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CLONES, SUBSTRATES AND ENVIRONMENTS FOR SEEDLINGS OF RUBBER TREE ROOTSTOCKS

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ABSTRACT: The objective of this study was to evaluate the growth of rubber clones GT1, PR255, RRIM600 grown on different substrates and environments for the production of rubber rootstocks. The experiment was conducted at the Universidade Estadual de Mato Grosso do Sul, Cassilândia University Unit, in Cassilândia-MS. The treatments were conducted in 3 environments: (A1): Sombrite®; (A2) Aluminet® and (A3) full sun, where the clones being tested were: GT1; PR 255 and RRIM 600, grown in substrates: pure soil; soil + cattle manure and commercial substrate usually used for forestry. The experimental design was a randomized block in a factorial 3 x 3 (three substrates x three clones), with 4 replicates with 10 seedlings of rubber trees per plot. The use of commercial substrate and soil + cattle manure in the ratio (7:1) favored the development of rubber rootstock seedlings with taller plants, more leave number, higher mass and higher quality index. For the formation of rootstocks, the variety RRIM600 was the one that showed better agronomic characteristics in the formation of rubber tree seedlings, with higher number of leaves, tallest plants, larger mass and higher quality index. The use of Sombrite® and Aluminet® screens contributed positively to the formation of rootstocks with larger root and above-ground masses. The plants produced in the Aluminet® screen showed higher number of leaves.

KEYWORDS: *Hevea brasiliensis*, GT1, PR255, RRIM600, screens.

INTRODUCTION

Rubber tree [*Hevea brasiliensis* (Willd. ex Adr. de Juss.) Muell.-Arg.] stands out for being the largest source of natural rubber in the world. This raw material that has been required in the production of many essential items, as hospital/pharmaceutical supplies, footwear, construction, agricultural and industrial machinery and auto parts (BRITO et al., 2011). It is a dual purpose crop, with the possible sale of latex and its timber, as well as other advantages such as seed oil extraction for biodiesel manufacturing. In the environmental issue is a species that can be used for areas that need reforestation.

The rubber cultivation is an agricultural activity that requires high initial investment, so the rubber plantation deployment strategy must be well planned, and for its success requires the use of best seedlings (GONÇALVES & BACCHIEGA, 2010). For the best performance in the formation of rubber tree seedlings, researches indicate that the choice of rootstock clone becomes essential for increasing productivity of the plantation.

The substrate is another factor that influences the quality of the seedling and must provide adequate physical, chemical and biological properties, good moisture retention and essential nutrients for full development of seedlings (GONÇALVES, 1995). GARCIA & VIEIRA (1994) evaluating related factors to germination and vigor of rubber seeds found that the proportion of soil + sawdust conventionally used in nurseries plants, resulted in inferior seedlings when compared to sand and vermiculite substrates.

The rubber tree seedlings are grown in nurseries plant installed on ground level, using soil as a substrate and plastic bags as a container. This production system has been replaced due to numerous problems as the difficulty for weed control, lack of selective herbicides for culture; plant site practices, pricking out and grafting that require the intensive use of labor-work in

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uncomfortable position. To address these faults in this production process it is propose the use of suspended nursery plant to a meter from soil (BRASIL, 2009).

The production of seedlings in protected nurseries have advantages over the traditional cultivation in open field, since they have the ability to protect plants against climate adversities and increases the efficient on water use and fertilizers. According to OLIVEIRA et al. (2012), plant ambience aims to identify the best weather conditions on production area and get better seedlings, yields and productivities. FARIA et al. (2013) reported that the use of structures to protect the seedlings is very important when they are well managed, providing high quality plants with better adaptation to the field. COSTA et al. (2010) explain that the different greenhouse environments use mostly the shading screens and plastic film.

