Parameters of Physiology, Nutrition and Quality of *Eugenia dysenterica* DC Seedlings Grown in Organic Substrates from the Agricultural Industry

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Abstract

Residues from agricultural activities might be used as substrates for production of seedlings. In this study it was aimed to evaluate the physiology, nutrition and quality of *Eugenia dysenterica* DC. seedlings grown in substrates derived from agricultural organic wastes. The wastes used were as follows: rice husks (RHs); fermented cattle manure (FCM); cattle manure compost (CMC), which contained corn silage and FCM; sugarcane bagasse (SCB); filter cake from sugar-alcohol mills (FC); and subsoil (SB). Four substrates were formulated from the wastes, namely SB+RH (1:1; v:v), SB+CMC (1:1), SB+FCM (3:1) and SCB+FC (3:2) and Bioplant® substrate was also used. The following characteristics were evaluated: the emergence and vigor percentages, biometric characteristics, seedling quality indices, gas exchange, chlorophyll *a* fluorescence and leaf nutrient levels. The seedling emergence was similar for all the substrates. The highest vigor was found in seedlings that were cultivated in SCB+FC, which was equal to the vigor observed in Bioplant®. In general, the use of the SB+CMC substrate gave better seedling results for the biometric and nutritional characteristics, followed by SCB+FC. Most of the biometric characteristics showed a correlation with photosynthesis, electron transport rate and effective quantum yield of photosystem II, thus showing that these characteristics can be alternatives to the traditional quality indexes used for seedlings. The use of CMC, SCB and FC was shown to be appropriate for the production and nutritional supply of seedlings' specie. The composition SB (subsoil)+CMC (1:1) proved to be the most suitable to produce *E. dysenterica* seedlings.

Keywords: cagaita tree, sugarcane bagasse, cattle manure, mineral nutrition, gas exchanges, Dickson quality

1. Introduction

The Cerrado (tropical savanna) is considered the second largest Brazilian biome and is home to a great diversity of plant species (Paiva Sobrinho et al., 2010). *Eugenia dysenterica* DC is a fruiting species endemic to the Cerrado, belongs to the Myrtaceae family and is popularly known as cagaita (Souza et al., 2013). Its fruit is consumed in whole form or processed into sweet and jams, and it is also used in ice creams, popsicles and beverages. This fruit is considered to be an important source of vitamins C and A (Cardoso et al., 2011). Souza et al. (2012) reported the high inhibitory activity of cagaita fruits for α-amylase and α-glucosidase, with the latter contributing to the control of diabetes. The extract of its leaves have shown anti-diarrheal activity, so that the plant offers promise as medication (Lima et al., 2010).

The cagaita has socioeconomic and ecological importance (Bailão et al., 2015). The cultivation of native species through seedlings propagation can be an effective way to avoid biodiversity loss in the Cerrado biome. The propagation of woody plant species is achieved by producing high quality seedlings; that is, healthy, well-nourished and robust plants. These characteristics contribute to the survival of plants after transplantation to the field, thus resulting in the success of orchard installations, even in reforesting situations (Gomes et al., 2003).

The starting point for obtaining quality seedlings can be the use of substrate combinations with appropriate chemical and physical characteristics, organic matter and water contents which will provide the best conditions for development, according to species necessities (Boene et al., 2013). The good substrate, to favor germination and emergence, must exhibit porosity degrees that allows seed hydratation and aeration, since at the beginning it mainly requires water and oxygen for their germination and emergence (Nogueira et al., 2003); must exhibit slow decomposition, has a high cation-exchange capacity, and be free from pathogens and seeds of undesired plants (Dantas et al., 2009). The substrates derived from organic waste of agricultural industries can present these characteristics and represent cost reduction for farmers, also being a sustainable way of allocating waste from milling processes. To obtain these characteristics is often necessary mix two or more components, which together will form a suitable substrate for seedlings formation (Araújo Neto et al., 2009).

Dornelles et al. (2014) studied the development of *Anacardium othonianum* Rizz seedlings (Cerrado cashew tree) in substrates formulated with Mecplant® (MP) + carbonized rice husk (CRH) in 7:1 (v:v proportion), fine-grained vermiculite (FGV), FGV+CRH (3:1), FGV+CRH (1:1), FGV+CRH (1:3), and sugarcane bagasse (SB) + sugarcane mill filter cake (FC) (3:2). They verified that the substrate SB+FC (3: 2) promoted the best nutrition for plants. Mota et al. (2016a) concluded that the use of cattle manure mixed with subsoil in 1:3 proportion (v:v), provided the best results for the biometric characteristics and dry matter production of *Pouteria gardneriana* (Cerrado's native plant, popularly called guapeva).

The quality indices that are used to evaluate the seedlings are ratios, such as the one between shoot height and diameter, root and shoot dry weights (DWs) and other possible ratios that indicate seedlings robustness. Dickson et al. (1960) created a quality index that considers the ratios mentioned above as well as the accumulation of total DWs. Analyses of physiological parameters (Mota et al., 2016b) and nutritional status (Mota et al., 2016a) has been used to complement the growth and development parameters of native Cerrado seedlings. However, there are few studies that evaluate the production of seedlings based on all these parameters. Thus, the objective of this study was to evaluate the growth, quality, nutrition, gas exchange and fluorescence of chlorophyll *a* in *E. dysenterica* seedlings produced in substrates formulated with organic wastes from agricultural industries inferring about their use on Cerrado reforestation areas.

