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CROP PRODUCTION

Substrates and containers influence the growth of *Campomanesia phaea* (O. Berg. Landrum) seedlings, an endangered Atlantic Rainforest species

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ABSTRACT. Native to the Brazilian Atlantic Rainforest, cambuci fruits can be consumed either *in natura* or in the processed form, and cambuci trees can be used to recover degraded areas. However, studies regarding cambuci germination and seedling development are scarce. The main aim of this study was to understand how different substrates and containers influence the growth of cambuci seedlings. To this end, two experiments were conducted: five different substrates were tested in the first experiment, and based on the multivariate analysis of the first experiment, the effect of combination of the best-performing substrates and three commonly employed plant growth containers on seedling development was investigated. Overall, good quality cambuci seedlings were obtained when they were grown in the commercial pine bark substrate and peat and perlite mixture in a 260 cm^3 container.

Keywords: cambuci; Myrtaceae; initial development; seedling production.

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Introduction

Cambuci [*Campomanesia phaea* (O. Berg. Landrum)] is a native Atlantic Rainforest species distributed throughout southeastern Brazil (CNCFlora, 2012; Lorenzi, 2014). It is endemic to the state of São Paulo and is found in the region from the Serra do Mar Mountain range to the Paulista Plateau (Lorenzi, Bacher, Lacerda, & Sartori, 2006). Despite its high acidity levels, cambuci fruits can be consumed either *in natura* or, more commonly, in a processed form, such as juice, liquor, and jellies, without losing any nutritional content (ascorbic acid, minerals and fiber) (Tokairin, Bremer Neto, & Jacomino, 2018a; Tokairin, Silva, Spricigo, Alencar, & Jacomino, 2018b; Vallilo, Garbelotti, Oliveira, & Lamardo, 2005). Moreover, cambuci trees, along with other arboreous Myrtaceae species, can also be used to recover degraded areas and as ornamental plants (Delgado & Barbedo, 2007; Demétrio, Oliveira Jacob, Ambrosano, Oliveira, & Rodrigues, 2021; Gomes, Oliveira, França, Dacoregio, & Bortoluzzi, 2015; Lattuada, Souza, & Gonzatto, 2010; Vallilo, Moreno, Oliveira, Lamardo, & Garbelotti, 2008, Vallilo et al., 2005; Zillo, Silva, Zanatta, Carmo, & Spoto, 2013).

In Brazil, orchards of native species are scarce and primarily established from seedlings, which leads to genetic variability between plants, consequently jeopardizing orchard development and longevity (Gentil & Minami, 2005). Cambuci seeds take time to germinate, resulting in irregular seedling production. Moreover, cambuci seeds have critical moisture content, at which the germination speed decreases and seed mortality rate increases (Lorenzi et al., 2006; Santoro, Brogio, Forti, Novembre, & Silva, 2020). Therefore, it is crucial to ensure production of excellent cambuci seedlings.

The use of agricultural substrates enhances germination process and root development and ensures uniformity in seedling size (Gondin, Silva, Alves, Dutra, & Elias, 2015). A suitable substrate should display good water retention (WR), aeration, and drainage; should be easy to handle and sterile (Kämpf, 2000); and should be free from microorganisms, toxic substances, and foul smells (Minami & Salvador, 2010).

Similarly, plant growth containers also affect germination, seedling quality, and root system formation (Abreu et al., 2005; Kämpf, 2000), and container size directly affects water and nutrient availability and operational nursery aspects (Dumroese, Luna, & Landis, 2009).

In this context, owing to the scarcity of information regarding the effects of different substrates and containers on cambuci germination and seedling development (Bianchini et al., 2016; Gomes, Oliveira, Ferreira, & Batista, 2016; Maluf & Pisciottano-Ereio, 2005; Rego, Nogueira, Kuniyoshi, & Santos, 2009), this study aimed to identify the main effects of different substrates and containers on initial cambuci seedling growth.

Material and methods

To better understand the effects of different agricultural substrates and container on initial cambuci seedling growth, two experiments were performed in an experimental area belonging to the Crop Science Department at the University of São Paulo (USP/ESALQ) in Piracicaba, São Paulo State, Brazil (latitude: 23°42'29" S, longitude: 47°37'45" W, and elevation: 546 m). The cambuci seeds used were extracted from ripe fruits randomly harvested from a commercial orchard in the municipality of Natividade da Serra, São Paulo, Brazil (latitude: 23°31'27" S, longitude: 45°27'12" O, and elevation: 720 m).

To remove seeds, the ripe fruits were cut into half, the pulp was placed in a sieve with sand, and both were rubbed together. The friction between the seeds and sand under running water completely removes the pulp from the seeds. Thereafter, the seeds were washed under running tap water and placed on a tissue paper for 24h for drying to remove excess water.