Techniques related to the cultivation of rubber tree seedlings using different nurseries plant substrates and clones; assist in the development of culture in terms of technology, thus ensuring seedlings with high potential of fixation for a vigorous and productive plantation, while minimizing costs to the producer with information that helps improve farming practices. Based on the above, this study aimed to evaluate the growth of cultivated rubber tree clones on different substrates and environments for the production of rubber rootstock in the municipality of Cassilândia-MS.

MATERIAL AND METHODS

The experiment was conducted at the State University of Mato Grosso do Sul - UEMS, University Unit of Cassilândia- UUC, located at latitude -19.1225º (= 19º07’21” S), longitude -51.7208º (= 51º43’15” W) and altitude of 516 m (Automatic Station CASSILANDIA-A742). According to the climatic classification of Köppen the region has rainy tropical climate (Aw) with rainy summer and dry winter (winter precipitation less than 60 mm). The soil of the experimental area was classified as Quartz-sand neosol.

Experiments were performed with rubber rootstock seedlings comparing substrates and clones in three environments. The substrates used were: pure soil; soil + cattle manure in the proportion of 7: 1 and commercial substrate for forest crops with chemical analysis (Table 1). It was evaluated the GT1, PR255 and RRIM 600 clones. The following environments were used: 1) a nursery plant with Sombrite® featuring galvanized steel frame, having 8.00 m wide by 18.00 m long and 3.50 m high, side closing at 45 degree angle, with black screen in all its extension, mesh with 50% shade; 2) a nursery plant with aluminized screen term-reflective Aluminet®, 50% shading, and side locks at 90 degree angle with black screen 50% shading, 3) unprotected environment (open air), considered full sun. Because there is no repetition of cultivation environments, each was considered an experiment.

For each environment was used experimental design in randomized blocks in a factorial 3 x 3 (three substrates and three varieties), with four replications of ten plants per plot.

Seeds of GT1, PR255 and RRIM600 clones were obtained from multi-clonal plantations belonging to Continental Farm, located in the municipality of Colombia - SP, collected in February 2014. After collecting, the seeds were stored in plastic polyethylene bags with small holes to avoid losing their germinating power during transport. Sowing was done in germination beds, and the seeds covered with sawdust, without fertilization. When the seedlings have reached the stage "stick" (characterized by the presence of aerial parts and about 3.0 cm tall) they were transplanted into plastic bags with dimensions of 15x33 cm with 2.36 liters volume. Subsequently, the plants were transferred to the three environments.

TABLE 1. Chemical characterization of substrates for cultivation *Hevea brasiliensis* rootstock. Cassilândia-MS, 2014.

Chemical	**pH**	**K^+**	**Ca^{+2}**	**Mg^{+2}**	**H+Al**	**BS**	**Al**	**CEC**	**P**
Characteristics	($CaCl_2$)	---------------------------cmol$_c$ dm^{-3}------------------------							mg dm$^-$
Pure soil	4.3	1.9	2	10	18	13.9	8	31.9	4
Soil + manure (7: 1)	4.7	0.22	0.84	0.96	0.26	34.2	0.26	5.9	45.8
Commercial	4.2	1	12.19	4.09	18.	48.7	0.45	35.5	380.9
Chemical	**Cu**	**B**	**Zn**	**S**	**Fe**	**Mn**	**V**	**m**	**OM**
Characteristics	--------------------------mg dm^{-3}--------------------						-------%-----		g dm^{-3}
Pure soil	0.9	0.09	0.1	8	6	19.3	44	37	5
Soil + manure (7: 1)	1.1	0.08	2.3	14.1	51	64.1	34.2	11.4	20.4
Commercial	0.4	0.97	9.9	380.9	259	98.4	48.7	2.5	207.3

H+Al = Acidity potential; BS = Base Sum; CEC = Cation exchange capacity; V = Base saturation; m = Saturation per Al; OM = Organic matter

