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Biomass of native seedlings rescued from a *Eucalyptus* stand in different seasons of the year

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ABSTRACT: The biomass production of plants originating from natural regeneration may undergo seasonal changes throughout the year, conditioned to changes in climatic factors. In view of the above, the objective of the research was to know the changes that can occur in the biomass of *Myrsine coriacea*, *Vernonia discolor*, and *Vernonanthura discolor* seedlings regenerated under *Eucalyptus* reforestation, throughout the seasons and by height classes. For this, 36 seedlings of each species were collected quarterly, that is, 12 individuals from each of the following height classes: 5.0 to 25.9 cm; 26.0 to 49.9 cm; greater than or equal to 50.0 cm. On rescue occasions, the following measurements were performed: total height, collar diameter, aerial dry mass and root dry mass. From these variables, the relationship between aerial dry mass/aerial moisture mass and the relation between root dry mass/root moisture mass was calculated. The dry mass contents, as well as the relationship between dry and moisture mass of plants rescued from the three species showed differences in the seasons. *Myrsine coriacea* presented better performance in autumn, for most of the analyzed variables, while *Vernonanthura discolor* performed better in winter. As for *Vernonia discolor*, it was not possible to define a season of the year with better performance for most variables.

Key words: native seedling production; seasonal changes; seedling rescue; water content

Biomassa de plântulas nativas resgatadas em um povoamento de *Eucalyptus* em diferentes estações no ano

RESUMO: A produção de biomassa de plantas oriundas de regeneração natural pode sofrer alterações sazonais ao longo do ano, condicionadas às alterações de fatores climáticos. Diante do exposto, o objetivo da pesquisa foi conhecer as mudanças que podem ocorrer na biomassa de mudas de *Myrsine coriacea*, *Vernonia discolor* e *Vernonanthura discolor*, regeneradas sob reflorestamento de *Eucalyptus* ao longo das estações do ano e por classes de alturas. Para tal, foram realizadas coletas trimestrais de 36 plântulas de cada espécie, ou seja, 12 indivíduos de cada uma das seguintes classes de alturas: 5,0 a 25,9 cm; 26,0 a 49,9 cm; maior ou igual a 50,0 cm. Nas ocasiões de resgate foram realizadas as seguintes mensurações: altura total, diâmetro do coleto, massa seca aérea e massa seca radicular. A partir dessas variáveis foi calculada a relação entre massa seca/massa úmida aérea e relação entre massa seca e massa úmida radicular. Os teores de massa seca, bem como as relações entre massa seca e úmida de plantas resgatadas das três espécies apresentaram diferença nas estações do ano. *Myrsine coriacea* apresentou melhor desempenho no outono, para a maioria das variáveis analisadas, enquanto *Vernonanthura discolor* obteve melhor desempenho no inverno. Já para *Vernonia discolor* não foi possível definir uma estação do ano com melhor desempenho para a maioria das variáveis.

Palavras-chave: produção de mudas nativas; alterações sazonais; resgate de plântulas; teor de água

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Introduction

Obtaining seedlings of varied native species and in sufficient quantities represents one of the main constraints to the implementation of high diversity plantations (Vidal, 2008; Brancalion et al., 2012). Considering only tree and shrub species, nurseries offer customers less than 50% of the species recorded in the areas studied (Vidal et al., 2020), indicating that there is a huge field of research to be explored.

The difficulty in obtaining seedlings with high diversity in forest nurseries has led to the loss of biological and genetic diversity in regeneration projects in degraded areas (Calegari et al., 2011). And considering that the continuous economic expansion implies in new enterprises such as hydroelectric plants, road construction, airports, and others that end up deforesting areas of natural forests, one of the alternatives would be the use of natural regeneration for the production of seedlings, through the transplant of seedlings (Vidal, 2008).