Experiment 1: Cambuci seedling development employing different substrates

The experiment was started in May, 2018 (fall). Cambuci seeds were sown in 72-cell (95 cm^3) styrofoam trays to evaluate the initial seedling growth using different substrates. Five different substrates were tested: commercial pine bark substrate (T1), medium-texture autoclaved sand (T2), peat and perlite (1:1, v/v) mixture (T3), medium granulometry expanded vermiculite (T4), and soil (T5).

This experiment was conducted following a completely randomized design comprising five treatments and four replicates. Each experimental unit consisted of 18 seeds, for a total of 360 seeds.

Experiment 2: Cambuci seedling development employing different substrates and plant-growth containers

The second experiment was started one year later in June, 2019 (winter). New ripe fruits were collected and the seeds were extracted following the same procedure reported previously.

Three differently sized containers were combined with the substrates that showed the best results in experiment 1. The three different containers used were as follows: 72-cell 95 cm^3 styrofoam trays and two differently sized tubes (53 and 260 cm^3).

The experimental design was a completely randomized factorial design (2 × 3), comprising two substrates and three containers, with four replicates each. Each replicate consisted of 18 seeds, with a total of 432 seeds.

Seedling evaluations

To determine the number of emerged seedlings (% emergence), each experimental unit was evaluated every 15 days for 120 days. Emergence was considered when the hypocotyl was maintained above the substrate level. This data was used to calculate the emergence speed index (ESI) according to Maguire (1962), using the following equation: $ESI = E_1/N_1 + E_2/N_2 + ... + E_n/N_n$, where E_1, E_2, and E_n are the number of emerged seedlings in each evaluation, and N_1, N_2, and N_n represent the days after sowing (DAS).

After 120 days, the height (H, cm), stem diameter (D, mm), number of leaves (NL, leaves per seedling), leaf area index (LAI, cm^2), fresh and dry weight from both root (RSFW and RSDW, respectively, g) and shoot systems (SSFW and SSDW, respectively, g), root system length (RSL, cm), root system surface area (RSSA, cm^2), root system average diameter (RSAD, mm), and root system volume (RSV, cm^3) were determined with the aid of proper equipment.

The height and stem diameter were measured using a millimeter ruler and a digital caliper (Zass Precision®), respectively. Fresh and dry weights were assessed using a precision scale (Ohaus® Explorer), and the dry matter weight was determined after drying the samples at 65°C for 72h in a forced air circulation oven (Tecnal®, Model TE-394/3). A leaf area integrator (LICOR®, Inc., model LI-3100) was used to determine the LAI. The remaining variables associated with the radicular system, *i.e.,* RSL, RSSA, RSAD, and RSV, were calculated by scanning the roots (Epson®, model XL10000) and analyzing the images using the WinRHIZO® software (Regent Instruments, 2013).

Data regarding the climatic conditions during the experiments (mean, maximum, and minimum air temperatures; monthly precipitation; and humidity) are presented in Table 1. Seedlings were watered daily as needed. Samples from different substrates were also subjected to physicochemical analysis in the laboratory (Table 2).

Table 1. Monthly precipitation (mm), humidity (%), and minimum, mean, and maximum temperatures (°C) during Experiment 1 in Piracicaba, São Paulo State, Brazil in 2018 and 2019.

Year	Month	Precipitation (mm)	Humidity (%)	Temperature (°C)		
				Minimum	Mean	Maximum
2018	May	12.95	71.45	12.97	19.94	27.81
	June	8.89	72.59	13.62	20.48	27.51
	July	2.54	62.48	10.66	19.40	28.64
	August	105.67	73.03	12.09	18.34	25.68
	September	54.30	71.01	14.85	21.25	28.73
	Means	36.87	70.11	12.84	19.88	27.67
2019	June	10.60	71.93	12.20	19.59	26.99
	July	51.30	71.00	9.42	17.71	25.99
	August	6.20	66.87	11.53	19.61	27.69
	September	55.60	67.17	15.11	22.93	30.75
	October	109.30	62.84	17.16	25.33	33.50
	Means	46.60	67.96	13.08	21.04	28.99

*Values available at the Climate Data Series from the 'Luiz de Queiroz' Campus in Piracicaba (SP), LEB, ESALQ, USP.

Table 2. Physicochemical characterizations of the assessed substrates on a wet weight basis. Piracicaba, São Paulo State, Brazil, 2018-2019.

Substate	pH	N total	P_2O_5	K_2O	S	Ca	Mg	C	C/N	Density	WR	EC
	$CaCl_2$	%	-------g dm^{-3}------			----------%--------				g cm^3	%	mS cm^{-1}
Pine bark substrate	5.8	0.65	2.30	1.36	0.56	1.53	1.01	22.8	35	0.47	85.6	1.77
Sand	6.6	0.29	7.12	0.71	1.07	0.22	0.05	0.30	1	1.78	14.3	0.12
Peat + perlite	5.8	0.73	0.18	0.54	0.43	1.51	0.34	22.9	31	0.18	190.5	3.57
Vermiculite	6.4	0.08	0.12	0.31	0.0	0.09	2.40	0.06	1	0.62	340.4	0.55
Soil	5.6	0.43	3.59	0.49	0.49	0.46	0.22	4.09	9	1.24	36.3	0.31

WR: water retention; EC: electrical conductivity. *Results from laboratory analysis conducted in the Soil Sciences Department, Piracicaba, São Paulo State, Brazil (LSO, ESALQ/USP).

