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Vermiculite and shading in the formation of 'Rangpur' lime rootstock

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Abstract

Rangpur' lime (*Citrus limonia* Osbeck cv. *Cravo*) is widely used as a rootstock for citrus seedlings, although factors such as luminosity and substrate composition are extremely important to obtain quality seedlings. The objective of this study was to analyze the formation of 'Rangpur' lime seedlings grown in different substrates and luminosities. A 3x4 factorial arrangement was used in an RBD with four replications of twelve plants. Three luminosities (full sun, 35% and 70% shading) and four substrate: vermiculite proportions were tested (S1-1: 0, S2-2: 1, S3-1: 2, and S4-0: 1). The seeds were sown in 280 cm^3 tubes and emergence was evaluated every two days for 30 days, and biometric assessments at 30, 60, 90, and 120 DAE by measuring the height, diameter, leaves, and mortality. Root length, shoot and root dry mass were measured at 30 and 120 days. Seeds in full sun showed high ESI values and a shorter time period, as well as high mortality at 120 DAE. Highly shaded environments cause etiolation and reduce biomass accumulation and the DQI. Seeds in S4 showed lower results for most variables. It is recommended to use 35% shading associated with the substrate proportions S1, S2, and S3.

Keywords: *Citrus limonia* Osbeck cv. *Cravo*, luminosity, radiation, seedlings, substrate

Introduction

Citriculture has both global and national importance, with Brazil being the largest fruit producer and the largest exporter of concentrated orange juice in the world (Souza et al., 2015). In this context, orange stands out as a fruit with high nutritional value, rich in sugars, acids, polysaccharides, and phytochemicals such as vitamin C and carotenoids, in addition to providing benefits due to the presence of natural antioxidants (Hendre et al., 2020).

Currently, most citrus plants produced in the country come from grafted seedlings, in greenhouses. The cultivation of citrus seedlings in protected environments favors the production of plants with high genetic and sanitary conditions (Nascimento et al., 2018). However, knowledge about germination ecophysiology and early seedling growth is essential to successfully produce quality seedlings (Mota et al., 2012). Thus, substrate formulation and appropriate shading in protected environments stand out as important factors to increase seedling quality (Silva et al., 2018).

The ideal substrate should provide nutrients, be made of light and porous materials, and have physical and chemical properties appropriate to the needs of the species to be propagated (Arrua et al., 2016). Usually, substrates that consist of a mixture of more than one material are more likely to meet the chemical and physical needs of plants (Silva et al., 2018). Thus, vermiculite has been widely used in substrates, although knowing its correct proportion is essential to ensure seedling quality and low production costs (Cruz et al., 2020).

Another important factor is the use of protected environments to harbor seedlings during early development since, in this stage, seedlings are sensitive to strong winds and high radiation (Arrua et al., 2016). Light intensity has a direct effect on seed germination,

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emergence, growth, and survival as well as on seedling formation (Martins et al., 2014). Shade nets are an alternative to reduce the thermal amplitude and total solar radiation on plants, resulting in increased production in some species, among them fruit trees (Silva et al., 2020). Currently, the production of seedlings directly in full sun is an option due to its superior performance, as less photooxidative damage results in a relatively low mortality rate and better growth after transplanting, when compared to seedlings grown in the shade (DaMatta et al., 2018).

Therefore, radiation-protected environments associated with suitable substrates provide an excellent seedling standard for field use, forming more uniform and productive orchards (Anjos et al., 2017). Currently, studies on the interaction between shading and substrate composition in the formation of seedlings are being carried out. Salles et al. (2019) observed significant interaction in the papaya seedling production under different shading levels and substrate compositions. Arrua et al. (2016) studied two environments and different substrates in mangaba seedlings. Anjos et al. (2017) observed different behavior of sweet pepper in different substrates and light environments. However, the effect of the interaction between luminosity and substrate in the formation of citrus seedlings is still little known.

Given the above, the authors aimed to evaluate the effect of different proportions of commercial substrate and vermiculite associated with three light intensities on the development of 'Rangpur' lime seedlings for rootstock production.

Materials and Methods

Local and Material

The experiment was carried out using 'Rangpur' lime seeds obtained by free pollination from an orchard located in Venda Nova do Imigrante - ES, Brazil (20° 20' 31.6" S and 41° 07' 39.8" W), which were taken to the Seed Analysis Laboratory of the Center for Agricultural Sciences and Engineering of the Universidade Federal do Espírito Santo (CCAE-UFES), in Alegre - ES, Brazil. The assembly was performed in a shade house located near the Seed Analysis Laboratory, whose daily temperature and radiation averages (Figure 1) were recorded during the experimental period. According to the Köppen climate classification, the experimental area (20° 75' S and 41° 49' W) has a Humid Tropical Climate (Aw), an elevation of 129 m, hot and humid summers, and an average rainfall of 1,200 mm year^{-1}.