Transposition of seedlings from natural regeneration presents itself as a viable and low-cost technique that can be used in a complementary way to seedling propagation in forest nurseries (Paula et al., 2013; Calegari et al., 2011). And among the main benefits associated with this technique is the production of seedlings of regional species not available in nurseries and the elimination of steps such as processing, storage, and pre-germination treatment (Vidal, 2008).

Seedling quality is a key factor in achieving successful forest implementation (Schorn et al., 2020). Extensive studies since the 1950s have shown that desirable morphological attributes contribute to seedling survival after they are transported to planting sites (Grossnickle, 2012). In this context, dry matter production has been considered one of the best parameters to characterize seedling quality (Maciel & Palomino, 2018). This parameter is quite consistent and used in evaluating seedlings for variations in external factors (Paula et al., 2013). According to Martinkoski et al. (2015), seedling growth is influenced by several factors, varying not only among species but also responding to environmental variations and ecological conditions. Air temperature and precipitation stand out as the climatic variables that have the greatest influence on the growth of various species.

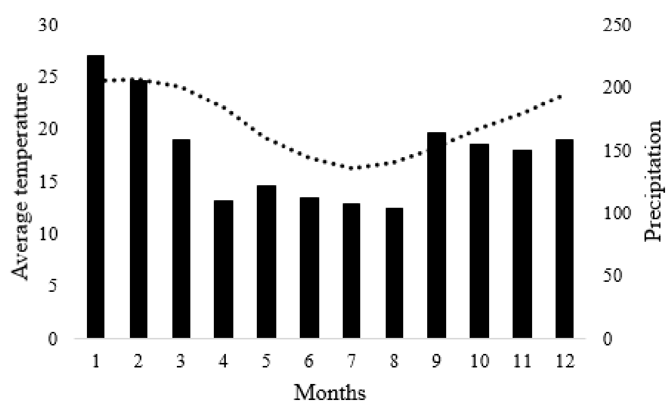
In view of the above, the objective of this study was to know the biomass performance, in different seasons of the year, of rescued seedlings of the species *Myrsine coriacea*, *Vernonia discolor*, and *Vernonanthura discolor*, in an area under *Eucalyptus* reforestation in the Tijucas River Valley in Santa Catarina, Brazil. To this end, the following hypotheses were established: a) there are differences in aerial and root dry mass of seedlings rescued in different seasons of the year; b) the ratio between dry mass/moisture mass of the rescued seedlings is higher in the seasons of lower vegetative development; c) rescued seedlings from different heights show different patterns in the dry mass/moisture mass ratio throughout the year.

Materials and Methods

Characterization of the area

The study was conducted in an experimental area in the locality of Moura (27° 14’ 18.56” and 48° 47’ 13.78”), in the municipality of Canelinha, SC, Brazil. The site has an altitude of 40 to 65 m and is inserted in the watershed of the Tijucas River. The vegetation in the region consists of submontane Ombrophilous Dense Forest (IBGE, 2012). The region climate is of type Cfb, according to the Köppen classification. The average annual temperature ranges from 15.3 to 24.2 °C and the average annual precipitation reaches 1,747 mm (Alvares et al., 2013). The monthly averages for temperature and precipitation in the region where the research was carried out are shown in Figure 1.

In the experimental area there was a reforestation with *Eucalyptus grandis* that was 18 months old at the time of the first collection. The reforestation had dense understory, with height varying from 0.05 to 1.50 m, containing herbaceous, shrub, and tree species. For the study, three native species were selected that had high frequency and density in the understory of similarly situated reforestations. We used species of pioneer character, according to Carvalho (2008), and with high potential for use in forest restoration projects: *Vernonia discolor* (Spreng.) H. Rob., *Myrsine coriacea* (Sw.) R. Br., and *Vernonanthura discolor* (Spreng.) H. Rob.



Source: Climate.Data (2022).

Figure 1. Average climatic data of monthly temperature and precipitation at the research site.