Statistical analyses

Statistical analyses were performed using SAS® v.9.4. software (SAS Institute Inc., Cary, NC, USA). All data were subjected to analysis of variance (ANOVA), and the treatment means were compared using Tukey's HSD test ($p < 0.05$). Principal component analysis (PCA) and cluster analysis (CA) were also performed. It is important to note that some variables were excluded from this analysis to avoid multicollinearity effects.

Results and discussion

Experiment 1

During this experiment, the mean air temperature was 19.8ºC (Table 1), which was lower than the optimal temperature (25°C) for seed germination of Myrtaceae species (Gomes et al., 2016). However, this did not affect cambuci seedling emergence, which began at 60 DAS, reaching more than 80% at 90 DAS and 94% at 120 DAS. These data were similar to those reported for jaboticaba (*Plinia* sp.) seeds, which showed 80.8% seedling emergence at 90 DAS (Danner et al., 2007).

At 60 DAS, seedling emergence rates of cambuci seeds sown in vermiculite (T4) were higher than those sown in other substrates, which is consistent with the findings of Maluf and Pisciottano-Ereio (2005), who indicated that vermiculite enhances cambuci seed germination. However, this result was in contrast to the results reported by Bianchini et al. (2016) who indicated that high seedling emergence occurs in the commercial pine bark substrate.

By the end of the experiment, 100% seedling emergence was observed for vermiculite (T4), which is significantly different from the emergence rate obtained for soil (T5) (88.9%) (Figure 1). According to Fachinello, Hoffman, and Nachtigal (2005), the use of soil can jeopardize the initial seedling growth, as soil exhibits high WR and compaction levels. However, Danner et al. (2007) did not report statistical differences in emergence using soil as a substrate for jaboticaba.

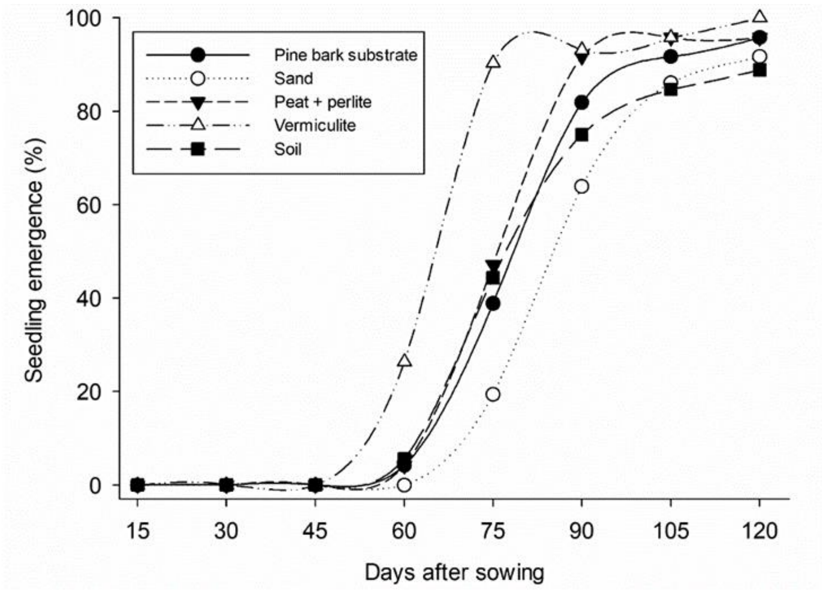

Figure 1. Mean fortnight percentages of cambuci (*Campomanesia phaea* O. Berg. Landrum) seedling emergence in different substrates for 120 days. Piracicaba, São Paulo State, Brazil, 2018.

Statistical differences between treatments were observed for ESI, H, D, NL, LAI, SSFW, SSDW, RSL, RSAD, RSSA, RSV, RSFW, and RSDW values.

Sowing in the vermiculite substrate (T4) resulted in higher ESI values, which were statistically different from the other substrates, except for the peat and perlite mixture (T3). These results are different from those reported by Danner et al. (2007), who observed higher ESI values for jaboticaba seedlings sown in the commercial Plantmax® (pine bark) substrate.

A higher ESI provides a competitive advantage to seedlings, as they are exposed to weather variations for a shorter duration during their initial developmental stages (Martins, Nakagawa, & Bovi, 1999). Popinigs (1985) reported that ESI values are directly correlated to substrate WR capacity. In the present study, vermiculite had higher WR levels (Table 2). Therefore, this higher water availability may provide a better environment for cambuci seed germination and emergence.