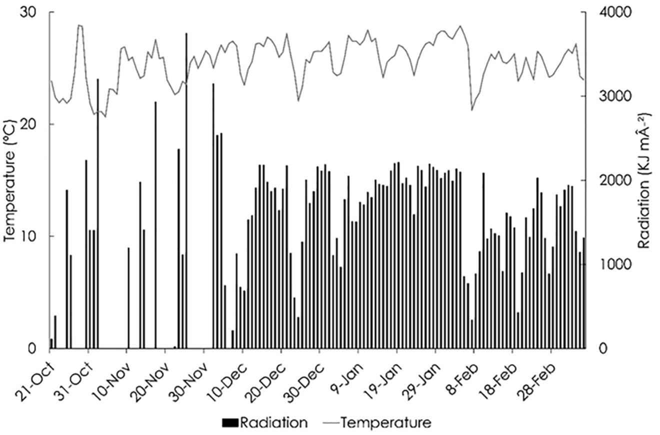

Figure 1. Average temperature and radiation during the experimental period (10/21/2020 to 01/9/2021). Source: INMET (2021)

Experimental design and Seeding

The treatments were conducted in a 3x4 factorial arrangement in an RBD with four replications of twelve plants. Treatments consisted of different luminosities obtained with black polyolefin fabrics with 50% light retention: L1 (full sunlight), L2 (35% shading), and L3 (70% shading), and four substrates: S1, S2, S3, and S4, which were put into 280 cm^3 tubes. Sowing was performed on October 21, 2020, using three seeds per tube at a depth of approximately 15 mm.

The substrates used were: Tropstrato HT®, composed of pine bark and vermiculite, PG Mix® 14.16.18, potassium nitrate, single superphosphate, and peat, whose chemical analysis revealed: pH (H_2O): 5.00; Al^{3+}: 0.50; Ca^{2+}: 15.00; Mg^{2+}: 5.00; Na^+: 0.21; K^+: 1.02 $cmol_c$ dm^{-3}; P: 111.00 mg dm^{-1}; C: 55.80, and OM: 96.20 g kg^{-1}; and coarse vermiculite, with a CEC of 200 mmolc kg^{-1} and 100% water retention capacity. Treatment composition varied according to the different substrate: vermiculite proportions: S1 (Tropstrato 1: vermiculite 0); S2 (Tropstrato 2: vermiculite 1); S3 (Tropstrato 1: vermiculite 2); and S4 (Tropstrato 0: vermiculite 1).

Irrigation was provided daily to maintain substrate moisture close to field capacity. Thinning was performed thirty days after emergence, leaving only one plant per tube.

Analysis

The variables analyzed were: emergence (E%), calculated at 30 days after sowing as the ratio between the number of emerged seedlings and the total number of seeds; emergence speed index (ESI), determined concomitantly with the daily emergence of seedlings ≥ 2 mm above the substrate level (Maguire 1962); number of seedlings per seed (NSS), calculated as the quotient between the total number of seedlings and the total number of germinated seeds; and polyembryony rate (PR), calculated as the ratio between the total number of seeds with two or more seedlings and the total number of germinated seeds. The following variables were analyzed at 30, 60, 90, and 120 days after sowing: plant height (H), measured from the substrate level to the apical bud with the aid of a graduated ruler; stem diameter (D), obtained with the aid of a digital caliper; number of leaves per plant (NL), determined by manual counting. The root length (RL), shoot dry mass (SDM), root dry mass (RDM), and total dry mass (TDM) were obtained after 120 days, when the seedlings were packed into Kraft paper bags and dried in a forced-air circulation oven at 60 °C for 72 hours, after determining the fresh mass. Finally, the following values were calculated at the end of the experiment: height/ root length ratio (H/RL), shoot dry mass/ root dry mass ratio (SDM/RDM), height/diameter ratio (H/D), mortality rate (MOR), and the Dickson Quality Index (DQI). The Dickson Quality Index (DQI) was determined according to Equation 1 (Dickson et al., 1960):

$$DQI: \frac{TDM\ (g)}{((H\ (cm))/(D\ (mm)))+((SDM\ (g))/(RDM\ (g)))} \qquad \text{Equation 1}$$

Statistic

For each emergence experiment, the ANOVA assumptions were tested using the Shapiro-Wilk tests for the normality of the residuals, while Bartlett's test was used for the homogeneity between the variances. The analysis of variance was performed when the assumptions were met and, when significant, the Scott-Knott test was used. Regression analysis was used for the emergence and ESI over time. All statistical analyses were performed using the R software (Team 2020).