2. Method

2.1 Plant Material

The *Eugenia dysenterica* DC fruits were harvested from healthy adult plants, in full production, at Gameleira Farm located in Montes Claros de Goiás, GO, Brazil (19°53′S latitude, 44°25′W longitude, altitude of 749 m). The fruits were transported to the Laboratory of Plant Tissue Culturing (LCTV) of the Goiano Federal Institute - Campus of Rio Verde (17°48′16″ S latitude, 50°54′19″ W longitude and 753 m altitude), where were pulped and the seed coats were removed to facilitate germination (Martinotto et al., 2007). The study was conducted in a greenhouse at the LCTV. Temperature and relative humidity inside the greenhouse were recorded using a DataLogger (NOVUS, Porto Alegre, Brazil) being the average values of temperature and relative humidity of 25.5 °C and 76%, respectively.

2.2 Chemical Properties of Substrates

The tested substrates were formulated from sugarcane bagasse (SCB), filter cake from sugar-alcohol mills (FC), fermented cattle manure (FCM), rice husks (RHs), cattle manure compost (CMC) (which contained corn silage and FCM), subsoil (SB) from a soil classified as Distroferric Red Latosol and the commercial substrate Bioplant®. According to information from the manufacturer, this last substrate is composed of *Pinus* bark, vermiculite, coir, aggregating agents and mineral supplements (NPK+micro). Five substrates were formulated and tested as follows: Bioplant®, SCB+FC at a 3:2 proportion (3:2), SB+RH (1:1), SB+CMC (1:1) and SB+FCM (3:1) whose chemical characteristics are shown in Table 1.

Table 1. Chemical analysis of macro and micronutrients of the substrates used in the study

Substrate	pH	OM	Ca	Mg	CEC	V	K	P	Fe	Mn	Cu	Zn	B
	H$_2$O	gkg^{-1}	-------- mg dm^{-3} -------			%	------------------------ mg dm^{-3} --------------------------						
Bioplant®	4.97	430	10.4	4.04	18.7	56	1207	3.7	132	52	1.66	24.5	2.14
SB[y]+RH[x](1:1)	6.42	22	1.1	0.37	2.6	55	3.3	20.8	39	24	1.76	3.8	0.17
SB+CMC[w](1:1)	6.60	118	4.3	6.90	12.6	86	950	170	103	62	2.33	22.6	0.56
SB+FCM[v](3:1)	6.40	25	3.6	3.5	8.5	82	543	82.3	58	35	4.75	17.2	0.53
SCB[u]+FC[t] (3:2)	7.02	600	2.3	0.61	5.3	70	667	21.9	153	81	2.32	30.8	2.29

Note. [y]Subsoil; [x]Rice husks; [w]Cattle manure compost (corn silage + fermented cattle manure); [v]Fermented cattle manure; [u]Sugarcane bagasse; [t]Filter cake from sugar-alcohol mill; [s]Base saturation index; [r]Organic matter; and [q]Effective cation exchange capacity.

2.3 Seedling Growth and Morphological Characteristics

Containers (tubes) of 288 cm^3 were filled with the substrates, and one seed was sown in each container. The spray irrigation was 12 mm day^{-1}, divided into two irrigations diary. Percent seedling emergence (PSE) was tested at two day intervals between emergence of the first seedling and the end of emergence; the emergence speed index (ESI) was calculated according to Maguire (1962).

After 128 days of seeding, were measured stem diameter (SD) and count the number of seedlings leaves (NL), which were separated in stems, leaves, and roots. The stem length (SL) and the leaf area (LA) was obtained. The stems, leaves, and roots were dried in a forced-air oven at 65 °C to constant weight, to obtain weights separately. The ratio between the SL and SD (SL/SD), the ratio between the root dry weight and shoot dry weight (R/S), and the Dickson quality index (DQI) were used as seedling quality parameters according to Dickson et al. (1960). Leaves from the plants of each replicate were combined and ground in a Willey mill to determine the micro and macronutrient content on nutrients according to Malavolta et al. (1997).