Cambuci seedlings sown in the commercial pine bark substrate (T3) had the smallest mean stem diameter (Table 3), similar to the findings reported by Caldeira et al. (2012) using teak (*Tectona grandis*). According to Gomes and Silva (2004), stem diameter is an extremely important variable for estimating survival rates of forest species because it indicates greater reserve levels, which ensure higher plant resistance and better soil fixation (Sturion & Antunes, 2000). In this study, the cambuci seedlings did not develop enough during the 120-day experimental period to be planted into the field, as only steam diameter values ranging from 5 to 10 mm are considered optimal for forest species to be planted into the field (Gonçalves, Santarelli, Moraes Netto, Manara, & Stape, 2000).

Table 3. Means of shoot system variables of cambuci seedlings grown in different substrates. Piracicaba, São Paulo State, Brazil, 2018.

Substrates	Shoot system variables						
	ESI*	H (cm) ^{ns}	D (mm)*	NL (l sd^{-1}) *	LAI (cm^{2}) ^{ns}	SSFW (g) ^{ns}	SSDW (g) ^{ns}
Pine bark substrate	0.20 b	6.64 a	1.52 c	7.85 a	11.31 a	0.246 a	0.067 a
Sand	0.18 b	6.36 a	1.61 bc	8.12 a	10.79 a	0.236 a	0.063 a
Peat + perlite	0.21 ab	6.65 a	1.70 a	8.18 a	11.23 a	0.256 a	0.067 a
Vermiculite	0.25 a	6.78 a	1.68 ab	8.26 a	9.41 a	0.231 a	0.059 a
Soil	0.19 b	6.52 a	1.63 ab	6.66 b	9.61 a	0.235 a	0.062 a
Means	0.19	6.59	1.63	7.81	10.47	0.240	0.063
P-value	0.0001	0.3905	<0.0001	<0.0001	0.0167	0.309	0.1547
CV (%)	7.05	18.09	11.79	19.67	37.15	29.82	31.84

(*)Means followed by the same letters in the columns are not significantly different according to Tukey's test (P-value < 0.05). Caption: Coefficient of variation (CV), emergence speed index (ESI), height (H), stem diameter (D), number of leaves (NL), leaf area index (LAI), shoot system fresh weight (SSFW), and shoot system dry weight (SSDW).

The mean number of leaves for seedlings sown in soil (T5) was less than seven leaves per seedling, which was statistically different from the other substrates (Table 3). By emerging rapidly, the shoot seedling system can soon begin photosynthesis and other physiochemical processes (Taiz & Zeiger, 2010). The low number of leaves observed in the seeds sown in the soil substrate may be due to the lower ESI observed for this substrate.

As stated previously, all root system variables were statistically different among treatments (Table 4). Overall, the means of the root system variables for the commercial pine bark (T1) and peat + perlite (T3) substrates were higher than those of sand (T2) and vermiculite (T4), whereas, soil (T5) presented intermediate values.

The intermediate trend for soil (T5) was noted for several variables, such as RSL, RSSA, RSV, and RSFW, whose means were significantly different from those obtained for pine bark (T1) and/or peat + perlite (T3), whereas RSAD and RSDW were not significantly different from those obtained for pine bark (T1) and/or peat + perlite (T3) (Table 4).

Table 4. Means of root system variables of cambuci seedlings grown in different substrates. Piracicaba, São Paulo State, Brazil, 2018.

Substrates	Root system variables					
	RSL (cm)*	RSAD (mm)*	RSSA (cm^2)*	RSV (cm^3)*	RSFW (g)*	RSDW (g)*
Pine bark substrate	159.83 a	0.368 a	18.499 a	0.171 a	0.327 a	0.038 a
Sand	112.07 b	0.319 c	11.133 b	0.088 b	0.178 c	0.027 cd
Peat + perlite	145.84 a	0.363 a	16.648 a	0.152 a	0.245 b	0.035 ab
Vermiculite	122.18 b	0.334 b	12.830 b	0.108 b	0.181 c	0.025 d
Soil	112.23 b	0.357 a	12.264 b	0.107 b	0.159 c	0.031 bc
Mean	130.43	0.348	14.275	0.129	0.218	0.0312
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
CV (%)	31.77	8.88	31.38	33.12	33.66	32.31

(*)Means followed by the same letters in the columns are not significantly different according to Tukey's test (P-value < 0.05). Caption: Coefficient of variation (CV), root system length (RSL), root system average diameter (RSAD), root system superficial area (RSSA), root system volume (RSV), root system fresh weight (RSFW), and root system dry weight (RSDW).

Substrates must ensure optimal seed germination and seedling development conditions, and certain characteristics such as low density, WR, aeration, and drainage are essential for the same (Kämpf, 2000; Wendling, Gatto, Paiva, & Gonçalves, 2002). Overall, cambuci seedlings displayed better root development in substrates containing higher organic carbon levels, intermediate WR capacity, and low-density (Table 2); the commercial pine bark substrate (T1) and peat + perlite mixture (T3) provided these conditions.