Results And Discussion

Emergence and emergence speed index

The seeds kept in full sun emerged at a higher rate and speed than those kept in the shade. At 15 days after emergence (DAE), the seeds kept in the shade showed a similar emergence to those kept in full sun (90%) (Figure 2A). Regarding the emergence speed index (ESI), the seeds kept in full sun showed a higher value in a shorter time (12 DAE), while the seeds kept under medium and high shading showed a lower ESI and a later peak of this value, which occurred at 17 DAE (Figure 2B).

These results differed from those in the study conducted by Marçal et al. (2014), who obtained higher emergence rates and ESI in 'Cleopatra' mandarin seeds maintained under shading levels of 55.82 and 51.97%, respectively. Martins et al. (2014) concluded that shadows

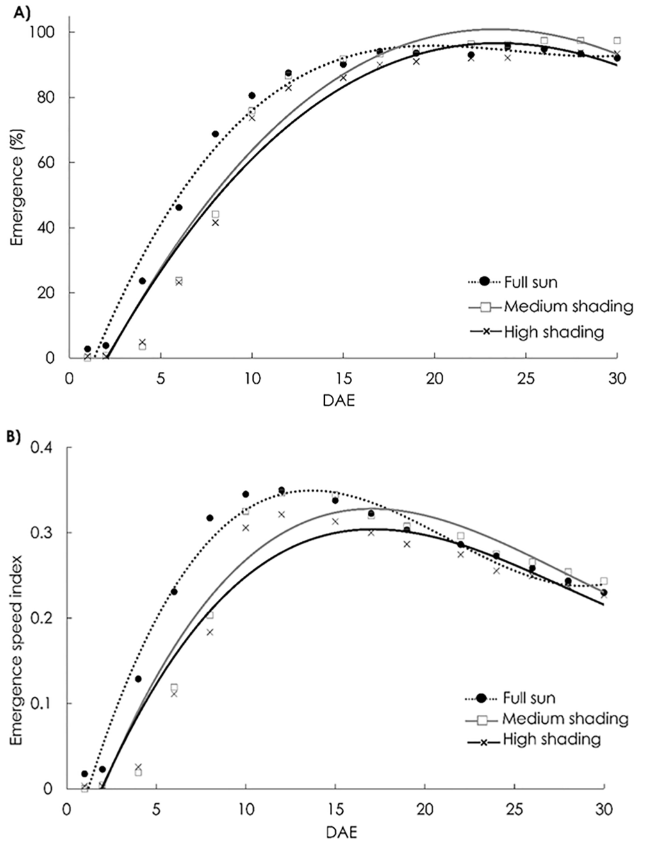

Figure 2. Emergence (A) and emergence speed index (ESI) (B) for 'Rangpur' lime seeds submitted to three luminous intensities, during 30 days.
Emergence: full sun: y = 0.0087x3 - 0.6379x2 + 15.032x - 19.353; R^2 = 0.98*; medium shading (35%): y = 0.0019x3 - 0.3163x2 + 11.667x - 23.164; R^2 = 0.96*; high shading (70%): y = 0.0018x3 - 0.3005x2 + 11.074x - 21.454; R^2 = 0.96*. Emergence speed index: full sun: y = 0.00006x3 - 0.0041x2 + 0.0755x - 0.0851; R^2 = 0.97*; medium shading (35%): y = 0.00003x3 - 0.0026x2 + 0.0605x - 0.1113; R^2 = 0.92*; high shading (70%): y = 0.00003x3 - 0.0023x2 + 0.0553x - 0.0991; R^2 = 0.92*
*Significant at the 5% probability level.

of 30 and 50% favored an increase in the percentage of emergence of citrus rootstocks.

The response to shading is a consequence of the adaptive plasticity of each species, representing an important ability for allowing a wider distribution and greater ease of handling (Mota, Scalon, and Heinz 2012). Furthermore, the substrate protects the seeds against the adverse effects caused by the thermal environment, such as temperature and relative humidity, reducing the effects on seed germination and emergence (Cecco et al., 2018).

Regarding the seeds subjected to the different substrate: vermiculite proportions, treatment S4 (vermiculite only) resulted in lower emergence and ESI. Substrates S1, S2, and S3, which had commercial substrate in their composition, behaved similarly regarding emergence (Figure 3A). For the ESI, these treatments also behaved similarly, although S1 (commercial substrate only) showed a higher mean value (Figure 3B). Vermiculite contributes to the formation of a more aerated substrate and with greater water retention capacity. However, its low nutrient content is compensated by the use of commercial substrates (Cecco et al., 2018). The mixture of vermiculite and substrate improves its physicochemical properties, which is essential for ensuring seedling quality and low production costs (Cruz et al., 2020).