Data collection and analysis

Quarterly collections of *V. discolor*, *M. coriacea*, and *V. discolor* were made in the understory of the *Eucalyptus* reforestation in April, June, and October 2020 and January 2021. On each occasion 36 seedlings of each species were collected, i.e. 12 individuals for each of the following height classes: 5.0 to 25.9 cm, 26.0 to 49.9 cm, and ≥ to 50.0 cm. For each height class and for each species, three sampling units containing four seedlings each were allocated.

The experiment was carried out under an entirely randomized design in a 3 × 4 factorial scheme. The factors studied were height classes and seasons. The analyses were performed using R Statistical Software (version 3.5.0) (R Development Core Team, 2008).

The seedlings were collected with the largest possible portion of soil close to the roots, transplanted on the same day into 5 L plastic bags with commercial Plantmax® substrate and kept in a forest nursery for 90 days from the initial collection date. In the nursery, the seedlings were kept in a bed under shade with 50% of light and irrigated through nebulization.

On the rescue occasions, the total height and collar diameter of each plant were measured. At the end of the 90-day period these same variables were measured again and the aerial dry mass (ADM) and root dry mass (RDM) parameters were evaluated.

To determine the dry mass, the seedlings were dried in a forced ventilation oven at 75 °C until they reached a constant weight. From these values, the ratio of aerial dry mass/aerial moisture mass and root dry mass/root moisture mass were calculated according to Araújo et al. (2018).

To verify the normality of the variables, the Shapiro-Wilk test was used ($p < 0.05$). A completely randomized design in a 3 × 4 factorial was used to compare means between seasons, for each height class and for each variable. The analyses were performed using R (version 3.5.0) (R Development Core Team, 2008).

Results

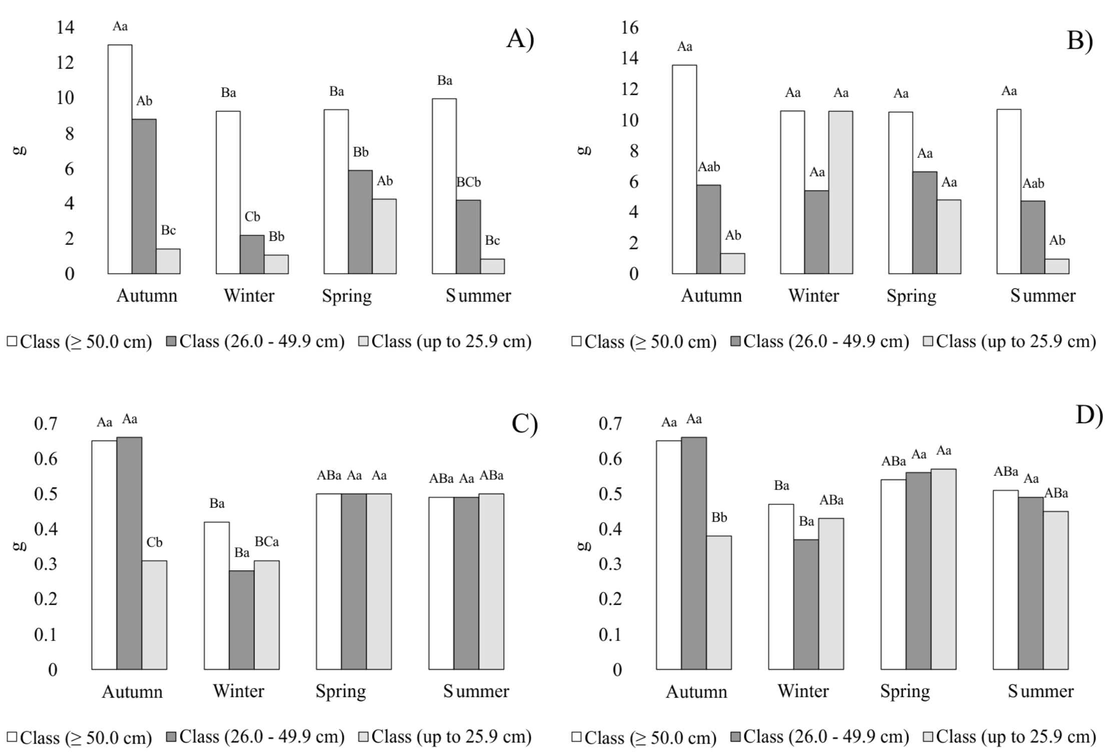
Results by species are presented, as well as the analysis of the variables across seasons and across the three classes of seedling heights.