2.4 Physiological Measurements

Seedling gas exchange, fluorescence, growth, quality, and water content, as well as substrate moisture content, were evaluated at 128 days after the seeding. Gas exchange was evaluated in the morning between 7:30 and 11:30 am with an LCi portable photosynthesis meter (ADC BioScientific, Great Amwell, United Kingdom), that generated the following variables: net CO$_2$ assimilation (A), transpiration (E), water use efficiency (A/E) obtained from the ratio between A and E, and stomatal conductance (g_s). The chlorophyll a fluorescence variables were obtained using a Mini-PAM modulated fluorometer (Walz, Effeltrich, Germany). The maximum quantum yield of photosystem II (PSII) (F_v/F_m) was calculated using the darkness parameters with the equation, $F_v/F_m = (F_m - F_0)/F_m$, where F_0 and F_m are the minimum and maximum fluorescence, respectively, of the dark-adapted plant tissue obtained before dawn. F_0 was obtained by lighting the plant tissue with a low-intensity modulated red light (0.03 μmol m^{-2} s^{-1}), and F_m was obtained with a saturating light pulse (6,000 μmol m^{-2} s^{-1}) lasting 0.8 seconds. The effective quantum yield of PSII (φPSII) was calculated using the equation φPSII = (F_m' $- F)/F_m$', on what F_m' and F are the maximum fluorescence and fluorescence, respectively, when the plant tissue is under actinic light with an intensity of 1,000 μmol m^{-2} s^{-1} for 50 seconds. The quantum yield of regulated non-photochemical energy dissipation of PSII (φNPQ) and the quantum yield of unregulated non-photochemical energy dissipation of PSII (φNO) were obtained according to Hendrickson et al. (2004). The apparent electron transport rate of PSII (ETR) was obtained from Bilger et al. (1995).

The relative water content (RWC) and substrate moisture content were measured before dawn at 4:30 am. To determine RWC, one leaf was collected per plant using a scalpel and was then immediately weighed on an analytical balance to obtain the fresh weight (FW). After obtaining the FW, the leaves were placed in a humid chamber, and the petioles were immersed in distilled water, remaining under these conditions for 24 hours at 25 °C, with compensation irradiance, to allow the leaves to reach maximum turgor. After the leaves reached maximum turgor, they were weighed to obtain the turgid weight (TW) and were then dried in a forced-air oven at 65 °C to constant weight, yielding the dry weight (DW). The RWC was calculated using the equation RWC = (FW – DW)/(TW – DW). To determine the relative moisture of the substrates studied, substrate samples were collected from the same tube containing the plants used for determining the RWC. Immediately after collecting the substrates, they were weighed on a semi-analytical balance to obtain wet weight (WW) and then dried in a forced-air oven at 105 °C to constant weight, yielding the substrate dry weight (DW). To obtain the relative moisture content of the substrate (WCDS) on a dry basis, the equation WCDS = (WW – DW)/DW was used.

2.5 Experimental Design and Statistical Analysis

The experiment followed a randomized block design with five replicates and 20 tubes per replicate for PSE and ESI, two for RWC and WCDC, one for gas exchange and fluorescence, and five for the other evaluations. The data were subjected to an analysis of variance and Tukey test. A significance level of 5% was used to discriminate the minimal significant differences among the means. For the correlations analysis, Pearson's Method was used. The statistical analysis was performed using the computational program SAEG 9.1.

3. Results

The ability of the substrates to absorb water differed among the substrates. The water content was greatest in the SCB+FC, followed by the others in the following order: Bioplant® > SB+CMC > SB+RH, and the substrate SB+FCM did not differ from the last two items (Table 2). Despite the difference in the water contents of the substrates, this characteristic did not influence the RWC of the *E. dysenterica* seedlings. The PES, which had a mean value of 97.2%, was not influenced by the tested substrates (Table 3). The cagaita tree seedlings had greater vigor when grown in the Bioplant® and SCB+FC substrates, followed by the SB+FCM and SB+RH substrates. Less vigor was observed in the SB+CMC treatment.

The gas exchange characteristics of the *E. dysenterica* seedlings, such as the liquid assimilation of CO_2 (*A*), transpiration (*E*), stomatal conductance (*g_s*), and the ratio between the intercellular and atmospheric concentration (*Ci/Ca*), did not differ in accordance with the tested substrates (Table 4). The mean values of *A*, *E*, *g_s* and *Ci/Ca* were 6.31 μmol m^{-2} s^{-1}, 2.23 mmol m^{-2} s^{-1}, 0.16 mol m^{-2} s^{-1} and 0.69 mol mol^{-1}, respectively.

Table 2. Water content in the dry base substrates (WCDS) and leaf relative water content (RWC) in *Eugenia dysenterica* DC. seedlings grown in different substrates

Substrate	WCDS	RWC
	------------------------------- g g^{-1} -------------------------------	
Bioplant®	1.73 b^z	0.95^{ns}
SB^y+RH^x(1:1)	0.35 d	0.96
SB+CMC^w(1:1)	0.60 c	0.96
SB+FCM^v(3:1)	0.51 cd	0.93
SCB^u+FC^t (3:2)	2.22 a	0.96
MSD^s	0.205	0.064

Note. ^{z}Means followed by the same letter do not differ from one another according to Tukey's test (p < 0.05); ^{y}Subsoil; ^{x}Rice husks; ^{w}Cattle manure compost (Corn silage + fermented cattle manure); ^{v}Fermented cattle manure; ^{u}Sugarcane bagasse; ^{t}Filter cake from sugar-alcohol mill; ^{s}Minimum significant difference; and ^{ns}Non-significant difference.