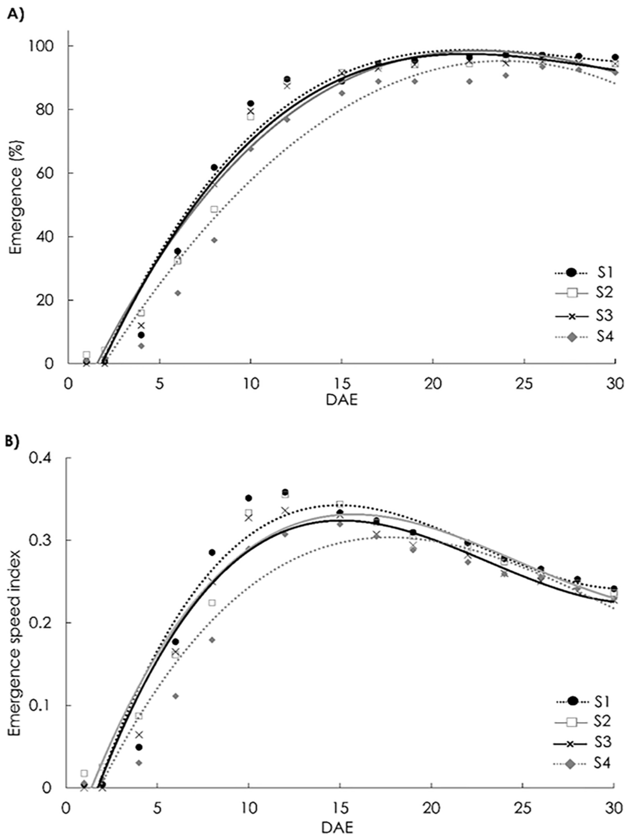

Figure 3. Emergence (A) and emergence speed index (ESI) (B) for 'Rangpur' lime seeds subjected to different substrates, during 30 days. S1(Tropstrato 1: vermiculite 0); S2 (Tropstrato 2: vermiculite 1); S3 (Tropstrato 1: vermiculite 2); S4 (Tropstrato 0: vermiculite 1).

Emergence: S1: y = 0.0066x3 - 0.5475x2 + 14.44x - 24.638; R^2 = 0.97*; S2: 2:1 y = 0.0036x3 - 0.3949x2 + 12.234x - 18.305; R^2 = 0.97*; S3: 1:2 y = 0.0059x3 - 0.5147x2 + 13.973x - 24.094; R^2 = 0.97*; S4: 0:1 y = 0.0003x3 - 0.2159x2 + 9.7171x - 18.257; R^2 = 0.96*. Emergence speed index: S1: y = 0.00005x3 - 0.0037x2 + 0.0734x - 0.116; R^2 = 0.94*; S2: y = 0.00004x3 - 0.0029x2 + 0.0621x - 0.081; R^2 = 0.95*; S3: 1:2 y = 0.00005x3 - 0.0034x2 + 0.0685x - 0.109; R^2 = 0.96*; S4: 0:1 y = 0.00002x3 - 0.0020x2 + 0.0511x - 0.087; R^2 = 0.93*.

*Significant at the 5% probability level.

Biometric characteristics
30 days after emergence

There was no significant effect of neither the individual treatments nor the interaction on seedling emergence at 30 days after the first plant emerged (Table 1). The polyembryony rate (PR) and the number of seedlings per seed (NSS) were only influenced by substrate composition, while the H/RL and SDM/RDM ratios were influenced by luminosity, and the root dry mass (MSR) was influenced by both factors. The height (H), root length (RL), number of leaves (NL), and shoot dry mass (SDM) showed a significant interaction at 5% probability.

The results of the variables whose interaction was not significant for the luminosity factor are shown in Table 2. The emergence, PR, and the number of plants per seed were not influenced by shading. Higher means were obtained in the full sun treatment for RDM, resulting in lower values for the H/RL and SDM/RDM ratios. The RDM of the plants in full sun was 150% higher than those under high shading (70%), corroborating the results obtained by Martins et al. (2014). These authors state that orange rootstocks have mechanisms and the ability to adapt to high luminosity environments while maintaining seedling quality. Regarding the different substrate: vermiculite proportions, higher PR and NSS values were obtained with substrates S2 and S3. The RDM was 44% lower in the treatment composed of vermiculite only (S4). At first, despite differences in the chemical and physical characteristics of the substrates, they may not be able to significantly change the emergence speed and percentage (Schäfer et al. 2005). This is also corroborated by Franco et al. (2007), in whose study no difference was observed for the seedling emergence of citrus rootstocks grown in different substrates.

Table 1. ANOVA F test values for the characteristics analyzed 30 days after emergence.

SDM/RDM		EME	PR	NNS	H	RL	NL	SDM	RDM	H/RL
19.38*	L	2.91^{ns}	0.43^{ns}	0.34^{ns}	15.15*	30.82*	84.37*	22.11*	35.18*	29.73*
0.5^{ns}	S	1.11^{ns}	3.70*	3.26*	10.02*	20.45*	12.00*	5.85*	3.77*	0.63^{ns}
0.44^{ns}	LxS	1.65^{ns}	0.75^{ns}	0.86^{ns}	2.81*	20.45*	6.17*	2.92*	1.13^{ns}	2.26^{ns}
26.3	CV(%)	6.95	9.65	9.59	17.11	12.44	11.49	25.34	28.25	18.95

L: luminosity; S: substrate; LxS: luminosity x substrate; CV: coefficient of variation.