Myrsine coriacea

Aerial dry mass (ADM) resulted in significantly different values among height classes within seasons (Table 1, Figure 2A). For the height class up to 25.9 cm the highest ADM was

Table 1. Results of variance analysis for aerial dry mass (ADM), root dry mass (RDM), aerial dry mass/aerial moisture mass (ADM/AMM), and root dry mass/root moisture mass (RDM/RMM) of rescued *Myrsine coriacea* seedlings in an *Eucalyptus* reforestation understorey.

Sources of variation	DF	Mean square	F	p > F
ADM				
Height classes (H)	2	217.9013	178.163	< 0.001
Seasons (E)	3	22.3255	18.254	< 0.001
H × E	6	9.1984	7.5209	< 0.001
Residue	24	1.2230		
RDM				
Height classes (H)	2	161.7313	8.4621	0.0017
Seasons (E)	3	20.4541	1.0702	0.3803
H × E	6	25.0715	1.3118	0.29
Residue	24	19.1125		
ADM/AMM ratio				
Height classes (H)	2	0.0418	6.9582	0.0041
Seasons (E)	3	0.0723	12.0543	< 0.001
H × E	6	0.0323	5.3788	0.0012
Residue	24	0.0060		
RDM/RMM ratio				
Height classes (H)	2	0.0248	4.4910	0.022
Seasons (E)	3	0.0398	7.2283	0.0013
H × E	6	0.0200	3.6371	0.0104
Residue	24	0.0055		



Averages followed by the same letter, capitalized between stations and lower case between height classes within stations, do not differ by Tukey test at 5% probability level.

Figure 2. Aerial dry mass (A), root dry mass (B), aerial dry mass/aerial moisture mass (C), and root dry mass/root moisture mass (D) of rescued *Myrsine coriacea* seedlings in the understory of a *Eucalyptus* reforestation.

observed in spring. For the upper classes, the highest values were observed in the autumn.

Regarding root dry mass (RDM), only in autumn and summer were statistically different values observed among height classes within seasons (Figure 2B). However, no significant differences were observed between seasons.

The aerial dry mass/aerial moisture mass ratio resulted in significantly different values between height classes only for autumn (Figure 2C). For the seasons, the ratio resulted in close values in spring and summer. Individuals taller than 26.0 cm performed better in autumn, although they did not differ statistically from the spring and summer. Individuals up to 25.9 cm, on the other hand, performed better in spring and summer.