Table 3. Percentage of emerged seedlings (PES) and emergence speed index (ESI) in *Eugenia dysenterica* DC. seedlings cultivated in different substrates

Substrate	PES	ESI
	%	-
Bioplant®	98^{ns}	0.36 a^z
SB^y+RH^x(1:1)	98	0.29 b
SB+CMC^w(1:1)	92	0.23 c
SB+FCM^v(3:1)	98	0.30 b
SCB^u+FC^t (3:2)	100	0.35 a
MSD^s	9.88	0.044

Note. ^{z}Means followed by the same letter do not differ from one another according to Tukey's test (p < 0.05); ^{y}Subsoil; ^{x}Rice husks; ^{w}Cattle manure compost (Corn silage + fermented cattle manure); ^{v}Fermented cattle manure; ^{u}Sugarcane bagasse; ^{t}Filter cake from sugar-alcohol mill; ^{s}Minimum significant difference; and ^{ns}Non-significant difference.

Table 4. Rate of liquid assimilation for CO_2 (A), transpiration (E), stomatal conductance (g_s) and the ratio between intercellular and atmospheric concentrations (Ci/Ca) in *Eugenia dysenterica* DC. seedlings cultivated in different substrates

Substrate	A	E	g_s	Ci/Ca
	$\mu mol\ m^{-2}\ s^{-1}$	$mmol\ m^{-2}\ s^{-1}$	$mol\ m^{-2}\ s^{-1}$	$mol\ mol^{-1}$
Bioplant[®]	5.34^{ns}	2.06^{ns}	0.14^{ns}	0.72^{ns}
SB[y]+RH[x](1:1)	4.02	1.56	0.09	0.71
SB+CMC[w](1:1)	7.79	2.39	0.17	0.65
SB+FCM[v](3:1)	7.15	2.65	0.23	0.69
SCB[u]+FC[t] (3:2)	7.23	2.48	0.18	0.68
MSD[s]	4.50	1.94	0.187	0.105

Note. [z]Means followed by the same letter do not differ from one another according to Tukey's test (p < 0.05); [y]Subsoil; [x]Rice husks; [w]Cattle manure compost (Corn silage + fermented cattle manure); [v]Fermented cattle manure; [u]Sugarcane bagasse; [t]Filter cake from sugar-alcohol mill; [s]Minimum significant difference; and [ns]Non-significant difference.

The characteristics of chlorophyll *a* fluorescence in the *E. dysenterica* DC. seedlings, as indicated by the minimum fluorescence adapted to the dark (F_0), maximum quantum yield of the PSII (F_v/F_m) and effective quantum yield of the PSII (ϕPSII), were not influenced by the tested substrates (Table 5). Their means were 212, 0.76 and 0.28, respectively. The ETR differed in two of the tested substrates; it was higher in plants that were cultivated in the SCB+FC substrate and lower in the SB+RH substrate, with means of 140.7 and 75.5 $\mu mol\ m^{-2}\ s^{-1}$, respectively.

Table 5. Minimum fluorescence adapted to the dark (F_0), maximum quantum yield of the PSII (F_v/F_m), effective quantum yield of the PSII (ϕPSII) and electron transport rate (ETR) in *Eugenia dysenterica* DC. seedlings cultivated in different substrates

Substrate	F_0	F_v/F_m	ϕPSII	ETR
	-	-	-	$\mu mol\ m^{-2}\ s^{-1}$
Bioplant[®]	209.4^{ns}	0.76^{ns}	0.23^{ns}	$90.90\ ab^{z}$
SB[y]+RH[x](1:1)	219.8	0.74	0.20	75.45 b
SB+CMC[w](1:1)	205.8	0.78	0.33	125.13 ab
SB+FCM[v](3:1)	221.6	0.74	0.32	123.58 ab
SCB[u]+FC[t] (3:2)	203.4	0.78	0.34	140.65 a
MSD[s]	25.68	0.043	0.154	59.54

Note. [z]Means followed by the same letter do not differ from one another according to Tukey's test (p < 0.05); [y]Subsoil; [x]Rice husks; [w]Cattle manure compost (Corn silage + fermented cattle manure); [v]Fermented cattle manure; [u]Sugarcane bagasse; [t]Filter cake from sugar-alcohol mill; [s]Minimum significant difference; and [ns]Non-significant difference.

The cultivation substrates had an influence on all the biometric characteristics that were evaluated in the *E. dysenterica* seedlings (Table 6). The SB+CMC substrate gave the seedlings highest values in all of the biometric characteristics, except for the DW of the roots (DWR), which had lower values than those of the Bioplant[®] substrate. In relation to the evaluated biometric characteristics, the seedlings cultivated in the SCB+FC substrate did not differ from those grown in the SB+CMC substrate. For the biometric characteristics, the lowest values were observed in the seedlings that were grown in the SB+RH substrate, except for the total dry weight (TDW), which showed no difference in the other substrates; and the dry weight of the roots (DWR), which was greater than that of the SB+FCM and less than that of the Bioplant[®]. Similar to the SB+RH, the latter substrate provided the cultivated seedlings with the lowest values for the biometric characteristics, except for the DWR, which was greater than that of the others, and the TDW, which was greater than that of the SB+FCM substrate.