EME: emergence; TP: polyembryony rate; NNS: number of seedlings per seed; H: height; RL: root length; NL: number of leaves; SDM: shoot dry mass; RDM: root dry mass; H/RL: height/root length ratio; SDM/RDM: shoot dry mass/ root dry mass ratio.

nsNot significant at the 5% probability level; *Significant at the 5% probability level.

Table 2. Emergency (EME); polyembryony rate (PR); number of seedlings per seed (NNS); root dry mass (RDM); height/root length ratio (H/ RL) and shoot dry mass/root dry mass (SDM/RDM) ratio for 'Rangpur' lime plants submitted to three light intensities and different substrates, 30 days after the emergency.

L	EME (%)	PR (%)	NNS	RDM (g)	H/RL	SDM/RDM
SP	92.01	18.12	1.24	0.015 a	0.29 b	3.25 b
MS	93.41	17.37	1.22	0.011 b	0.42 c	3.44 a
HS	97.39	19.74	1.25	0.006 c	0.49 a	3.37 a
S	EME (%)	PR (%)	NNS	RDM (g)	H/RL	SDM/RDM
S1	96.52	15.75 b	1.19 b	0.011 a	0.37	4.55
S2	94.44	21.51 a	1.29 a	0.012 a	0.41	2.90
S3	94.46	22.22 a	1.29 a	0.012 a	0.39	3.14
S4	91.67	14.15 b	1.17 b	0.008 b	0.43	2.82

L: luminosity; FS: full sun; MS: medium shading (35%); AS: high shading (70%).

S: substrate; S1(Tropstrato 1: vermiculite 0); S2 (Tropstrato 2: vermiculite 1); S3 (Tropstrato 1: vermiculite 2); S4 (Tropstrato 0: vermiculite 1).

Means followed by the same letter in the same column not different from each other by the Scott-Knott test at a level of 5% probability.

The number of embryos and polyembryony are determined by several factors, such as the cultivar, the pollinator species, the nutritional status of the parent plant, and water availability (Rodrigues et al., 1999). Therefore, it can be inferred that the higher polyembryony rate and, consequently, the higher number of seedlings per seed obtained with substrates S2 and S3 are related to the improvement of the physicochemical conditions of the substrate, increasing its water retention.

The highest height means were obtained with medium shade and substrates S2 and S3 (Table 3). There was no significant difference between the substrate proportions used in full sun. Under 70% shading, the best result was obtained with the S3 treatment. There was a direct relationship between increased seedling height and increased shading, in agreement with the studies conducted by Marçal et al. (2014) and Martins et al. (2014). Regarding the substrate, Guimaraes et al. (2011) observed higher height and RL in the plants of *Erythrina velutina* subjected to a mixture of commercial substrate and vermiculite instead of the isolated use of each component, which is probably related to substrate moisture and aeration, promoting greater seedling growth, development, and vigor.

Table 3. Height (H), root length (RL), number of leaves (Nl) and shoot dry mass (SDM) for 'Rangpur' lime plants submitted to three light intensities and different substrates, 30 days after the emergency.

S	H (cm)			RL (cm)		
	FS	MS	HS	FS	MS	HS
S1	4.08 Aa	5.51 Aa	4.79 Ba	14.57 Aa	14.14 Aa	10.51 Bb
S2	4.27 Ab	6.57 Aa	3.94 Bb	14.87 Aa	13.56 Aa	8.58 Cb
S3	4.33 Ab	6.34 Aa	5.77 Aa	14.71 Aa	13.82 Aa	13.48 Aa
S4	3.21 Aa	3.76 Ba	4.17 Ba	10.43 Ba	11.25 Ba	6.58 Cb
S	NL			SDM (g)		
	FS	MS	HS	FS	MS	HS
S1	5.00 Aa	3.75 Ab	2.28 Ac	0.036 Aa	0.017 Bb	0.041 Aa
S2	5.00 Aa	4.06 Ab	2.30 Bc	0.037 Aa	0.015 Bb	0.044 Aa
S3	4.62 Aa	3.62 Ab	3.41 Bb	0.029 Aa	0.031 Aa	0.037 Aa
S4	3.43 Ba	3.00 Ba	2.50 Bb	0.023 Aa	0.016 Bb	0.025 Ba

L: luminosity; FS: full sun; MS: medium shading (35%); AS: high shading (70%).