According to Scalon and Jeromine (2013), substrate organic matter levels increase water drainage, and consequently, aeration. Nevertheless, increased aeration and drainage cannot be considered to be beneficial for seedling development under all circumstances. For example, the seedlings of several Myrtaceae family species, such as 'cagaita' (*Eugenia dysenterica*) which is a native Brazilian savanna species, develop better in soils containing no organic matter (Sobrinho et al., 2010) because their habitat commonly lacks organic matter in the soil (Scalon & Jeromine, 2013). Moreover, 'uvaia' seedlings (*Eugenia pyriformis*), a species belonging to the same botanical family as cambuci, have been reported to develop better in both sand and vermiculite (Medeiros, Costa, Curi, Moura, & Tadeu, 2010).

Regarding WR and density values, Pio et al. (2005) observed that a commercial substrate with similar density values to the commercial pine bark substrate (T1) applied herein produced better quality 'Sabará' jaboticaba seedlings. In contrast, sand (T2), which exhibited lower WR and organic matter values, produced inferior cambuci seedlings, consistent with the findings reported by Danner et al. (2007) for jaboticaba seedlings (*Plinia* sp.).

The associations between the evaluated variables and the substrates employed were displayed in the PCA. Three principal components (PC I, PC II, and PC III) explained 93.26% of the total variance in the data.

The variables with the highest weights in PC I were LAI, RSL, RSSA, RSAD, RSV, SSDW, and RSDW, whereas ESI, H, D, and NL were the most significant in PC II; in PC III a contrast was observed between NL and LAI versus H and RSAD. To facilitate PCA and cluster plot data visualization, PC I, II, and III were named 'general seedling aspects', 'emergence and shoot system' and 'foliar aspects vs. plant height and root diameter, respectively. The PC plots were combined (I × II, I × III, and II × III) to indicate similarities on a multivariate level.

CA based on the average method was then applied to cluster each treatment based on their similarities.

The I × II plot explained most of the variance (79.05%) in data (Figure 2A). The first and fourth quadrants in this plot comprised better 'general seedling aspects' obtained in the different treatments, which included the commercial pine bark substrate (T1) and peat + perlite mixture (T3) in this case, although both treatments exhibited intermediate 'emergence and shoot system' values.

In contrast, seedlings grown in sand (T2), vermiculite (T4), and soil (T5) were inferior. However, the vermiculite substrate (T4) was displaced in the plot owing to higher ESI values, which is an important variable for PC II, as discussed previously.

The second plot, I × III, which explained 67.75% of the observed variance, also reinforced better-quality seedling growth in the commercial pine bark substrate (T1) and peat + perlite mixture (T3) than in sand (T2), vermiculite (T4), and soil (T5) (Figure 2B). Finally, the third plot (II × III) is not discussed because it explained very low percentage of variance (39.91%) (Figure 2C).

CA indicated that sand (T2) and vermiculite (T4) were the most similar substrates and formed a single cluster, followed by the commercial pine bark substrate (T1) and peat + perlite mixture (T3), which formed a second cluster. The soil (T5) was added to the first cluster. The dendrogram (Figure 2D) confirmed the previously discussed univariate analysis trends; the treatments showing the highest (T1 with T3) and lowest (T2 with T4) means were grouped separately. Finally, although soil (T5) exhibited some parameters with higher means, it was still placed in the lower value group.