Table 6. Stem length (SL), root collar diameter (RCD), number of leaves (NL), dry weight of stem (DWS), dry weight of leaves (DWL), dry weight of roots (DWR) and total dry weight (TDW) in *Eugenia dysenterica* DC. seedlings cultivated in different substrates

Substrate	SL	RCD	NL	DWS	DWL	DWR	TDW
	cm	mm	------------------------------------- g -------------------------------------				
Bioplant[®]	3.04 c[z]	0.98 c	2.08 b	0.020 b	0.145 cd	0.599 a	0.765 a
SB[y]+RH[x](1:1)	3.02 c	0.95 c	2.44 b	0.018 b	0.129 d	0.466 b	0.613 ab
SB+CMC[w](1:1)	5.21 a	1.26 ab	5.04 a	0.038 a	0.348 a	0.367 bc	0.753 ab
SB+FCM[v](3:1)	3.96 bc	1.33 a	3.88 a	0.037 a	0.226 bc	0.339 c	0.602 b
SCB[u]+FC[t] (3:2)	4.96 ab	1.13 b	4.76 a	0.041 a	0.272 ab	0.404 bc	0.716 a
MSD[s]	1.17	0.15	1.20	0.0095	0.084	0.110	0.16

Note. [z]Means followed by the same letter do not differ from one another according to Tukey's test (p < 0.05); [y]Subsoil; [x]Rice husks; [w]Cattle manure compost (Corn silage + fermented cattle manure); [v]Fermented cattle manure; [u]Sugarcane bagasse; [t]Filter cake from sugar-alcohol mill; [s]Minimum significant difference; and [ns]Non-significant difference.

Most of the biometric characteristics evaluated in the *E. dysenterica* seedlings showed a correlation with the gas exchanges and chlorophyll *a* fluorescence (Table 7). The SL, NL, dry weight of the stems (DWS) and dry weight of the leaves (DWL) showed a positive correlation with the A, $\phi PSII$ and ETR. The F_v/F_m showed a positive correlation with the biometric characteristics of SL, NL, DWL and the TDW. The latter characteristic also showed a negative correlation with the F_0. Of the three quality indices evaluated (DQI, R/S and SL/RCD), the SL/RCD showed a positive correlation with A, F_v/F_m, $\phi PSII$ and ETR and a negative correlation with F_0. The R/S ratio showed a negative correlation with $\phi PSII$ and ETR. The DQI did not have a correlation with the gas exchanges or chlorophyll *a* fluorescence (Table 7).

Table 7. Matrix of Pearson's linear correlation between the characteristics of gas exchange and chlorophyll *a* fluorescence and the biometric characteristics and quality of *Eugenia dysenterica* DC. seedlings grown in different substrates

	A^z	E^y	g_s^x	Ci/Ca^w	F_0^v	F_v/F_m^u	$\phi PSII^t$	ETR^s
SL[r]	0.452*	0.303	0.255	-0.264	-0.332	0.491*	0.548*	0.529*
RCD[q]	0.212	0.074	0.100	-0.386	0.249	-0.077	0.322	0.326
NL[p]	0.530*	0.399	0.382	-0.187	-0.351	0.524*	0.632*	0.593*
DWS[o]	0.444*	0.317	0.313	-0.246	-0.151	0.323	0.527*	0.519*
DWL[n]	0.436*	0.277	0.240	-0.326	-0.272	0.482*	0.511*	0.487*
DWR[m]	-0.156	-0.080	-0.162	0.167	-0.205	0.076	-0.225	-0.231
TDW[l]	0.228	0.169	0.051	-0.108	-0.453*	0.501*	0.223	0.196
SL/RCD[k]	0.409*	0.310	0.232	-0.105	-0.559*	0.616*	0.437*	0.418*
R/S[j]	-0.346	-0.212	-0.235	0.346	0.024	-0.194	-0.410*	-0.429*
DQI[i]	-0.253	-0.156	-0.214	0.110	0.055	-0.128	-0.296	-0.285

Note. *Significant Pearson's correlation at the 5% probability level; [z]Liquid assimilation of CO_2; [y]Transpiration; [x]Stomatal conductance; [w]*Ci/Ca* ratio; [v]Minimum fluorescence adapted to the dark; [u]Maximum quantum yield of the PSII; [t]Effective quantum yield of the PSII; [s]Electron transport rate; [r]Stem length; [q]Root collar diameter; [p]Number of leaves; [o]Dry weight of stem; [n]Dry weight of leaves; [m]Dry weight of roots; [l]Total dry weight; [k]Ratio between length and diameter of stem; [j]Ratio between dry weight of the shoot and the root system; and [i]Dickson quality index.

The DQI was higher in plants cultivated in the Bioplant[®] substrate than in plants cultivated in the SB+CMC, SB+FCM and SCB+FC substrates (Table 8). In relation to the other substrates, the Bioplant[®] and the SB+RH substrate provided the seedlings that were cultivated in them with a higher ratio between the dry weight of the root system and the shoot (R/S). The SL/RCD was greater in the seedlings cultivated in the SCB+FC and SB+CMC substrates than in those cultivated in the other substrates.

