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Morphophysiological responses of *Ilex paraguariensis* seedlings to different substrates and fertilizations

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ABSTRACT
The increasing demand for *Ilex paraguariensis* St. Hill (yerba mate) by-products generated the need for producing a greater quantity of seedlings and improve their quality. Thus, the objective of this work was to evaluate the efficiency of different substrates combined with base fertilization for *Ilex paraguariensis* seedlings, through morphophysiological parameters. The experiment was conducted in a completely randomized design, in a 5 x 3 factorial arrangement, and the treatments consisted of five substrate formulations combined with two fertilizers, and a control without fertilization, in November 2013. The *I. paraguariensis* seedlings produced in the substrate consisted of subsurface soil, cattle manure and charred rice hulls (2:2:1) presented the greatest heights (53.36 cm), stem diameter (6.80 mm), leaf area (692.52 cm^2), root dry weight (6.2 g) and total dry weight (16.4 g). The efficiency of the different fertilizations used was similar regarding the seedling growth. The physiological parameters did not differ between treatments. The substrate composed of subsurface soil, manure and charred rice hulls (2:2:1) and fertilization with urea (0.7 g L^{-1}) single superphosphate (8.0 g L^{-1}) and potassium chloride (0.4 g L^{-1}) is recommended for producing *I. paraguariensis* seedlings.

Palavras-chave:
erva-mate
composto orgânico
espécie florestal

Parâmetros morfofisiológicos como resposta ao substrato e adubação em mudas de *Ilex paraguariensis*

RESUMO
A demanda crescente pela utilização de subprodutos de *Ilex paraguariensis* St. Hill (erva-mate) gerou a necessidade de se produzir mudas em maior quantidade e melhor qualidade. Assim, este estudo objetivou identificar a eficiência de substratos alternativos em combinação com adubação de base em mudas de *Ilex paraguariensis*, por meio da avaliação de parâmetros morfofisiológicos. Como tratamentos utilizou-se um fatorial 5 x 3 com cinco formulações de substrato combinadas a dois tipos de adubação, mais uma testemunha sem adubação, em novembro de 2013. O substrato composto por terra de subsolo, esterco bovino e casca de arroz carbonizada (2:2:1) apresentou as maiores médias para altura (53,36 cm), diâmetro do coleto (6,80 mm), área foliar (692,52 cm^2), massa seca radicular (6,2 g) e total (16,4 g). As adubações utilizadas proporcionaram eficiência semelhante no desenvolvimento das mudas; já os parâmetros fisiológicos não diferiram entre os tratamentos. Sugere-se, então, para a produção de mudas de *Ilex paraguariensis*, o uso do substrato composto por terra de subsolo, esterco bovino e casca de arroz carbonizada (2:2:1) e adubação com ureia (0,4 g L^{-1}), superfosfato simples (8,0 g L^{-1}) e cloreto de potássio (0,7 g L^{-1}).

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Introduction

The species *Ilex paraguariensis* (Aquifoliaceae), popularly known as yerba mate, plays an important socio-economic role in the southern region of Brazil (Bernardi et al., 2005). Leaves of yerba mate are dried to prepare a traditional tea, which is consumed by millions of people in South America, sometimes replacing the coffee, due to its stimulant effect (Oliveira & Waquil, 2015; Bergottini et al., 2015).

In recent years, the use and export of *I. paraguariensis* by new sectors, such as the pharmaceutical industry, cosmetics and beverage was intensified. Many studies have been conducted on this species due to its economic importance (Wendling et al., 2006; Horbach et al., 2011; Santin et al., 2013), aiming to increase the productivity of yerba mate, from the seedling production to the establishment in the field.