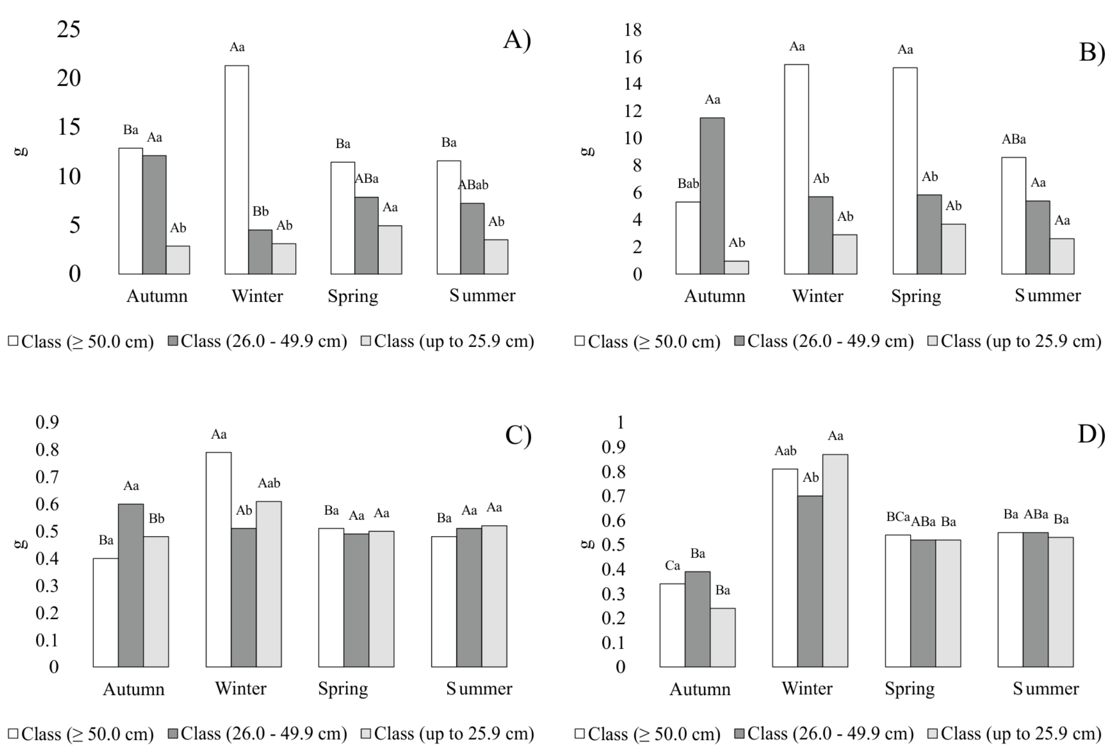
The root dry mass/root moisture mass ratio also showed a statistically significant difference between height classes only for autumn (Figure 2D). The ratio showed significantly different averages among the seasons. Individuals with heights greater than 26.0 cm showed better performance in the autumn, although this did not differ statistically from the spring and summer. For individuals up to 25.9 cm tall, the root dry mass/root moisture mass ratio was higher in the spring, differing statistically only in the autumn.

Vernonanthura discolor

Aerial dry mass (ADM) resulted in significantly different values among height classes in the autumn, spring, and summer (Table 2, Figure 3A). *Vernonanthura discolor* seedlings with heights ≥ 50.0 cm showed significantly higher ADM values in winter. On the other hand, in the seedlings with height between 26.0 and 49.9 cm, the highest values were found in the autumn, not significantly different from the values found in the spring and summer. And in seedlings up to 25.9 cm, no statistical differences were observed between seasons.

Table 2. Results of the analysis of variance for aerial dry mass (ADM), root dry mass (RDM), aerial dry mass/aerial moisture mass (ADM/AMM), and root dry mass/root moisture mass (RDM/RMM) of rescued *Vernonanthura discolor* seedlings in an *Eucalyptus* reforestation understory.

Sources of variation	DF	Mean square	F	p > F
		ADM		
Height classes (H)	2	341.6707	30.6892	< 0.001
Seasons (E)	3	10.5814	0.9504	0.432
H × E	6	45.4869	4.0857	0.006
Residue	24	11.1333		
		RDM		
Height classes (H)	2	227.5061	12.0434	< 0.001
Seasons (E)	3	17.5840	0.9308	0.441
H × E	6	44.9353	2.3787	0.06
Residue	24	18.8904		
		ADM/AMM ratio		
Height classes (H)	2	0.0299	2.9812	0.0698
Seasons (E)	3	0.0873	8.7105	< 0.001
H × E	6	0.0521	5.1944	0.002
Residue	24	0.0100		
		RDM/RMM ratio		
Height classes (H)	2	0.0018	0.2201	0.804
Seasons (E)	3	0.3858	47.475	< 0.001
H × E	6	0.0251	3.083	0.022
Residue	24	0.0081		



Averages followed by the same letter, capitalized between stations and lower case between height classes within stations, do not differ by Tukey test at 5% probability level.