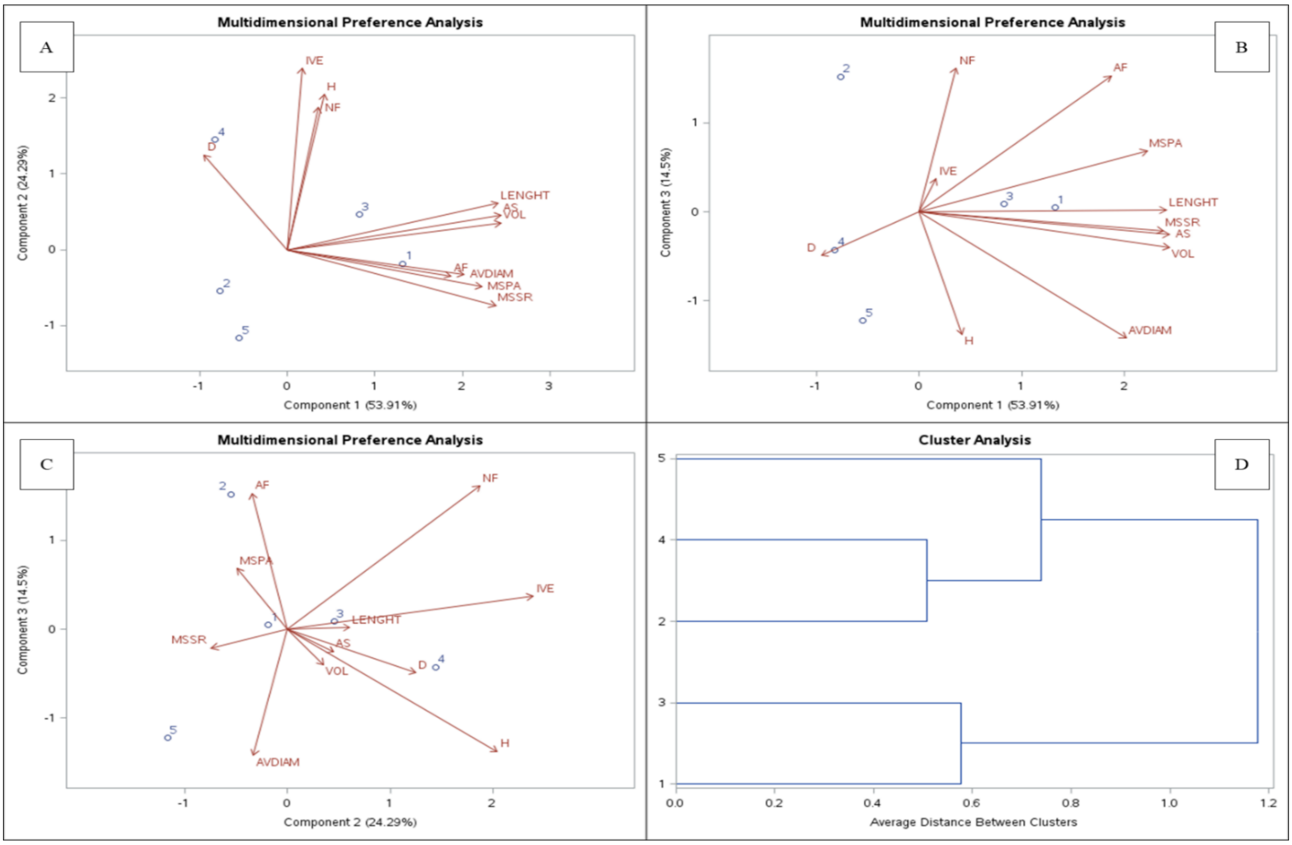

Figure 2. Variable distribution and influence of each applied treatment: (A) PC I and PC II; (B) PC I and PC III; (C) PC II and PC III; (D) Cluster analysis based on multivariate distances. Caption: (1) Pine bark substrate, (2) Sand, (3) peat + perlite (1:1, v/v), (4) vermiculite, and (5) soil.

Notably, root system variables were decisive for the multivariate analysis, comprising the main similarity factor among all tested substrates due to their PCA weights, especially PC I (data not shown).

Experiment 2

All variables evaluated in this experiment showed statistically significant differences. Notably, some variables were transformed to satisfy the statistical analysis assumptions.

Seedling emergence began at 60 DAS, and significant differences were observed among the treatments (Figure 3). Overall, seeds grown in 260 cm^3 containers exhibited higher ESI values (Table 5) and, consequently, faster emergence rates than those grown in other containers. However, no differences were observed in emergence upon comparing the tested substrates.

The ESI values obtained in this experiment were lesser than those obtained in experiment 1 and reported in the literature (Santoro et al., 2020). The ESI values for the substrate mix were expected to be higher than those of the commercial substrate owing to specific physical characteristics, such as lightness, slightly acidic pH, and high WR capacity of peat (Ristow, Antunes, & Carpendo, 2012) associated with perlite porosity (Bortolozzo et al., 2007). However, this was not the case.