The evaluation was conducted five months after transplanting, analyzing the characteristics: plant height from the collar to the apex (cm), collar diameter (mm), number of leaves and height / diameter ratio (H / D). At the end of the trial period was the collection of rootstock that passed by dehydration in an oven with forced air at 70 ° C until reaching constant mass in order to determine its shoot dry mass and root. The Dickson quality index was calculated with height values (H), collar diameter (DIAM), shoot dry mass (SDM), root dry mass (RDM) and total dry matter (TDM) through equation:

$$DQI = \frac{TDM\ (g)}{\frac{H\ (cm)}{DIAM\ (mm)} + \frac{SDM\ (g)}{RDM\ (g)}}$$

Air temperatures (ºC), the relative humidity of air (%) and solar radiation (W m^{-2}) were monitored (Figure 1). The determinations of micrometeorological characteristics inside the protected environment (screen house) were performed from specific sensors coupled to a "datalloger" Delta T Devices, GP2 model, in the region of protected environments. The system was programmed to perform readings at 10 seconds of intervals, averaging every minute. For global radiation was estimated daily average from 8:00 a.m. to 6:00 p.m. For the external environment the air temperature values, relative humidity and solar radiation (8:00 to 6:00 p.m.) were acquired from automatic station for collection data from Cassilândia A742 of INMET-SONABRA.

Initially the results for the three tests (environments) were subjected to analysis of variance by F test with comparison of means by Tukey test at 5% probability. Later, there was the analysis of variance of substrates and clones, with the evaluation of the residual mean square (BANZATTO & KRONKA 2013), in order to analyze the experiments together and find the most suitable environment for the production of rubber tree seedlings. It was used the statistical program Sisvar 5.3 (FERREIRA, 2010), and the averages compared by Tukey at 5% probability.