Figure 3. Aerial dry mass (A), root dry mass (B), aerial dry mass/aerial moisture mass (C), and root dry mass/root moisture mass (D) of rescued *Vernonanthura discolor* seedlings in an *Eucalyptus* reforestation understory.

Root dry mass (RDM) resulted in significantly different values among height classes in the autumn, spring, and summer (Figure 3B). Between seasons, significant differences were observed only for class ≥ 50.0, in which a greater production of RDM was observed in winter and spring, differing significantly only in autumn (Figure 3B).

The aerial dry mass/aerial moisture mass ratio resulted in slightly close values between the height classes in spring and summer. The highest values of this ratio for seedlings up to 25.9 cm and for those ≥ 50.0 cm, overall, occurred in winter (Figure 3C). For the 26.0 to 49.9 cm class, no statistically significant difference was observed between the seasons.

The root dry mass/root moisture mass ratio also resulted in slightly close values between the height classes in spring and summer (Figure 3D). Overall, the results obtained showed significantly higher values for this variable in winter.

Vernonia discolor

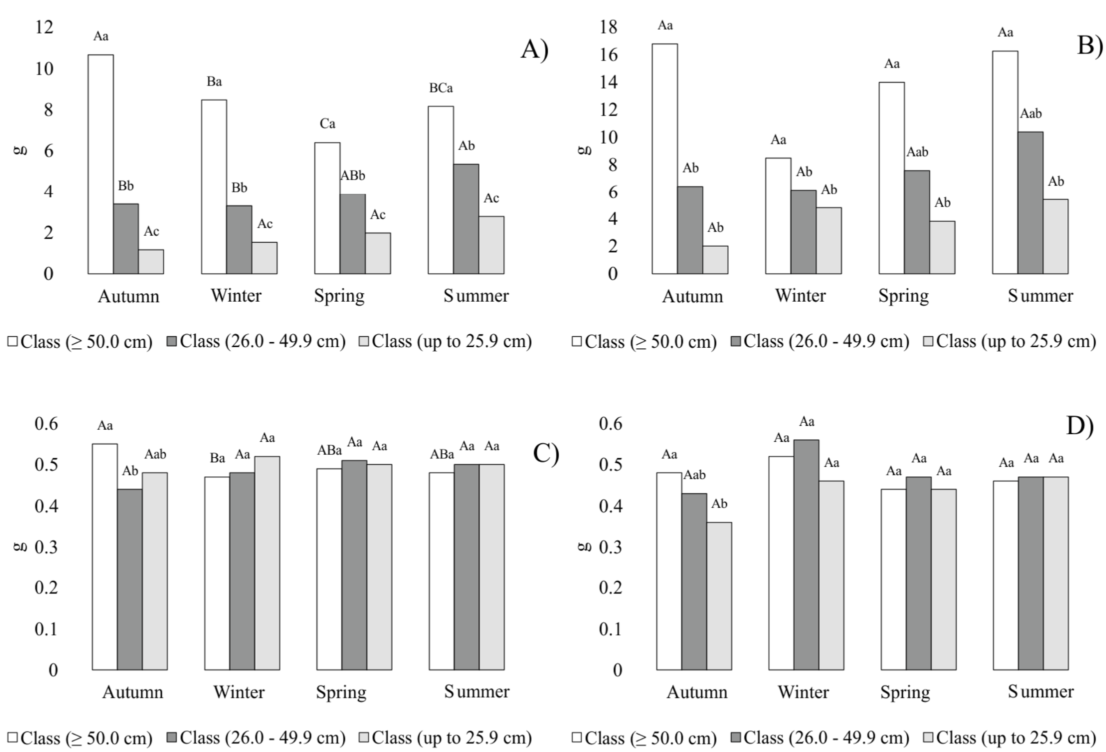
Aerial dry mass (ADM) resulted in significantly different values among height classes within seasons (Table 3, Figure 4A). Seedlings with height ≥ 50.0 cm produced higher biomass in the autumn. The seedlings with heights between 26.0 and 49.9 cm produced higher values in the summer, although they did not differ statistically from the spring. While for the up to 25.9 cm class, no statistically significant difference was observed between the seasons.

