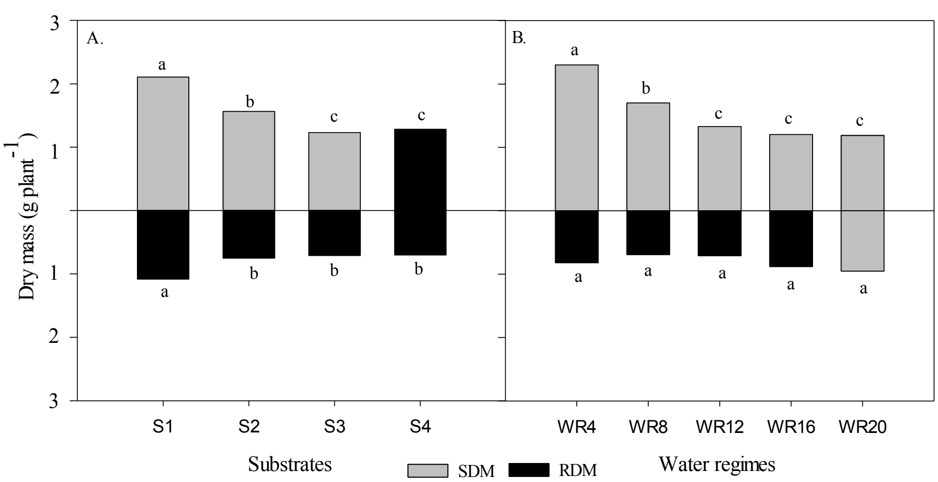

Figure 2. Effect of different water regimes (A) and substrates (B) on shoot dry mass (SDM) and root dry mass (RDM) of *Eugenia uniflora* seedlings at 120 days after sowing. Averages followed by the same letter do not differ at 5 % probability ($P < 0.05$) of error by the Tukey test. S1: Commercial substrate (100 % CS), S2: 80 % CS + 20 % carbonized rice husk (CRH), S3: 60 % CS + 40 % CRH and S4: 40 % CS + 60 % CRH. WR4: 4 mm day^{-1}, WR8: 8 mm day^{-1}, WR12: 12 mm day^{-1}, WR16: 16 mm day^{-1} and WR20: 20 mm day^{-1}.

Efecto de diferentes regímenes hídricos (A) y sustratos (B) sobre la biomasa de la parte aérea (BAP) y biomasa de la raíz (BR) de plantas de *Eugenia uniflora* a los 120 días después de la siembra.

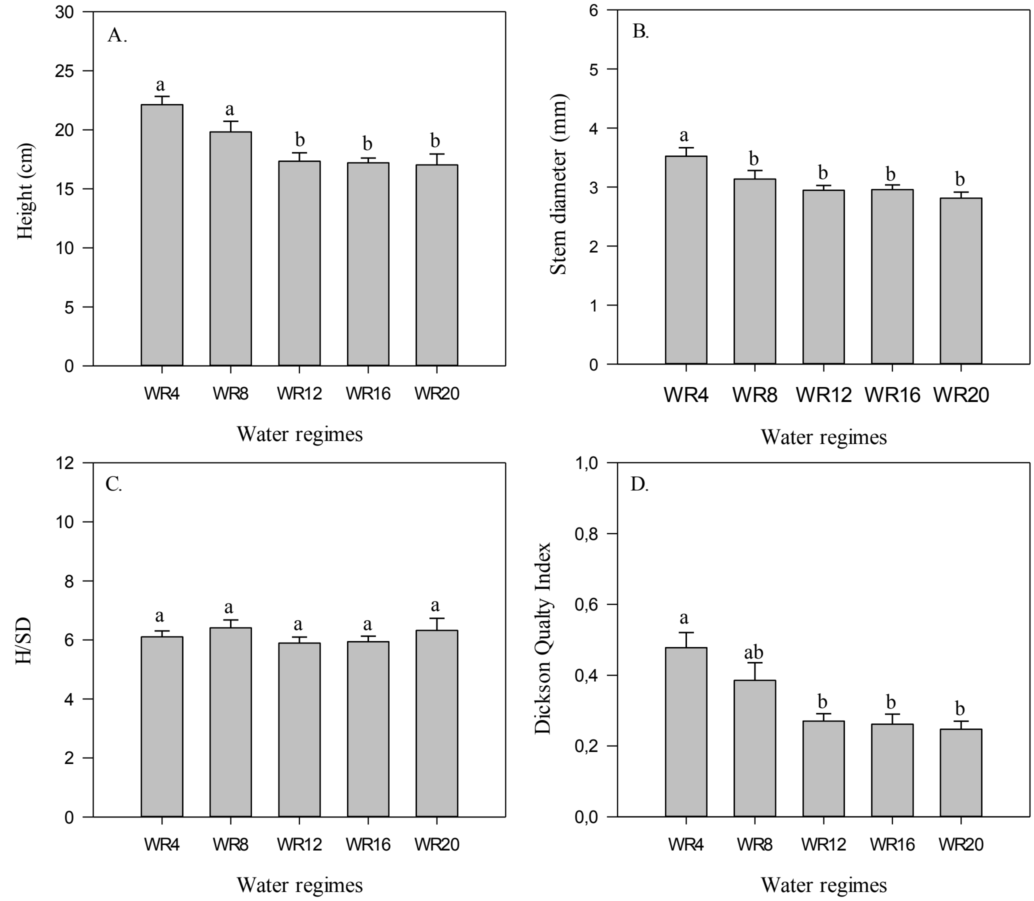

Figure 3. Height (A), stem diameter (B), height/diameter ratio (C) and Dickson Quality Index (D) of *Eugenia uniflora* seedlings as a function of different water regimes at 120 days after sowing. Averages followed by the same letter do not differ at 5 % probability of error by the Tukey Test. WR4: 4 mm day^{-1}, WR8: 8 mm day^{-1}, WR12: 12 mm day^{-1}, WR16: 16 mm day^{-1} and WR20: 20 mm day^{-1}. Vertical bars indicate standard deviation.