Table 8. Ratio between stem length and root collar diameter (SL/RCD) and between the dry weights of the roots and shoots (R/S) as well as the Dickson quality index (DQI) in *Eugenia dysenterica* DC. seedlings grown in different substrates

Substrate	SL/RCD	R/S	DQI
	cm mm^{-1}	g g^{-1}	-
Bioplant[®]	3.12 b[z]	3.80 a	0.23 a
SB[y]+RH[x](1:1)	3.18 b	3.34 a	0.19 ab
SB+CMC[w](1:1)	4.11 a	1.16 b	0.15 b
SB+FCM[v](3:1)	3.03 b	1.44 b	0.17 b
SCB[u]+FC[t] (3:2)	4.42 a	1.59 b	0.15 b
MSD[s]	0.91	0.74	0.05

Note. [z]Means followed by the same letter do not differ from one another according to Tukey's test (p < 0.05); [y]Subsoil; [x]Rice husks; [w]Cattle manure compost (Corn silage + fermented cattle manure); [v]Fermented cattle manure; [u]Sugarcane bagasse; [t]Filter cake from sugar-alcohol mill; [s]Minimum significant difference; and [ns]Non-significant difference.

The highest leaf nitrogen (N) levels were provided by the SB+CMC and SCB+FC substrates (the SCB+FC value did not differ from that of the SB+FCM), while the lowest leaf N levels were observed in the Bioplant[®] and SB+RH substrates (Table 9). For phosphorous (P), the Bioplant[®] and SCB+FC substrates provided the highest leaf concentrations in relation to the other substrates. In the SB+RH substrate, the leaf P concentration was lower than the others were and did not differ from the SB+CMC; the latter did not differ from the SB+FCM substrate. The leaf potassium (K) concentrations were highest in the Bioplant[®] and SB+RH substrates. The latter showed no difference in relation to the SB+CMC and SB+FCM substrates, and these two were similar to the SCB+FC, which in turn had a lower concentration than the first two.

Table 9. Levels of macro and micronutrients in the leaf tissue of *Eugenia dysenterica* DC. seedlings cultivated in different substrates

Substrate	N	P	K	Ca	Mg	S	B	Fe	Cu	Mn	Zn
	g kg^{-1}						mg kg^{-1}				
Bioplant[®]	19.0c[z]	5.28a	13.76a	10.78b	2.98b	1.02a	171.2a	162.8[ns]	5.2[ns]	67.8b	16.2[ns]
SB[y]+RH[x](1:1)	15.6d	0.88c	10.24ab	11.12b	3.10b	0.24b	129.4c	168.4	5.0	53.4b	11.8
SB+CMC[w](1:1)	26.4a	1.92bc	7.04bc	25.68a	7.04a	1.06a	138.4b	164.0	4.8	38.4b	12.6
SB+FCM[v](3:1)	24.4b	2.80b	9.44bc	14.82b	7.56a	1.32a	122.6d	165.0	4.0	30.2b	12.8
SCB[u]+FC[t] (3:2)	26.0ab	4.24a	5.92c	35.98a	6.98a	1.18a	106.4e	121.8	5.0	170.8a	17.6
MSD[s]	1.95	1.35	4.04	10.32	2.12	0.33	4.83	80.12	1.23	42.50	7.00

Note. [z]Means followed by the same letter do not differ between one another according to Tukey's test (p < 0.05); [y]Subsoil; [x]Rice husks; [w]Cattle manure compost (Corn silage + fermented cattle manure); [v]Fermented cattle manure; [u]Sugarcane bagasse; [t]Filter cake from sugar-alcohol mill; [s]Minimum significant difference; and [ns]Non-significant difference.

Compared to the other substrates, there were higher leaf levels of calcium (Ca) in seedlings cultivated in the SCB+FC and SB+CMC substrates. In relation to the other substrates, the foliar magnesium (Mg) levels were lower in the plants cultivated in the Bioplant[®] and SB+RH substrates; the leaf sulfur (S) concentration was lower in the seedlings cultivated in the SB+RH substrate. The SCB+FC substrate provided the highest leaf concentration of manganese (Mn). For boron (B), the leaf concentrations were different in all of the substrates (which are arranged in descending order) as follows: Bioplant[®] > SB+CMC > SB+RH > SB+FCM > SCB+FC. In the *E. dysenterica* seedlings, the leaf concentrations of iron (Fe), copper (Cu) and zinc (Zn) were not influenced by the tested substrates.

The analyses of correlations between the leaf nutrient levels and the biometric characteristics and seedling quality show that N had the highest number of correlations with the following characteristics (Table 10): positive

for SL, RCD, NL, DWS, DWL and SL/RCD; negative for DWR, R/S and DQI; and there was no correlation for TDW. After N, the other elements that had the highest number of correlations were K and Mg. K had a positive correlation with DWR, R/S and DQI, and a negative correlation with SL, NL, DWS, DWL and SL/RCD. Mg had a positive correlation with SL, RCD, NL, DWS, DWL and SL/RCD. The leaf Ca levels had a negative correlation with the quality indices of R/S and DQI, and a positive correlation with SL, NL, DWS, DWL and SL/RCD. The S levels generally had positive correlations (with SL, RCD, NL, DWS and DWL), but there was one negative correlation (with R/S).

Of the micronutrients, B had the highest number of correlations, which included negative correlations with SL, NL and DWS and positive correlations with DWR, R/S and DQI. After B, Cu had the highest number of correlations; the only negative ones were with RCD, NL and DWS. The nutrients P, Mn and Zn had a correlation with DWR, SL/RCD and TDW, respectively, while Fe displayed no correlations.