Root dry mass (RDM) resulted in significantly different values among height classes within seasons (Figure 4B). However, between stations the ADM did not show significant differences between height classes.

Table 3. Results of the analysis of variance for aerial dry mass (ADM), root dry mass (RDM), aerial dry mass/aerial moisture mass (ADM/AMM), and root dry mass/root moisture mass (RDM/RMM) of rescued *Vernonia discolor* seedlings in an *Eucalyptus* reforestation understory.

Sources of variation	DF	Mean square	F	p > F
		ADM		
Height classes (H)	2	134.3693	199.0484	< 0.001
Seasons (E)	3	3.3282	4.9302	0.0083
H × E	6	5.0049	7.4140	< 0.001
Residue	24	0.6751		
		RDM		
Height classes (H)	2	516.6208	31.1442	< 0.001
Seasons (E)	3	13.0220	0.7850	0.514
H × E	6	11.5745	0.6978	0.654
Residue	24	16.5880		
		ADM/AMM ratio		
Height classes (H)	2	0.0009	0.7367	0.4892
Seasons (E)	3	0.0000	0.0249	0.9946
H × E	6	0.0043	3.5566	0.0116
Residue	24	0.0012		
		RDM/RMM ratio		
Height classes (H)	2	0.0069	3.0764	0.0647
Seasons (E)	3	0.0057	2.5202	0.0820
H × E	6	0.0024	1.0473	0.4203
Residue	24	0.0023		

The aerial dry mass/aerial moisture mass ratio between height classes resulted in close values in all four seasons (Figure 4C). There were significant differences only in the class greater than 50.0 cm, in which the highest biomass production



Averages followed by the same letter, capitalized between stations and lower case between height classes within stations, do not differ by Tukey's test at 5% probability level.

Figure 4. Aerial dry mass (A), root dry mass (B), aerial dry mass/aerial moisture mass (C), and root dry mass/root moisture mass (D) of rescued *Vernonia discolor* seedlings in an *Eucalyptus* reforestation understory.

was found in the autumn, although it did not differ statistically from the spring and summer.

The root dry mass/root moisture mass ratio resulted in close values between height classes in summer and spring (Figure 4D). However, between the stations the ratio did not show a statistically significant difference.

Discussion

In different species it is possible to find high variation in biomass allocation. In general, the younger seedlings, here considered those of smaller heights, aggregated higher contents of dry mass in spring, a period in which there was an increase in temperature, precipitation, and photoperiod (Figure 1). As for larger seedlings, in general, these values were more expressive in the autumn (Figure 3A). Therefore, the results suggest that distinct climatic variables in these seasons, characterized by temperatures, precipitation, and luminosity, associated with the phenological events of the species, especially the distinct vegetative development rhythm in spring and autumn, may contribute to the performance obtained by the seedlings of the species. According to Oliveira et al. (2019), photoassimilate partitioning patterns can vary depending on the species, age, and climatic conditions that seedlings are subjected to.

The performance of the seedlings in root dry mass (RDM) showed distinct results compared to the ADM. Overall, RDM was highest in the ≥ 50.0 cm class. Plants with well-developed root systems have a greater chance of survival in the field (Freitas et al., 2012). For a larger root biomass provides a better performance of the seedlings due to the greater ease of support and greater area for absorption of water and nutrients (Almeida et al., 2005). Therefore, plants with higher RDM will be more effective for forest management due to their ability to acclimatize after transplanting.