Several factors affect the seedling growth during the nursery phase, especially the substrate and fertilization, which can be more or less technified. Physical and chemical characteristics must be considered in studies on substrates to obtain a component combination that promotes the best plant growth. Moreover, organic residues are the major components of substrates, because their chemical attributes, which may reduce fertilization costs (Cunha et al., 2005). In addition, they improve the physical properties of substrate, increasing the water retention capacity, aeration and the availability of nutrients for the seedlings (Trazzi et al., 2013). Base fertilization must be evaluated together with the substrate, since this is a critical factor for producing healthy seedlings (Jacobs & Landis, 2009; Richardson et al., 2014).

These studies aim to find suitable combinations to produce high quality seedlings, enabling them to satisfactorily grow in the field and endure adverse biotic conditions. According to Akpo et al. (2014), the seedling survival in the field depends on good knowledge and practices of nursery management.

The evaluation of the quality of seedlings can be carried out through morphological and physiological parameters (Cooke & Suski, 2008; Campoe et al., 2014.). Therefore, the different parameters must be considered for the determination of the most suitable input to produce seedlings, in order to obtain more consistent results.

Several studies have been carried out to improve the yerba mate production, however, little information to assist producers of seedlings, who depend on locally available, low-cost inputs, is found in the literature. Thus, the objective of this study was to evaluate the efficiency of different substrates combined with base fertilization for *Ilex paraguariensis* seedlings, through morphophysiological parameters.

Material and Methods

The experiment was conducted in November 2013 at the Federal University of Santa Maria (UFSM) (29°43'13"S, 53°43'14"W), Santa Maria, Rio Grande do Sul (RS), Brazil, in partnership with the Technical Assistance and Rural Extension Enterprise of Rio Grande do Sul (EMATER-RS). According to the Köppen classification, the local climate is Cfa, with well-distributed rainfall and monthly mean rainfall of 100 to 170 mm (Alvarez et al., 2013).

The seedlings of *Ilex paraguariensis* were produced from seeds (Cambona 4), in polyethylene bags (300 cm^3) containing subsurface soil. The seedlings were transplanted to 10x20-cm (diameter x height) polyethylene bags with side and base holes, under different treatments, 60 days after planting, when the seedlings had about 11.0 cm of height and 2.6 mm of collar diameter.

The seedlings were maintained in a shade house after transplanted, with 50% of the natural light for 180 days. An automated micro-sprinkler irrigation system was used during this period, with four applications a day, for a total water depth of 12.00 mm.

The experiment was conducted in a completely randomized design, in a 5 x 3 factorial arrangement, with five substrate formulations combined with two fertilizers, and a control without fertilization, totaling fifteen treatments with three replications and ten plants per plot.

The substrates used consisted of subsurface soil (S), cattle manure (C) and charred rice hulls (R) (SCR) at a ratio of 2:2:1 (v/v/v); SCR at a ratio of 2:1:1; subsurface soil (S), pig manure (P) and charred rice hulls (R) (SPR) at a ratio of 2:2:1; SPR at a ratio of 2:1:1; and a control treatment (CT) with only subsurface soil. The base fertilization consisted of controlled release fertilizer (CRF) minipril (Osmocote® 15-09-12 6 g L^{-1}) (F1); conventional fertilization of early release with urea (0.7 g L^{-1}), single superphosphate (8.0 g L^{-1}) and potassium chloride (0.4 g L^{-1}) (F2); and a control treatment with no fertilizer (NF).

Substrate samples were analyzed in the Laboratory of Horticulture Substrate Analysis of the Federal University of Rio Grande do Sul, to verify their physical and chemical properties, according to the Normative Instruction No.17 of the Ministry of Agriculture, Livestock and Supply (Brasil, 2007) (Table 1).