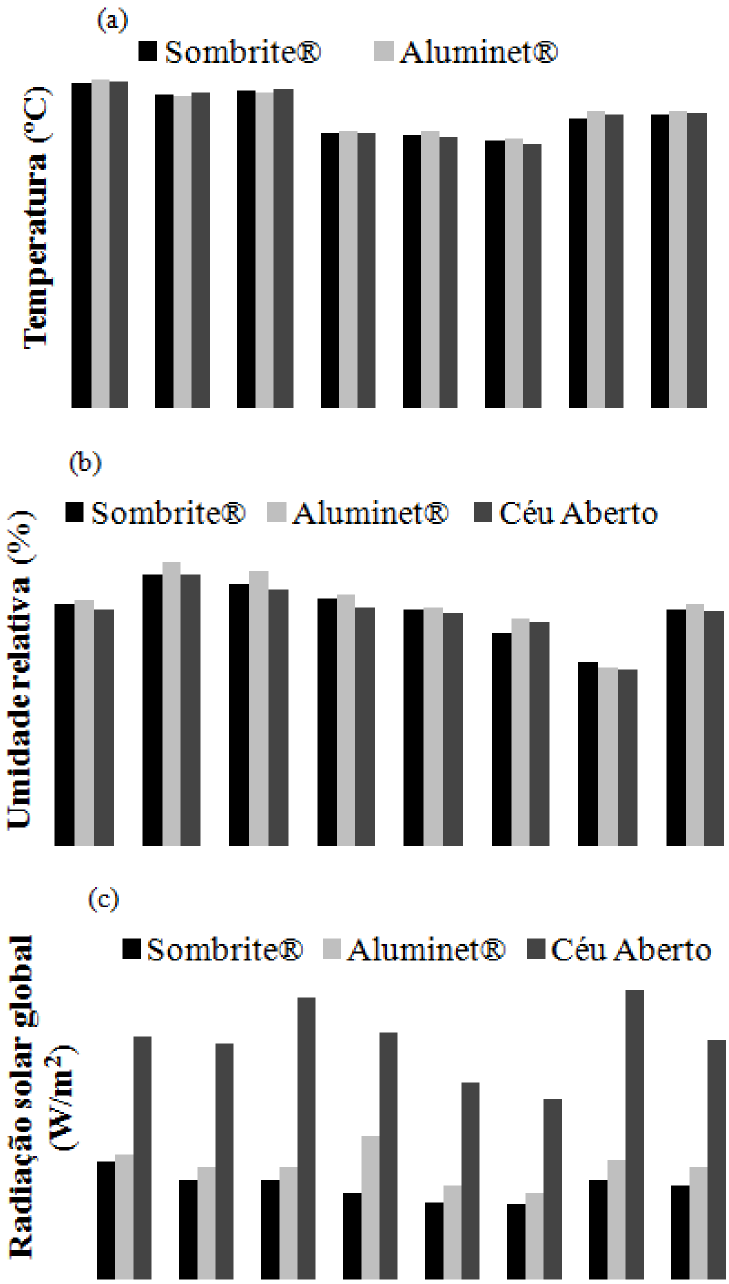

FIGURE 1. Temperature (a), Relative humidity (b) and solar radiation (c) recorded in the growing environments during the trial period.

RESULTS AND DISCUSSION

The ratio between the highest and the lowest residual mean square from the analysis of substrates and clones in three farming environments for plant height, collar diameter, number of leaves, H/D ratio, shoot dry mass, dry mass of the root system and Dickson quality index were, respectively, 3.98; 3.17; 1.64; 1.92; 3.87; 3.24 and 1.60. For all feature, the relationship between the residual mean squares (RMS) was lower than seven, allowing the implementation of joint analysis of the experiments (BANZATTO & KRONKA, 2013).

There was no interaction of substrates with clones factors and environments to height and collar diameter; the same was true for environments that showed no interaction between the two factors for root dry mass (RDM) and Dickson quality index (DQI).For these variables factors were evaluated separately (Table 2).

TABLE 2. Height, collar diameter, root dry mass and Dickson quality index of rubber tree clones grown on different substrates. Cassilândia, MS, 2014.

Substrates	Height (cm)	Collar diameter (mm)
Commercial substrate	44.35 a	5.45 a
Soil + manure	44.43 a	5.42 a
Pure soil	39.93 b	5.08 b
C.V.(%)=	10.67	7.57
Environments	Root dry mass (g)	DIQ
Sombrite ®	1.57 ab	0.40 a
Aluminet ®	1.78 a	0.40 a
Open Air	1.45 b	0.44 a
C.V.(%)=	25.39	23.21

* Means followed by the same letter in the columns do not differ by 5% Tukey test.

The commercial substrates and soil + manure (7: 1) provided average height 10.05% higher than the pure soil. The use of manure substrate is capable of promoting high quality seedlings, as they provide improvements in physical conditions such as aeration and drainage, as well as providing nutrients according to OLIVEIRA et al. (2014a) and DIAS et al. (2009). The pure soil has fewer nutrients (Table 1) and consequently plants with lower heights resulting in collar diameter 7.0% lower when grown on pure soil (Table 2). These results corroborate with ZIETEMANN & ROBERTO RUFFO (2007), which studied the development of guava plants (*Psidium guajava* L) in substrates composed of pure soil (Oxisol), mixture of soil + sand + organic matter (manure) (2 : 1: 1), and Plantmax® and coconut fiber Sococo®, which found that the pure soil (Oxisol) showed lower growth of the seedlings.

The use of pure soil possibly influenced negatively the development of rootstocks to present low levels of phosphorus, sulfur, manganese, iron, organic matter (Table 1). However, the organic residue addition to this soil favors the growth of rootstocks. Organic residues when passing through the decomposition process offer to the soil organic matter provide numerous benefits for forest species, because there is an increase in moisture retention ability provides nutrients to the substrate and reduces the apparent bulk density, increasing porosity of the environment (GONÇALVES et al., 2000). To SILVA et al. (2010) the best results for the amount of leaves per plant in yellow passion fruit seedlings (*Passiflora edulis f. flavicarpa* Deg) were obtained in the substrates containing the proportion of soil + manure (2:1 v:v).