S: substrate; S1(Tropstrato 1: vermiculite 0); S2 (Tropstrato 2: vermiculite 1); S3 (Tropstrato 1: vermiculite 2); S4 (Tropstrato 0: vermiculite 1).

Means followed by the same letter in the same column not different from each other by the Scott-Knott test at a level of 5% probability.

The use of the vermiculite-based substrate resulted in shorter root length regardless of the luminosity to which the plants were subjected (Table 3). Longer root lengths were obtained in full sun and medium shading in treatments S1, S2, and S3, whereas, in high shading, substrate S3 provided the best result. The seedlings that emerged from seeds kept in full sun in substrates S1, S2, and S3 showed a higher number of leaves, whereas, under high shading, there was a reduction in this variable of up to 55% per plant regardless of the substrate. However, for SDM, the treatments corresponding to full sun and high shading (except S4) resulted in higher means. Pure vermiculite provided worse NL and SDM results. This reduction in the number of leaves was also observed by Cruz et al. (2020) with eucalyptus, in which the treatment consisting of 75% vermiculite, 25% soil, and daily irrigation frequency resulted in fewer leaves. In fruits *Passiflora alata*, the highest mean values of shoot biomass were found in seedlings developed under low light intensities (Freitas et al., 2015). However, Martins et al. (2014) found no differences for the SDM of 'Rangpur' lime seedlings grown under different light intensities, while greater root, shoot, and total dry matter accumulations were obtained in citrus rootstocks grown with a nutrient-rich commercial substrate (Fochesato et al., 2006).

120 days after emergence

Table 4 shows the significance for the variables analyzed at 120 days after emergence. There was a significant interaction for the H, SD, NL, MOR, SDM, RDM, TDM, H/RL, and DQI.

Table 4. ANOVA F test values for the characteristics analyzed 120 days after emergence.

	H	SD	NL	RL	H/SD	MOR
L	6.75*	148.36*	45.78*	10.47*	66.95*	15.47*
S	30.70*	44.18*	45.48*	2.43*	4.57*	3.64*
LxS	5.35*	16.49*	3.87*	2.02ns	0.58ns	2.70*
CV(%)	10.65	6.59	14.23	3.09	11.94	117.7
	SDM	RDM	TDM	H/RL	SDM/RDM	DQI
L	34.74*	64.09*	67.91*	10.43*	13.99*	68.43*
S	42.92*	20.04*	47.13*	32.59*	6.55*	13.24*
LxS	6.48*	8.68*	10.48*	4.29*	2.02ns	6.86*
CV(%)	20.65	24.14	18.18	10.77	25.72	27.88

L: luminosity; S: substrate; LxS: luminosity x substrate; CV: coefficient of variation.

H: height; SD: stem diameter; NL: number of leaves; RL: root length; H/SD: height/stem diameter ratio; MOR: mortality; SDM: shoot dry mass; RDM: root dry mass; TDM: total dry mass; H/RL: height/root length ratio; SDM/RDM: shoot dry mass/ root dry mass; DQI: Dickson Quality Index.

nsNot significant at the 5% probability level; *Significant at the 5% probability level.

Regarding height and the H/CR ratio, the 'Rangpur' lime plants showed statistically similar means when subjected to substrates S1, S2, and S3 at the three light intensities studied (Table 5). This differs from the behavior up to 90 DAE, showing that elongation occurred during early seedling growth, which may reflect the adaptive plasticity of plants to shading conditions as these variables are dependent on the habitat that the species occupies (Mota, Scalon, and Heinz 2012). (Rodrigues et al., 2015), studying the behavior of several rootstock genotypes, obtained similar values of height and number of leaves under 25% shading.

Table 5. Height (A), stem diameter (SD), number of leaves (NL), mortality (MOR), shoot dry mass (SDM), root dry mass (RDM), total dry mass (TDM), H/RL ratio and Dickson Quality Index (QDI) for 'Rangpur' lime plants submitted to three light intensities and different substrates, 120 days after the emergency.