Table 1. Physical and chemical attributes of substrates and organic compounds used for the production of *Ilex paraguariensis* seedlings

Substrate	DD ($kg\ m^{-3}$)	TP	AE	AW	RW	pH* (H_2O)	EC ($mS\ cm^{-1}$)
		(%)					
SCR 2:2:1 (v/v/v)	748.4	69.0	25.2	14.0	29.3	6.4	0.6
SCR 2:1:1 (v/v/v)	797.9	69.6	20.5	23.1	25.9	5.9	0.5
SPR 2:2:1 (v/v/v)	701.9	72.7	29.6	20.1	22.1	5.7	1.6
SPR 2:1:1(v/v/v)	847.3	71.1	22.2	23.6	23.1	5.5	0.9
CT	1197.1	52.9	10.2	18.0	24.6	5.4	0.1
CM	204.2	91.8	51.1	4.7	37.1	6.3	1.8
PM	439.8	78.3	35.9	16.1	26.2	6.1	3.7

SCR - Subsurface soil, cattle manure and charred rice hulls; SPR - Subsurface soil, pig manure and charred rice hulls; CT - Control treatment with subsurface soil; CM - Cattle manure; PM - Pig manure; * Determined in water, dilution 1:5 (v/v); EC - Electrical conductivity in solution 1:5 (v/v); DD - Dry density; TP - Total porosity; AE - Aeration space; AW - Available water; RW - Remaining water.

The cattle and pig manure used were subjected to a biological stabilization process before incorporated to the substrates.

Ten seedlings of each plot were measured, regarding their height (H), with a graduated (cm), and stem diameter (SD), with digital caliper rule (mm), at 180 days after the transplanting (DAT) of the seedlings. The shoots of the seedlings were separated from the roots, considering as shoots the portion above the substrate (leaves, branches and stem). The roots were washed in running water over a 0.84-mm mesh sieve to remove the substrate.

The leaves were placed on a white paper (21 x 29.7 cm), pressed with a transparent glass, photographed with a digital camera (SONY Cyber-shot 8.1 megapixel) with zoom of 1.4 at a height of 50 cm, and the images were processed in the software Image J to obtain the leaf area (LA). Subsequently, the samples of shoots and roots were stored in paper bags, dried in an oven at 65 ± 2 °C to constant weight and weighed on an analytical balance to verify the shoot (SDW), root (RDW) and total (TDW) dry weight of the plants.

The physiological parameters relative contents of chlorophyll "a", "b" and total were evaluated with a chlorophyll-meter (ClorofiLOG, CF 1030, Falker Agricultural Automation, Brazil) in two points of the middle third of the fifth leaf from the plant apex, and expressed in Falker Chlorophyll Index (FCI).

The evaluations of chlorophyll "a" fluorescence were performed at 8:00 and 10:00 h, on sunny days. The fifth leaf from the plant apexes were collected, wrapped in aluminum foil for 30 min for dark adaptation and analyzed using a portable modulated light fluorometer (JUNIOR-PAM, Walz, Germany), which provides the maximal fluorescence (F_m) and maximum quantum yield (F_v/F_m).

The data were tested for the assumption of normality in error distribution (Shapiro-Wilk) and homogeneity of variance (Bartlett), using the software *Action*. The data were then subjected to analysis of variance (ANOVA) and when the significant effect of treatment was shown by the F test, the means were compared by the Scott-Knott test ($p \leq 0.05$). The statistical software Sisvar (Ferreira, 2011) was used to analyze the data.

Results and Discussion

The substrate and fertilization factors significantly affected all morphological variables. The substrate SCR 2:2:1 presented higher values of seedling height (H) and stem diameter (SD) than the other treatments, promoting a better plant growth with averages of 53.36 cm (H) and 6.80 mm (SD) (Table 2). The SDW, RDW and TDW values presented similar trend, since all of them were favored by the use of the substrates SCR 2:2:1 and SPR 2:1:1. The use of the substrate CT resulted in the lowest seedling growth, especially of the shoots (Table 2).

Wendling et al. (2006) evaluated different types of substrate for seedling production and emphasized the use of cattle manure as beneficial to the plant growth (H and SD), which was also observed in this present study; and best cost benefit ratio of the substrate consisting of cattle manure (40%) and sawdust (60%), which contributed to the seedling growth, reaching approximately 12.0 cm of height and 2.30 mm of stem diameter at 180 days after transplanting to 100-cm^3 tubes. Lourenço et al. (1999) also found higher average values of SDW (1.63 g) and RDW (0.53 g), evaluating the effectiveness of substrates consisted of subsurface soil and cattle manure in 600-cm^3 containers. These values were lower than those found in the present study due to their smaller container volumes, which may have restricted the seedling growth, denoting the importance of combining the substrate and fertilization to an appropriate container size.