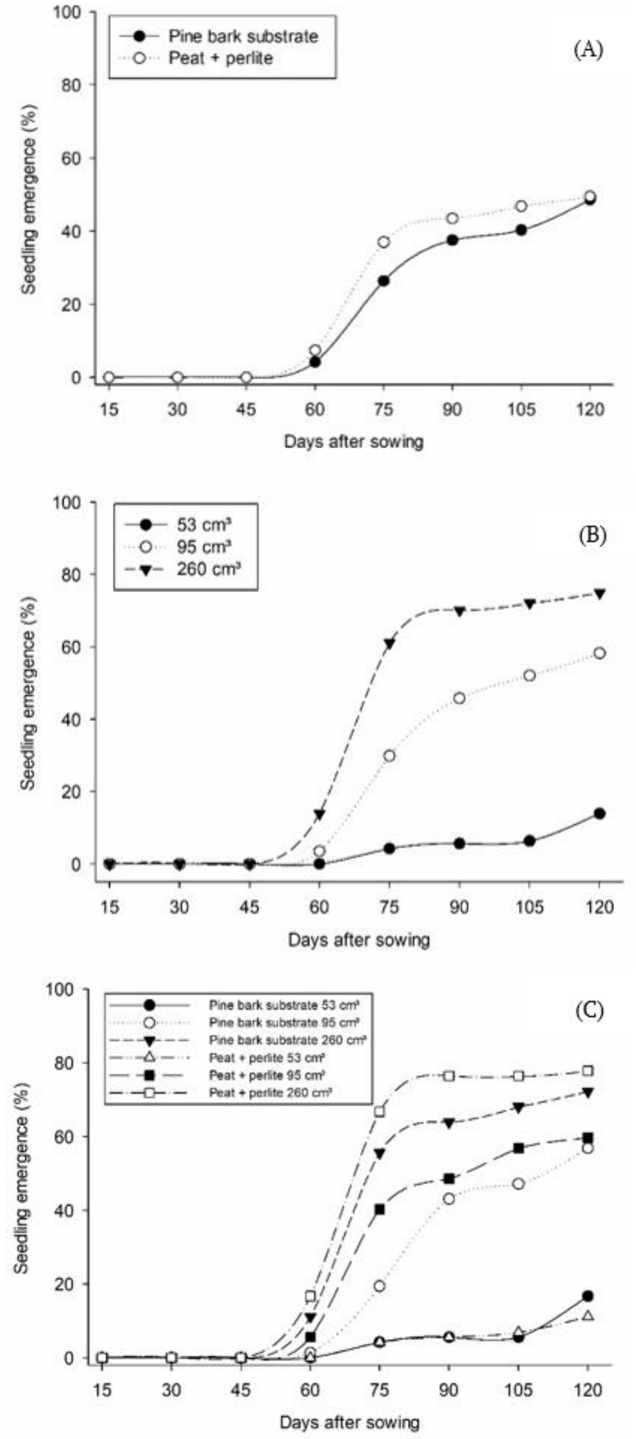

Figure 3. Mean fortnight percentages of cambuci (*Campomanesia phaea* O. Berg. Landrum) seedling emergence (%) employing different substrates (A), containers (B), and their combinations (C) for 120 days. Piracicaba, São Paulo State, Brazil, 2019.

Table 5. Emergence speed index (ESI) of cambuci seedlings sown in different substrates and containers. Piracicaba, São Paulo State, Brazil, 2019.

Variable	ESI*			
Substrate	Container (cm³)			Mean
	53	95	260	
Pine bark substrate	0.03 c	0.12 b	0.19 a	0.12
Turfa + perlite	0.02 c	0.15 b	0.21 a	0.12
Mean	0.03 c	0.13 b	0.20 a	0.12
P-value	<0.001			
CV (%)	21.62			

(*)Means followed by the same letters (lowercase in columns and uppercase in rows) do not significantly differ based on Tukey's test (P-value < 0.05).

The 260 cm^3 container, regardless of the substrate, resulted in better seedling aspects for most of the assessed variables, *i.e.,* higher SSDW, NL, LAI, H, RSL, RSSA, and RSV values. However, the RSAD was lower and the NL did not differ from those observed in the 53 cm^3 containers (Tables 6 and 7).

Table 6. Means of shoot system variables of cambuci seedlings sown in different containers and substrates. Piracicaba, São Paulo State, Brazil, 2019.

Variable	H (cm) *				D (mm)*3			
Substrate	Container (cm^3)			Mean	Container (cm^3)			Mean
	53	95	260		53	95	260	
Pine bark substrate	5.62 Bb	6.60 Aa	6.83 Aa	6.36 A	1.61	1.51 B	1.56	1.56
Peat + perlite	5.18 Bb	5.25 Bb	5.99 Ba	5.50 B	1.61 ab	1.66 Aa	1.56 b	1.60
Mean	5.41 c	5.92 b	6.40 a	5.92	1.61	1.58	1.56	1.58
P-value	<0.001				0.004			
CV (%)	21.48				22.68			
Variable	LAI (cm^2) *1				NL[2]			
Substrate	Container (cm^3)			Mean	Container (cm^3)			Mean
	53	95	260		53	95	260	
Pine bark substrate	6.75 Bc	9.37 Ab	12.83 Aa	9.61 A	6.90	7.21	7.40	7.17
Peat + perlite	6.75 Bb	6.60 Bb	10.53 Ba	8.08 B	7.36	6.95	7.64	7.31
Mean	6.75 c	7.97 b	11.56 a	8.82	7.12 ab	7.08 b	7.53 a	7.24
P-value	<0.001				0.0081			
CV (%)	35.67				53.50			
Variable	SSFW (g)*				SSDW (g) *1			
Substrate	Container (cm^3)			Mean	Container (cm^3)			Mean
	53	95	260		53	95	260	
Pine bark substrate	0.243 Bc	0.321 Ab	0.411 Aa	0.324 A	0.058	0.062	0.071	0.063 A
Peat + perlite	0.232 Bb	0.245 Bb	0.311 Ba	0.265 B	0.049	0.055	0.073	0.059 B
Mean	0.237 c	0.283 b	0.355 a	0.294	0.054 c	0.058 b	0.072 a	0.062
P-value	<0.001				<0.001			
CV (%)	26.92				15.82			

(*)Means followed by the same letters, lowercase in rows and uppercase in columns, do not differ significantly according to Tukey's test (P-value < 0.05). (ns) not statistically significant. (1) Data transformed as: $f(x) = \sqrt{x}$. (2) Friedman's non-parametric test. (3) Data transformed as $f(x) = x^2$. Caption: Shoot system fresh weight (SSFW), shoot system dry weight (SSDW), number of leaves (NL), leaf area index (LAI), height (H), and stem diameter (D).