The root dry mass was higher for plants grown under Aluminet® (22.75%) screen house when compared to open air. The environment in the open air exposed the rootstock to climatic conditions (Figure 1), for example, direct radiation on plant and wind, and pest attack as “mandarová” (*Erinnyis ello*) and white-mite (*Polyphagotarsonemus latus*) and diseases caused by fungus *Fusarium* spp. and *Pythium* ssp. which caused lower growth of the rootstock and higher costs on plant protection products. SILVA et al. (2013) reported that it is not advisable the production of coffee seedlings in full sun, regardless of the type of substrate used, as it was seen the worst seedlings compared to those produced in screen house.

It is observed in the environment with aluminized screen (Aluminet®) availability of 47% of external radiation and to Sombrite® of 39.3%.This radiation limitation in individual screen environments did not affect the growth of rootstocks, as probably the amount available was above the photic saturation point for rubber tree. Aluminet® screen house showed high relative humidity, which favored a lower evapo-transpiration and better root development. The temperatures were very similar in the three farming environments (Figure 1).

For DQI there was no significant difference when comparing environments, however, the rootstocks were less vigorous when grown in pure soil, not reaching necessary conditions to go to the field (Table 2). For this parameter, all the environments presented similar quality seedlings, however, the seedlings conducted in Aluminet® had more robust root system, providing better fixation in the field.

The rootstock with higher number of leaves was conducted in Aluminet® (Table 3). SCALON et al. (2001), SENEVIRATHNA et al. (2003), LIMA JUNIOR et al. (2005) reported that the decrease in brightness takes the plants to adapt and expand its leaf area, which enables more efficient interception of light, thereby ensuring an increase in carbon gain.

The Aluminet® environment led to the clones larger number of leaves than the environment in full sun (Table 2), which is consistent with SENEVIRATHNA et al. (2003) and CONFORTO et al. (2011) in which *Hevea brasiliensis* plants showed higher number of leaves in shaded environments than in full sun. SENEVIRATHNA et al. (2003) reported also that the CO_2 absorption saturation occurs at a lower density photosynthetic radiation flux in shaded plants. In the environment with aluminized screen there was higher relative humidity and radiation sufficient to saturate the assimilation of CO_2 without harming the photosynthetic efficiency (Figure 1), environmental conditions that propitiated the best seedlings. DOSSEAU et al. (2007) also reported that at greater shadowing conditions; with higher number of leaves *Tapirira guianensis* Alb with bigger leaf area. COSTA et al. (2014) found best seedlings of *Acrocomia Aculeata* in Aluminet® screen when compared to Sombrite® and the greenhouse with low density polyethylene while COSTA et al. (2012) found no differences between the individual screen houses to the seedlings “baruzeiro” (*Dipteryx alata*).

TABLE 3. Leaf number and height/diameter ratio of rubber tree rootstocks cultivated in different environments and substrates. Cassilândia, MS, 2014.

Environments	Number of Leaves	Rate (H/D)
Sombrite®	18.87 b	8.327 b
Aluminet®	20.65 a	8.756 c
Open air	18.52 b	7.185 a
Clones	Number of Leaves	Rate (H/D)
GT1	18.61 b	8.094 a
PR255	18.96 b	8.027 a
RRIM 600	20.46 a	8.146 a
Substrates	Number of Leaves	Rate (H/D)
Commercial substrate	19.83 a	8.180 a
Soil+ manure (7:1)	19.58 ab	8.212 a
Pure soil	18.63 b	7.876 a
CV(%)	10.10	9.18

* Means followed by the same letter in the column do not differ by the Tukey5% test.

In the environment open air the plants showed the smallest height/collar diameter ratio (H / D). The growth in height must be accompanied by growth in collar diameter, fact verified in the three cultivation environments (Table 3). This growth associated ensures less chance of overturning plants when transplanted to the field during strong winds. In greenhouse environments, even plants showing higher H/D ratio (Table 3) did not differ in collar diameter (Table 4) and showed greater root dry mass (Table 2), with higher quality plants.