The volume of cattle manure used in the substrate SCR 2:2:1 was favorable for the seedling growth, since important physical characteristics of the substrate, such as dry density (DD) (748.4 kg m^{-3}), aeration space (AE) (25.2%), and remaining water (RW) (29.3%) (Table 1) were improved. The conditions of this treatment probably provided organic matter and nutrients in adequate amounts, since according to Caldeira et al. (2008), some organic compounds have great importance in providing nutrients to plants.

The DD (748.4 kg m^{-3}) of the substrate SCR 2:2:1 was above the values recommended by Kämpf (2005) (400 to 500 kg m^{-3}), however not limiting the plant growth, which was probably because adequate AE (25.2%) of the substrate, which according to Penningsfeld (1983), must be at about 30% to enable an appropriate supply of oxygen to the roots.

In the present study, the substrates with cattle manure showed appropriate values of remaining water (RW), indicating a good water storage capacity (Table 1). According to Verdonck & Gabriels (1988), the RW represents the wastewater after the substrate is subjected to 100 hPa voltage, which must have values of 25 to 30%. Moreover, according to Schmitz et al. (2002), substrates with very low RW values may indicate a lower water storage capacity, requiring more frequent watering for the seedlings.

The available water (AW) of all substrates was lower than the values recommended by De Boodt & Verdonck (1972) (24-40%), however, it was not a limiting factor for the *Ilex paraguariensis* seedling growth. This result was probably

Table 2. Mean height (H), stem diameter (SD), leaf area (LA), shoot (SDW), root (RDW) and total (TDW) dry weight of *Ilex paraguariensis* seedlings subjected to different substrates and fertilization at 180 days after transplanting

Substrate	H (cm)	SD (mm)	LA (cm^2)	SDW	RDW (g)	TDW
SCR 2:2:1 (v/v/v)	53.36 a*	6.80 a	692.52 a	10.2 a	6.2 a	16.4 a
SCR 2:1:1 (v/v/v)	44.98 b	6.00 b	545.25 a	6.5 b	2.6 b	9.1 b
SPR 2:2:1 (v/v/v)	43.98 b	5.74 b	463.64 b	8.7 b	2.9 b	11.6 b
SPR 2:1:1 (v/v/v)	45. 47 b	5.97 b	602.02 a	10.8 a	4.9 a	15.7 a
CT	35. 25 c	5.32 b	304.58 c	5.8 b	3.5 b	9.3 b

SCR = subsurface soil, cattle manure and charred rice hulls; SPR = subsurface soil, pig manure and charred rice hulls; CT = control treatment with subsurface soil. *Means followed by the same letter in the column do not differ by the Scott-Knott test ($p < 0.05$)

Table 3. Mean height (H), stem diameter (SD), leaf area (LA), shoot (SDW), root (RDW) and total (TDW) dry weight of *Ilex paraguariensis* seedlings subjected to different fertilization at 180 days after transplanting

Fertilization	H (cm)	SD (mm)	LA (cm^2)	SDW	RDW (g)	TDW
F1	49.34 a*	6.16 a	716.95 a	11.98 a	4.90 a	16.88 a
F2	47.38 a	6.35 a	562.30 a	9.75 a	4.89 a	14.64 a
NF	35.10 b	5.38 b	307.15 b	4.51 b	2.29 b	6.80 b

F1 - Controlled release fertilizer (CRF) (NPK 15:09:12 6 g L^{-1}); F2 - Conventional fertilization of early release with urea (0.7 g L^{-1}), single superphosphate (8.0 g L^{-1}) and potassium chloride (0.4 g L^{-1}); NF - Control treatment with no fertilizer. *Means followed by the same letter in the column do not differ by the Scott-Knott test ($p < 0.05$)

due to the low water requirement at the initial growth of this species, since other important features, such as AE and RW were within the recommended range. Schmitz et al. (2002) evaluated substrate formulations consisting of soil, sand, turf, charred rice hulls and decomposed residues of acacia (*Acacia mearnsii* De Wild) and found no combination with AW values within the ideal range.