Table 10. Matrix of the Pearson's linear correlation between the leaf mineral levels and the biometric characteristics and quality of *Eugenia dysenterica* DC. seedlings according to different substrates

	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
SL[z]	0.736*	-0.025	-0.638*	0.685*	0.641*	0.467*	-0.442*	-0.342	-0.085	0.286	0.193
RCD[y]	0.670*	-0.034	-0.342	0.369	0.805*	0.599*	-0.331	-0.502*	0.229	-0.162	0.157
NL[x]	0.724*	-0.095	-0.715*	0.576*	0.621*	0.444*	-0.519*	-0.447*	-0.221	0.209	0.032
DWS[w]	0.772*	0.088	-0.626*	0.601*	0.724*	0.592*	-0.522*	-0.404*	-0.093	0.229	0.202
DWL[v]	0.781*	-0.060	-0.665*	0.572*	0.638*	0.476*	-0.339	-0.286	-0.126	0.096	0.086
DWR[u]	-0.580*	0.406*	0.570*	-0.391	-0.705*	-0.170	0.603*	0.275	0.132	0.088	0.371
TDW[t]	0.083	0.396	0.014	0.100	-0.169	0.259	0.321	0.023	0.031	0.195	0.487*
SL/RCD[s]	0.502*	0.036	-0.582*	0.667*	0.321	0.212	-0.382	-0.114	-0.259	0.518*	0.188
R/S[r]	-0.841*	0.192	0.686*	-0.595*	-0.851*	-0.458*	0.607*	0.345	0.100	-0.044	0.044
DQI[q]	-0.579*	0.394	0.629*	-0.507*	-0.618*	-0.080	0.604*	0.229	0.211	-0.095	0.373

Note. *Significant Pearson's correlation at the 5% probability level; [z]Stem length; [y]Root collar diameter; [x]Number of leaves; [w]Dry weight of stem; [v]Dry weight of leaves; [u]Dry weight of roots; [t]Total dry weight; [s]Ratio between length and diameter of stem; [r]Ratio between dry weight of the root system and the shoot; and [q]Dickson quality index.

4. Discussion

The Bioplant[®] and SCB+FC substrates, which had the highest water content values, also had the highest levels of organic matter (Table 1). Organic matter is a component that contributes to water absorption, and among its benefits are improvements to the soil structure. The presence of subsoil in the substrates contributed to a reduction in the water content, together with the fact that the substrates had low levels of organic matter in relation to the Bioplant[®] and SCB+FC. Another component that influences the water absorption capacity comprises RHs. The substrate containing this material had the lowest RWC. According to Guerrini & Trigueiro (2004), the increase in the RH content in the substrates was inversely proportional to the water retention capacity in the same substrates. The lack of difference in the RWC in the *E. dysenterica* DC leaves shows that, despite the difference in the RWC in the substrates, they were able to satisfy the water requirements of the plants.

The positive correlation between the emergence speed index (ESI) of the seedlings and the water content in the substrates indicates that the amount of water absorbed by the substrate influenced the seedling vigor. The ESI found in the present study were higher than those found by Nietsche et al. (2004) of 0.08 and 0.1 for small and large cagaita seeds grown in different substrates, respectively. However, those authors did not report removing the coats from the seeds, a procedure that is believed to reduce the time to germination and emergence. In the present study, the seed coat was removed by scarification to overcome the suposed native seed dormancy. The percentage of emerged cagaita plants (PSE) was higher than observed by Souza et al. (2001), who reported 80.6%. Martinotto et al. (2007) reported that the removal of the integuments from the *E. dysenterica* seeds favored *in vitro* germination, reaching close to 90%.

As observed for the RWC, the evaluated substrates did not have an influence on gas exchange, and even variations in the water contents of the substrates were not enough to influence the *A, E,* g_s and *Ci/Ca*. Lemos-Filho (2000) reported g_s values of 0.26 mol m^{-2} s^{-1} during the wet season, which are higher than those

observed in the present study. The value closest to the one observed by the author was provided by the SB+FCM substrate (0.23 mol m^{-2} s^{-1}). Neves et al. (2009) observed an A value of 12 μmol m^{-2} s^{-1} and an E of 1.2 mmol m^{-2} s^{-1} in *Eugenia uniflora* plants that were cultivated hydroponically. In comparison to the values reported in the present study, the A values were higher and the E values and Ci/Ca were lower than those reported by Neves et al. (2009) for *E. uniflora*. The lack of difference between the F_v/F_m mean values in the present study and the similarity with the values observed (0.78) by Lemos-Filho (2000) do not indicate evidence of damage in the photochemical phase. The differences between the ETR values of the plants grown in the SCB+FC and SB+RH substrates may have contributed to the difference in the photosynthesis values between them, although the result was not significantly different. Lemos-Filho (2000) found an ETR value of 200 μmol m^{-2} s^{-1}, while in the present study, the ETR was no more than 140 μmol m^{-2} s^{-1}.