Of the three studied varieties, clone RRIM 600 showed the highest number of leaves differing from GT1 and PR255 materials (Table 3), however the height/diameter ratio for any of the plants showed a significant difference between them. By the H/D ratio it is possible to highlight that all clones were not able to present damping in the field, however, RRIM 660 would have higher photosynthetic capacity as function of greater number of leaves.

For interaction between varieties and environments, the height was higher in RRIM 600 variety grown in screen house environments with Sombrite® and Aluminet® (Table 4) which did not occur in open air, probably due to exposure of plants to natural environmental weather. Plant under these conditions exhibit rapid allocation of photo-assimilates for shoot because they are not under adverse environmental conditions and uses less energy for their physiological functions. ORTEGA et al. (2006) analyzing the development on red arrack seedlings (*Psidium cattleianum*) and ARAÚJO & DANTAS (2014) “pinhão-manso” seedlings (*Jatropha curcas* L.) on different shadings verified that the lowest average height were found in plants subjected to full sun conditions, as well as SILVA et al. (2013) for coffee (*Coffee arábica*).

The Clone RRIM600 stood out with the highest growth in Sombrite® and Aluminet® environment (Table 4). MACEDO et al. (2003) in a study involving newly established plants in field of six different rubber tree clones introduced in the region of Lavras, Minas Gerais, noted that the RRIM 600 clone was superior to the others ones (IAC15, GT1, PR255, IPA1 and PB235) in the evaluations of height, showing suitable to local conditions.

TABELA 4. Interactions between clones and environments (C x E) and clones and substrates (C x S) to height (cm), collar diameter (mm), root dry weight (g), shoots dry weight (g) and Dickson index quality (DQI). Cassilândia, MS, 2014.

	Interaction (C x E)		
Clones	**Height (cm)**		
	Sombrite®	**Aluminet®**	**Open air**
GT1	40.77 aB	42.69 aB	35.99 bA
PR255	42.90 aB	42.61 aB	37.27 bA
RRIM600	50.27 aA	53.67 aA	39.97 bA
CV (%)= 10.67			
	Collar diameter (mm)		
	Sombrite®	**Aluminet®**	**Open air**
GT1	4.88 aB	4.94 aB	4.98 aB
PR255	5.17 aB	5.00 aB	5.19 aB
RRIM600	6.11 aA	5.95 abA	5.61 bA
CV (%)= 7.57			
	Shoot dry mass (g)		
	Sombrite®	**Aluminet®**	**Open air**
GT1	2.04 abB	2.42 aB	1.81 bB
PR255	1.77 aB	2.12 aB	1.82 aB
RRIM600	3.36 aA	3.90 aA	2.44 bA
CV (%)= 25.47			
	Interaction (C x S)		
	Shoot dry mass (g)		
	Pure soil	**Soil+manure (7:1)**	**Commercial**
GT1	1.48 bB	2.12 aB	2.67 aB
PR255	1.53 bB	1.96 abB	2.23 aB
RRIM600	2.21 bA	3.55 aA	3.93 aA
CV (%)= 25.47			
	Root dry mass (g)		
	Pure soil	**Soil+manure (7:1)**	**Commercial**
GT1	0.96 bB	1.31 bB	1.94 aB
PR255	1.08 bAB	1.21 abB	1.56 aB
RRIM600	1.45 bA	2.27 aA	2.64 aA
CV (%)= 25.39			
	Dickson Quality Index		
	Pure soil	**Soil+manure (7:1)**	**Commercial**
GT1	0.26 bB	0.34 bB	0.48 aB
PR255	0.27 bB	0.33 abB	0.39 aB
RRIM600	0.37 cA	0.58 bA	0.68 aA
CV (%)= 23.21			

* Means followed by the same lowercase and uppercase in the line in the column do not differ by 5% Tukey test.