These results may be related to the water requirements of each species in different growth stages. According to Ferraz et al. (2005), to assess the physical characteristics of the substrate that limit and those that favor the growth of each species is important to select the best ones for each purpose.

The lowest AE and TP and highest DD values were found in the treatment CT (Table 1), which probably hindered the seedling growth (Table 2). Thus, the use of low dry density components, such as cattle manure is recommended to avoid such problems.

According to Wendling & Brondani (2015), in addition to the subsurface soil and cattle manure, other renewable materials must be evaluated as substrates for yerba mate seedlings, such as coconut fiber and other organic compounds. However, the formulation of a substrate with desirable physical characteristics must take into account the components availability and costs, which are limiting factors for the use of coconut fiber as a substrate component in the Southern Brazil, for instance.

The fertilizations used in the experiment equally favored the seedling growth, contributing to shoot and root growth, since the morphological variables presented no statistical difference between the fertilizers F1 and F2, which provide higher averages than the treatments with no fertilization (Table 3). Brachtvogel & Malavasi (2010) found similar results of SD, SDW and TDW for *Peltophorum dubium* (spreng.) Taub seedlings with controlled release fertilizers and early release ones.

According to Wu & Liu (2008), controlled release fertilizers gradually provide nutrients to plants, meeting their nutritional needs. However, in the present study this advantage was not observed in any parameters, probably due to the use of polyethylene bags, which combined with the proper irrigation management, reduced the leaching of the fertilization F2, providing a longer availability of nutrients to the seedlings.

Base fertilization is essential for biomass accumulation, since deficiencies of essential nutrients compromise the plant metabolism, reducing the seedlings growth. The use of conventional fertilization (F2) for yerba mate seedlings is recommended based on the results found, since it provided an adequate seedling growth at lower costs.

The physiological parameters relative content of chlorophyll "a", "b" and total were similar among treatments, presenting means of 34.26 (a), 12.58 (b) and 46.8 (total) FCI. The method used was simple and non-destructive, allowing the immediate evaluation of the FCI values. The factors evaluated had no effect on the relative contents of chlorophyll, despite some treatments have presented lower growth. According to Ritchie et al. (2010), evaluations of physiological attributes are important to identify quality problems of plants that are not easily assessed.

The chlorophyll "a" fluorescence showed also no significant effect of treatments, with general means of 1435 (F_m) and 0.702 (F_v/F_m). According to Zanandrea et al. (2006), low F_m values indicate power dissipation in the antenna of photosystem II (PSII), decreasing its assimilation. Araújo (2007) also related the variable F_v/F_m to the ability of the PSII in capturing radiant energy and transfer it to photochemical reactions, which in an optimal condition has values near 0.83.

The reduced growth caused by some treatments in the present study did not negatively affect the physiological parameters, thus, despite the lower growth of the seedlings, these treatments did not represent a stress condition to the plants, denoting the importance of evaluating the morphological and physiological parameters to assess the quality of seedlings.

Conclusion

1. The substrate consisted of subsurface soil, cattle manure and charred rice hulls (2:2:1) was the most efficient for the production of *Ilex paraguariensis* seedlings regarding the plant growth. The addition of a base fertilization with urea (0.7 g L^{-1}), single superphosphate (8.0 g L^{-1}) and potassium chloride (0.4 g L^{-1}) is recommended to this substrate for reducing the production costs.

2. The physiological parameters showed no differences between the treatments evaluated, however, they must be considered to evaluate *Ilex paraguariensis* seedlings, since they show stress situations that may not be evident through the morphological parameters.

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