The length and diameter stem growth as well as the NL, and consequently the higher values for the dry weights of the stems and leaves, were favored by the substrates containing CMC, SCB and FC in relation to the RH component and Bioplant®. By contrast, the growth of the root system was greater in the Bioplant®, and it was influenced by the greater accumulation of the DWR. Morgado et al. (2009) found superior biometric characteristics when using SCB+FC, regardless of the ratio between them, during the production of sugarcane seedlings. The use of cattle manure has also shown good results in species such as *Acacia sp.* (Cunha et al., 2006), *Enterolobium contortisiliquum* (Araújo & Paiva Sobrinho, 2011) and *Harconia speciosa* (Silva et al., 2009); and *E. dysenterica* seedlings grown in combined substrates in the presence of cattle manure have shown better values for A, E, gs, Fv/Fm and ETR as well as better biometric characteristics (Mota et al., 2016a). However, in the present study, cattle manure was more promising when it was composted with corn silage.

Despite A not being influenced by the tested substrates, it was the only gas exchange characteristic to show a correlation with most of the biometric characteristics, and all were positive. The positive correlations between the biometric characteristics and the chlorophyll a fluorescence characteristics, which are linked to the photosynthetic potential with ETR, φPSII and Fv/Fm, together with A, demonstrate that the alterations of these characteristics influence the growth of the *E. dysenterica* seedlings.

In the analysis of the seedling quality indices, the Bioplant® substrate was observed to be associated with higher values for these indices, which suggests better quality and a better chance of survival after transplant to the field, given that it had the highest DQI and R/S and the lowest SL/RCD. The higher R/S shows that the plants invested photoassimilates into root system growth. According to Hunt (1990), the DQI for conifer seedlings should be greater than 0.20; values higher than this level were observed only for seedlings that were grown in Bioplant®. The lower SL/RCD indicates that the seedlings that were grown in the Bioplant® substrate are robust and less likely to suffer from etiolation. The correlation between SL/RCD and A as well as with the chlorophyll a fluorescence characteristics demonstrates that these values can be used to evaluate the quality of *E dysenterica* seedlings, especially fluorescence due to its status as a quick and simple evaluation method. The correlation between R/S, φPSII and ETR also contributed to this method, strengthening the use of chlorophyll a fluorescence.

However, when analyzing SL/RCD to account for the NL and the plant height, the seedlings grown in the Bioplant® only had two leaves, while those grown in SB+CMC and SCB+FC had approximately five leaves. If we consider that *E. dysenterica* has two opposite leaves per node (Silveira et al., 2013), the plants show one node in the Bioplant® substrate and three nodes in SB+CMC and SCB+FC. In addition, the seedlings grown in the Bioplant® have a height close to 60% of the height of those grown, for example, in SB+CMC, which produced the greatest height in absolute terms. If we also calculate the NL per centimeter of stem, we have 0.7 leaves/cm for plants grown in Bioplant®, while for the SB+CMC and SCB+FC substrates, which had higher SL/RCD values, we find 1 leaf/cm, thus confirming that these latter substrates were not responsible for etiolation in the seedlings. A similar characteristic occurs in the R/S, given that Bioplant® provided 3.8 times greater accumulation of dry weight in the root system than in the shoot, considering that the species prioritizes the development of the root system to the detriment of the shoot when young (Silveira et al., 2013).

Once the SCB+FC and SB+CMC substrates showed high leaf nutrient levels such as N, Ca, Mg, S as well as P and Mn in the case of the previous, a larger NL and greater height and better equilibrium in the distribution of photoassimilates and growth of the shoot, these substrates had the capacity to meet the nutritional needs of the plants more efficiently than the Bioplant® and SB+RH substrates. However, the correlations between the leaf nutrient levels and the biometric characteristics show that the nutrients N, Ca, Mg and S were the most limiting for the growth of the *E. dysenterica* plants. By contrast, when the foliar levels of the nutrients K, B and Cu increased, there were reductions in plant growth.

The foliar levels of N, Mg and S of 15, 3 and 0.4 g kg^{-1}, respectively, that were found by Melo & Haridasan (2009) in *E. dysenterica* plants are less than the levels observed in the present study, regardless of the treatment. In the comparison between the leaf macronutrient levels found by those authors and those for the plants grown in the SCB+FC and SB+CMC substrates, the levels are lower, except for the K level in the SCB+FC substrate. The superior performance of the *E. dysenterica* plants in the SCB+FC and SB+CMC substrates may be related to the mineralization of the sources of organic matter.

The influence of the leaf nutrient levels on the quality indices given by the positive and negative correlations, although they were not clearly defined and, despite the effect of the nutritional levels found here (especially when they were individualized) on the quality of the seedlings, demonstrates the importance of proper nutrition for obtaining quality seedlings.

5. Conclusion

The use of cattle manure compost (CMC), sugarcane bagasse (SCB) and filter cake from sugar-alcohol mills (FC) have shown to be appropriate for the production and nutritional supply of *E. dysenterica* seedlings. In general, the composition SB (subsoil) + CMC (1:1) proved to be the most suitable substrate for production of *E. dysenterica* seedlings also improving seedlings success in the field allowing its use on reforesting situations.

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