Meanwhile, the peat and perlite combination in the 95 cm^3 styrofoam containers resulted in larger stem diameters (D) compared to that in the commercial substrate in the same container. These findings differ from those of other studies on jaboticaba (*Plinia* sp.), in which seedling growth in larger containers resulted in higher diameter values (Danner et al., 2007).

According to Malta, Oliveira, Almeida, and Santos (2017), larger containers ensure better water and nutrient uptake, and consequently, good shoot system development. In the present study, both the LAI and SSFW were higher when grown in 260 cm^3 containers, and the highest means were obtained for the pine bark substrate.

Souza et al. (2001) reported that 'cagaiteira' (*Eugenia dysenterica* DC.) seedlings exhibit better shoot system development when sown in larger containers using a substrate mixture supplemented with a chemical fertilizer.

Cambuci seedlings grown in 260 cm^3 containers using the commercial pine bark substrate showed the highest RSL, RSSA, and RSV values compared to those of all other treatment combinations. Nevertheless, the highest RSAD means was noted for seedlings grown in 53 cm^3 containers (Table 7). Containers act as physical barriers for the root system; hence, container size limits the length of roots, and consequently, their surface area, whereas, it results in increased root diameter.

Overall, larger containers were found to be beneficial for both shoot and root system development of cambuci seedlings. Additionally, most of the assessed variables were higher in seedlings grown on commercial pine bark substrate.

In addition to being a fruit crop, the cambuci tree is also a forest species, and 260 cm^3 containers are generally commercially employed for this species.

Table 7. Means of root system variables of cambuci seedlings sown in different containers and substrates. Piracicaba, São Paulo State, Brazil, 2019.

Variables	RSFW (g) [*4]				RSDW (g) [*1]			
Substrate	Container (cm^3)			Mean	Container (cm^3)			Mean
	53	95	260		53	95	260	
Pine bark substrate	0.123 Ba	0.087 Bb	0.109 Bab	0.105 B	0.022 a	0.021 b	0.026 a	0.023
Peat + perlite	0.134 Bab	0.094 Bb	0.165 Aa	0.131 A	0.026 a	0.021 b	0.024 a	0.023
Mean	0.128 a	0.091 b	0.140 a	0.118	0.024 a	0.021 b	0.025 a	0.023
P-value	<0.001				<0.001			
CV (%)	13.42				19.11			
Variables	RSL (cm) [*1]				RSSA (cm^2) [*1]			
Substrate	Container (cm^3)			Mean	Container (cm^3)			Mean
	53	95	260		53	95	260	
Pine bark substrate	90.16 Ac	124.12 Ab	161.83 Aa	125.08 A	9.78 Bc	12.32 Ab	15.81 Aa	12.56 A
Peat + perlite	79.29 Aa	80.57 Ba	97.17 Ba	86.27 B	10.32 Bab	8.69 Bb	11.28 Ba	10.08 B
Mean	84.95 c	102.17 b	126.04 a	105.15	10.04 b	10.49 b	13.26 a	11.28
P-value	<0.001				<0.001			
CV (%)	20.89				20.10			
Variables	RSAD (mm) [*5]				RSV (cm^3) [*1]			
Substrate	Container (cm^3)			Mean	Container (cm^3)			Mean
	53	95	260		53	95	260	
Pine bark substrate	0.35 Ba	0.32 Bb	0.33 Ba	0.33 B	0.085 b	0.098 Ab	0.132 Aa	0.104
Peat + perlite	0.42 Aa	0.34 Ac	0.37 Ab	0.37 A	0.108 a	0.075 Bb	0.105 Ba	0.095
Mean	0.38 a	0.33 c	0.35 b	0.35	0.096 b	0.086 b	0.117 a	0.099
P-value	<0.001				<0.001			
CV (%)	8.96				20.98			

[*]Means followed by the same letters, lowercase in rows and uppercase in columns, do not differ significantly according to Tukey's test (P-value < 0.05). [ns] not statistically significant. [1] Data transformed as: $f(x) = \sqrt{x}$. [4] Data transformed as: $f(x) = \sqrt[4]{x}$. [5] Data transformed as: $f(x) = 1/x$. Caption: Root system fresh weight (RSFW), root system dry weight (RSDW), root system volume (RSV), root system length (RSL), root system surface area (RSSA), and root system average diameter (RSAD).

Conclusion

Vermiculite leads to early emergence of cambuci seedlings; however, the commercial pine bark substrate and peat and perlite mixture ensure better root and shoot system quality. Overall, 260 cm^3 containers combined with the above-mentioned substrates provided a better environment for the development of cambuci seedling.

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