The study developed had an important limitation related to the rescue of the seedlings. Although this was done with the aid of tools such as shovels and hoes, there was some difficulty in extracting the root system with integrity. This fact was observed more frequently in seedlings with greater heights. Therefore, the determination of RDM may have the values underestimated due to the loss of root biomass at the time of collection and handling (Avelino et al., 2021).

The aerial dry mass/aerial moisture mass ratio, for the three species studied, resulted in slightly close values between the height classes in spring and summer. The results show that in the taller seedlings there were greater changes in the aerial dry mass/aerial moisture mass ratio between the seasons, but it was not possible to observe a clear relationship between these changes with the seasons. According to Ryan et al. (2010), forest biomass production varies according to resource availability, photosynthesis processes, respiration, carbon compartmentalization, belowground flow, leaf production, and others.

The root dry mass/root moisture mass ratio also resulted in slightly close values between the height classes in spring and summer. These results present a convergence to the distribution of temperature and precipitation values (Figure 1), evidencing the possible effect of these climatic variables on the relationship between aerial dry mass/aerial moisture mass of the seedlings.

Tree planting has been the main way to achieve artificial regeneration of tropical forests in recent decades. As concerns about global deforestation increase, planting programs will also increase (Dionisio et al., 2019). However, for the production of seedlings of native forest species there is still little research due to the great diversity of plant species found in Brazil. This gives rise to the great need for studies regarding techniques that facilitate the production of seedlings of these species with quality, practicality, and efficiency, thus improving survival in the field and consequently reducing production costs (Freitas et al., 2022). According to Fonseca et al. (2002), programs for the implementation, recomposition and revitalization of native forests will only have guaranteed success when the methods and systems employed by nurserymen prioritize the production of seedlings with quality and low cost. In this context, seedling production of forest species is one of the most important activities in reforestation, as poor seedling quality can compromise the success of subsequent plantations (Dionisio et al., 2019). On the other hand, reforestation with seedlings of native tree species that emphasize biodiversity conservation may not be successful due to lack of knowledge about these species (Aimi et al., 2020). Therefore, the knowledge of morphological parameters in the quality of seedlings of native species can guarantee the success in the production and supply of seedlings in recovery and restoration projects of degraded areas.

The study, in general, represents an advance in knowledge about seedling rescue. A technique that presents an important alternative for the production and supply of seedlings of native forest species, aiming mainly at recovery projects and restoration of degraded areas. Considering that in reforestations for timber production the presence of native species in the understory is temporary, the use of individuals of these species can rapidly increase the supply of available seedlings and at lower costs, considering that the period the seedlings remain in the nursery is shorter.

Conclusions

Overall, the aerial dry mass, root dry mass, and dry mass/moisture mass ratios of rescued plants of the three species studied showed differences across seasons.

Myrsine coriacea performed better in autumn, for most of the variables analyzed, while *Vernonanthura discolor* performed better in the winter. As for *Vernonia discolor*, it was not possible to define a season of the year with better performance for most variables.

The dry mass/moisture mass ratio, in general, was higher in the seasons of lower vegetative development (winter) only for *Vernonanthura discolor*.

Seedlings of *Myrsine coriacea* and *Vernonanthura discolor*, rescued with different aerial heights, show different patterns in the dry mass/moisture mass ratio in autumn and winter.

Compliance with Ethical Standards

Author contributions: Conceptualization: LAS; Data curation: LAS; Formal analysis: LAS; Funding acquisition: LAS, TABF; Investigation: LAS, TABF, RB; Methodology: LAS, TABF, RB; Project administration: LAS; Resources: LAS, TABF; Supervision: LAS, TABF; Validation: LAS; Visualization: LAS, KFS; Writing – original draft: LAS, KFS; Writing – review & editing: KFS, LAS.

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