	H (cm)			SD			NL		
	FS	MS	HS	FS	MS	HS	FS	MS	HS
S1	9.85 Aa	9.86 Aa	9.62 Aa	1.93 Aa	1.45 Ab	1.20 Ac	12.81 Aa	8.75 Ab	7.75 Ac
S2	9.98 Aa	10.36 Aa	8.94 Aa	2.02 Aa	1.42 Ab	1.18 Ac	13.56 Aa	9.12 Ab	7.18 Ac
S3	8.90 Aa	9.72 Aa	9.53 Aa	1.73 Ba	1.35 Ab	1.20 Ac	10.50 Ba	8.25 Ab	7.81 Ab
S4	4.22 Bb	7.88 Ba	7.80 Ba	1.17 Ca	1.23 Ba	1.09 Aa	5.81 Ca	4.56 Ba	4.56 Ba
	MOR			SDM			RDM		
	FS	MS	HS	FS	MS	HS	FS	MS	HS
S1	10.00 Ba	0.00 Aa	5.00 Aa	0.39 Aa	0.27 Ab	0.19 Ab	0.28 Aa	0.19 Ab	0.08 Ac
S2	7.50 Ba	0.00 Aa	7.50 Aa	0.43 Aa	0.32 Ab	0.17 Ac	0.33 Aa	0.16 Ab	0.07 Ac
S3	20.00 Ba	7.50 Ab	2.50 Ab	0.27 Ba	0.24 Aa	0.16 Ab	0.23 Ba	0.13 Bb	0.12 Ab
S4	40.00 Aa	0.00 Ab	10.00 Ab	0.08 Ca	0.15 Ba	0.08 Ba	0.09 Ca	0.11 Ba	0.06 Aa
	TDM			H/RL			QDI		
	FS	MS	HS	FS	MS	HS	FS	MS	HS
S1	0.68 Aa	0.47 Ab	0.27 Ac	0.55 Aa	0.59 Aa	0.58 Aa	3.36 Ba	2.22 Ab	0.88 Ac
S2	0.76 Aa	0.48 Ab	0.25 Ac	0.57 Aa	0.61 Aa	0.54 Aa	4.38 Aa	1.78 Ab	0.81 Ac
S3	0.50 Ba	0.37 Bb	0.28 Ab	0.52 Aa	0.57 Aa	0.58 Aa	3.01 Ba	1.48 Ab	1.34 Ab
S4	0.17 Ca	0.26 Ba	0.14 Ba	0.24 Bb	0.47 Ba	0.44 Ba	1.35 Ca	1.30 Aa	0.66 Aa

L: luminosity; FS: full sun; MS: medium shading (35%); AS: high shading (70%).

S: substrate; S1(Tropstrato 1: vermiculite 0); S2 (Tropstrato 2: vermiculite 1); S3 (Tropstrato 1: vermiculite 2); S4 (Tropstrato 0: vermiculite 1).

Means followed by the same letter in the same column not different from each other by the Scott-Knott test at a level of 5% probability.

The values of stem diameter, NL, SDM, RDM, and TDM behaved similarly, showing the highest means in full sun and with treatments S1 and S2 (Table 5). However, the seedlings in full sun continued to show high mortality, especially when associated with the 100% vermiculite substrate. In full sun environments, the surface layer of the substrate may dry out due to the greater light incidence, leading to water stress (Marçal et al., 2014). Thus, although the 'Rangpur' lime seedlings can grow in full sun, the high mortality rate associated with this environment makes it unfeasible to nurserymen.

The Dickson Quality Index (DQI) was higher in the seedlings grown in full sun and with substrate S2. Shading at 70% provided values lower than 1 for this variable with substrates S1, S2, and S3. There is no ideal DQI value since this index may vary depending on the species, crop management, substrate type and proportion, container volume, and, mainly, the age at which the seedling is evaluated (Caldeira et al., 2013a). However, the use of vermiculite only results in a lower DQI, which is corroborated by Silva et al. (2017), who obtained a linear reduction of the DQI with higher vermiculite proportions in seedlings of timbaúva (*Enterolobium contortisiliquum*) and angico-vermelho (*Parapiptadenia rigida*).

The H/SD ratio (also called the robustness index) was higher at 70% luminosity and with treatments S1, S2, and S3, as shown in Table 6. This ratio represents the plant growth balance and should range from 5.4 to 8.1 (Araújo et al. 2017). Thus, only the seedlings in full sun showed a H/SD ratio lower than ideal (4.66). According to Arrua et al. (2016), stem diameter is an important quality parameter to prevent the seedlings from tipping due to insufficient thickness and height after planting in the field. Therefore, high H/SD ratios represent taller seedlings whose growth is not accompanied by an increase in stem diameter, which may imply the presence of etiolation due to the imbalance in light intensity (Silva et al., 2020). However, one should not use a quality indicator alone to define seedling quality (Silva et al., 2017).

Table 6. Root lenght (RL), height/stem diameter ratio (H/SD) and shoot dry mass/ root dry mass ratio (SDM/RDM) for 'Rangpur' lime plants submitted to three light intensities and different substrates, 120 days after the emergency.

L	RL	H/SD	SDM/RDM
FS	17.53 a	4.66 c	1.21 b
MS	16.88 b	6.92 b	1.28 b
HS	16.73 b	7.67 a	1.98 a
S	RL	H/SD	SDM/RDM
S1	16.96 b	6.58 a	1.76 a
S2	17.03 b	6.61 a	1.96 a
S3	16.80 b	6.76 a	1.51 b
S4	17.41 a	5.72 b	1.25 b

L: luminosity; FS: full sun; MS: medium shading (35%); AS: high shading (70%).