Altura (A), diámetro del cuello (B), relación altura / diámetro (C) e Índice de Calidad Dickson (D) de plantas de *Eugenia uniflora* en función de diferentes regímenes hídricos a los 120 días después de la siembra.

material (Gasparin *et al*. 2014, Aimi *et al*. 2019), it was not shown to be suitable for the production of *E. uniflora* seedlings in 110 cm³ tubes.

All substrates tested denoted seedlings with H/SD within the range proposed by Gonçalves *et al*. (2005). Also known as the robustness coefficient, this attribute represents the balance in the growth of seedlings in the nursery and firmness of their aerial parts, where very high values (≥ 10) indicate less robust seedlings (Araujo *et al*. 2018). Dutra *et al*. (2018) and Araujo *et al*. (2018) point out that the H/SD ratio that is considered ideal for any given species cannot be generalized to all others, since each has specific morphological characteristics of H and SD. However, this attribute can be a good indicator to analyze different treatments applied to a given species.

As the proportion of CRH increased, the dry mass weight of *E. uniflora* seedlings decreased. The highest accumulation of dry mass in *E. uniflora* seedlings on S1 (sphagnum peat) can possibly be attributed to less nutrient leaching, as it corroborates the results for the physical attributes of the tested substrates.

Regarding the irrigation regimes, the results indicate that *E. uniflora* is able to use water efficiently, allowing superior growth even with reduced irrigation. The lower water requirement of *E. uniflora* is probably related to the adaptability of the species, since it is found in different environments, occurring across the Pampas biome, in the Atlantic Forest and in forest areas in the Cerrado Biome (*sensu lato*), as well as in semi-arid regions from the Brazilian northeast (Bourscheid *et al*. 2011, Bezerra *et al*. 2018).

Complementarily, according to Bezerra *et al*. (2018), *E. uniflora* has a marked tolerance to drought, developing well in semi-arid conditions, provided that a minimum amount of water is provided. Thus, in the production of seedlings of the species, water regimes of 4 to 8 mm day^{-1}

are sufficient for their adequate development and growth, until they are ready for hardening and dispatch at 120 days after sowing.

Evaluating the effect of different substrates and irrigation regimes on the production of *Parapiptadenia rigida* seedlings, Dutra *et al*. (2016) also found that the use of 4 mm day^{-1} was sufficient for the production of quality seedlings. Kelling *et al*. (2017) found that irrigation regimes ranging from 4 mm day^{-1} in the initial phase to 8 mm day^{-1} in the rest of the production period provided the best responses in *Cordia trichotoma* plants produced in 110 cm^3 tubes using sphagnum peat substrate.

On the other hand, Turchetto *et al*. (2020) observed that in the production of *Enterolobium contortisiliquum* seedlings, irrigation regimes of 8 and 12 mm day^{-1} are necessary to obtain adequate development. Dutra *et al.* (2018) found that *Luehea divaricata* seedlings submitted to different substrates and irrigation schemes had high water demand during the production phase, requiring 16 mm day^{-1} of water. Thus, the importance of verifying the particular water requirements of each species is emphasized, to optimize productive activity and the rational use of water in nurseries.

At the same time, the Dickson Quality Index (DQI) is considered an interesting indicator for seedlings, since it considers the robustness and balance of the seedling biomass distribution. According to Gomes and Paiva (2011), DQI allows the quality classification of seedlings based on the relationship of their morphological attributes, in which the higher index values indicate the better seedling quality. Thus, in the present study, the values obtained when 4 mm day^{-1} (0.48) and 8 mm day^{-1} (0.38) regimens are used as satisfactory for *E. uniflora* seedlings (figure 3D).

The positive results of using the WR4 water regime (4 mm day^{-1}) in the production of *E. uniflora* seedlings are confirmed by analyzing the shoot dry matter data. Seedlings grown under WR4 showed the best averages (2.30 g plant^{-1}) (figure 2B), a result approximately 95 % higher than that of seedlings grown under WR20 (20 mm day^{-1}). This information will contribute to the advancement of knowledge about the production of *E. uniflora* seedlings, and may also serve as a basis for the creation of protocols for grouping of species of similar performance, thereby contributing to reducing wastage of water, leaching of nutrients and the appearance of diseases in forest nurseries caused by too much water.

CONCLUSIONS

The formulated hypothesis: smaller water regimes and substrates with intermediate proportions of rice husk enhances the development of seedlings of *E. uniflora* in nursery is partially confirmed.

The use of a substrate composed solely of sphagnum peat under water regimes of 4–8 mm day^{-1} allows for rapid growth of *Eugenia uniflora* seedlings in a nursery.

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