The collar diameter ranged from 4.88 to 6.11 mm (Table 4) depending on the clone and the environment, not being able to grafting that according to GONÇALVES et al. (2001) the rootstocks are considered suitable for green-grafting with a minimum diameter of 10 mm which is measured 5 cm from the soil (7-8 months). The RRIM600 clone in all environments generated higher averages of collar diameter, demonstrating satisfactory performance, being the closest to receive the graft, probably requiring less time in the suspended nursery.

In the assessment SDM the GT1 clone performed better when grown in Aluminet® than open air. Usually in the traditional farming system (full sun), the plants show symptoms of nutritional deficiency, due to the leaching of substrates by the rains, affecting directly the accumulation of dry matter (BIRRENKOTT et al., 2005; GODOY & COLE 2000).

SILVA et al. (2007) testing the influence of different levels of shading on “cupuaçu” seedlings (*Theobroma grandiflorum*), found higher total dry matter increment (TDM) than those who remained exposed in 50% shading environment, presenting difference from those grown at 0% and 90%. It was found that the efficiency in the use of light incidence generated higher production of photo-assimilates and consequently higher TDM of the plant, similar to this study.

Within the three environments analyzed (Table 4) highlights the RRIM 600 clone due to higher values on dry matter accumulation on shoot.

For shoot dry mass (Table 4), the GT1 clones, RRIM 600 showed better aerial development indices when grown in soil + manure and commercial substrate. As CUNHA et al. (2005) in a study with seedlings of ipê-roxo (*Handroanthus impetiginosus*), reported that soil from subsoil + organic compost, have provided satisfactory results in its development, however when using subsoil only it could not meet the nutritional requirement of the plants, leading to lower performance, as occurred in this trial. In the present study the pure soil (Table 1) showed lower amount of nutrients which influenced in the least amount of aerial and total dry mass in the plant.

When the substrates were analyzed separately is remarkable the superiority of the RRIM 600 clone than the others (Table 4), showing on various parameters of this study to be a suitable alternative for the production of rootstock. Dry mass analyzed on the root system (Table 4), the commercial substrate promoted greater root development in the GT1 clone, however for the RRIM 600 clone commercial substrate and soil + manure influenced the expansion of the root system. BRAGA JÚNIOR et al. (2010) found that substrates made from (top soil + cattle manure and humus + sand, both in the proportion of 3: 1, respectively) significantly increased the dry root mass values in juazeiro seedlings (*Ziziphus joazeiro*) because the manure and sand as well as providing nutrients, aeration aid and water availability to the plant.

It can be inferred that the low porosity of the pure soil, compared to other substrates, has caused the difficulty of expanding roots creating a low performance parameter of the root dry mass. LOPES et al. (2008) reported that the growth of seedlings is directly affected when the total porosity (TP) is not at optimal levels, hindering expansion of roots. Other low expansion factor related to the roots can be connected to the high observed amount of Al^{+3} presents in pure soil (Table 1). Aluminum as Al^{+3} is demonstrably toxic to plants, and its initial symptom is the reduction of root growth.

For DQI (Table 4) the GT1 and RRIM600 clones performed better when grown in commercial substrate. In clone PR255 commercial substrate was superior to pure soil. According to PAULINO et al. (2011) evaluating the growth of “pinhão-manso” seedlings found that commercial substrate Plantmax® provided seedlings quality to be transplanted in the field. The highest average related to DQI generated in this experiment when analyzing the commercial substrates and soil + manure were RRIM600 rootstocks, leading to consider high quality seedlings, able to be used for future composition of rubber crops.

CONCLUSIONS

1) The use of commercial substrate and soil + cattle manure in the ratio (7: 1) favored the development of rubber rootstock seedlings.

2) The variety RRIM 600 for the development of rootstocks was the one that showed better agronomic characteristics in the formation of rubber tree seedlings.

3) The use of Sombrite® and Aluminet® screen houses contributed positively to the formation of rootstocks.

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