S: substrate; S1(Tropstrato 1: vermiculite 0); S2 (Tropstrato 2: vermiculite 1); S3 (Tropstrato 1: vermiculite 2); S4 (Tropstrato 0: vermiculite 1).

Means followed by the same letter in the same column not different from each other by the Scott-Knott test at a level of 5% probability.

The SDM/RDM ratio was higher in the high luminosity treatments, while treatments S1 and S2 showed a higher value for this variable (Table 6). When analyzing substrate composition, it is seen that a higher RL mean was obtained in substrate S4. Caldeira et al. (2013a) stated that the SDM/RDM ratio is an important quality parameter for seedling evaluation as a shoot mass much higher than the root mass may damage plant support and/or water absorption, with the ideal value being close to 2. The seedlings subjected to 70% shading and substrates S1 and S2 showed better results for this parameter.

Overall, the substrate composed of vermiculite only (S4) showed lower means for most variables, demonstrating that its individual use is harmful to the production of 'Rangpur' lime seedlings. As an inert mineral with variable structure and low density, vermiculite should be used along with another substrate to promote greater aeration and porosity (Caldeira et al., 2013b). However, the use of vermiculite can be disadvantageous depending on the proportion or dose used (Silva et al., 2017).

Principal component analysis

The principal component analysis was performed to assist in interpreting and studying the relationships between the variables (Figure 4). Axis 1 explains 51.11% of the variability, while axis 2 accounts for 30.19%, totaling 81.3% of the total data variation, which is above the level considered adequate for explaining the data (70%). In axis 1, the DQI, SD, SDM, RDM, TDM, and NL variables are strongly correlated. The height and H/RL ratio variables are inversely correlated with MOR, while the H/SD and SDM/RDM ratios are inversely correlated with RL.

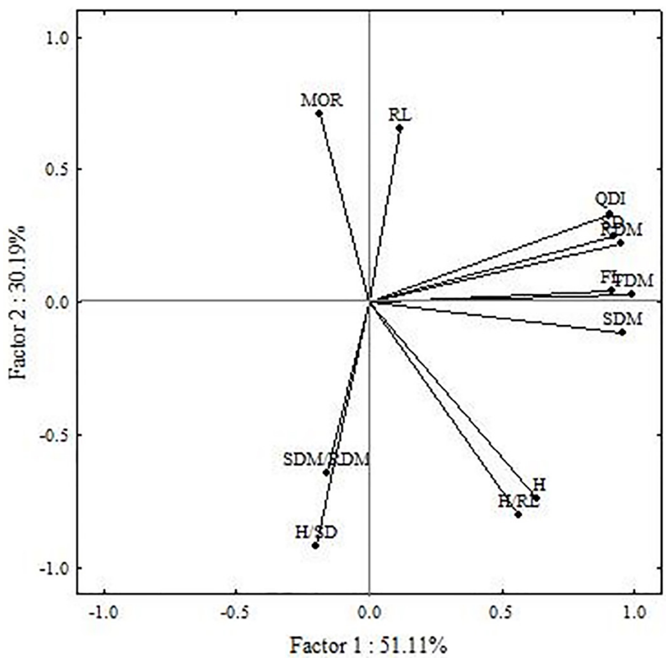

Figure 4. Principal component analysis of 'Rangpur' lime seedlings after 120 days of the emergency.
H: height; SD: stem diameter; NL: number of leaves; RL: root length; H/SD: height/stem diameter ratio; MOR: mortality; SDM: shoot dry mass; RDM: root dry mass; TDM: total dry mass; H/RL: height/root length ratio; SDM/RDM: shoot dry mass/ root dry mass; DQI: Dickson Quality Index.

This result is similar to that found by Camara et al. (2017), who observed that the height, diameter, shoot dry mass, root dry mass, and DQI are correlated in the production of seedlings of *Colubrina glandulosa* seedlings. Araújo et al. (2017) observed that the DQI is related to RDM, while stem diameter is related to SDM in seedlings of paricá (*Shizolobium amazonicum*).

Conclusions

'Rangpur' lime seeds in full sun showed a higher emergence speed index and a shorter time period (12 days), in addition to higher values of root dry mass, number of leaves, and stem diameter (30 DAE). However, high mortality was verified at 120 days after emergence. Thus, the full sun treatment can be used during germination and early seedling formation, although it is not recommended after 30 DAE. High shading environments (70%) lead to increased height, causing seedling etiolation and reducing biomass accumulation, resulting in seedlings with low DQI values.

Regarding the seeds subjected to the different substrate: vermiculite proportions, the S4 treatment (vermiculite only) resulted in lower results for most variables regarding both the emergence and formation of 'Rangpur' lime seedlings.

Based on the correlations obtained for the DQI, SD, SDM, RDM, TDM, and NL variables, 35% shading associated with substrates S1, S2, and S3 is recommended for the formation of 'Rangpur' lime seedlings.

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