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SOIL SCIENCE

Doses and critical phosphorus level for yerba mate (*Ilex paraguariensis* St. Hil.) clones

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ABSTRACT: In Brazil, the optimal dose of phosphorus in the cultivation of yerba mate (*Ilex paraguariensis* St. Hil.) clones has yet to be defined. This study verified the productivity of yerba mate clones in response to the application of P doses and determined the critical level of the nutrient in soil and yerba mate leaves. The experiment was developed in Itaiópolis-SC, in humic cambisol, from 2013 to 2020. Five doses of the nutrient were evaluated in two clones (BRS-BLD Aupaba and F2) of yerba mate. Fertility was defined by planting/post-planting (0, 2.3; 4.5; 6.8, and 9.0 g plant^{-1}), canopy formation 1 (0, 10, 20, 30, 40, and 50 g plant^{-1}), canopy formation 2 (0, 15, 30, 45, and 60 g plant^{-1}), and production (0, 16.7; 33.3; 50.0; 66.7 kg ha^{-1}). Harvests took place between July 2015 and January 2020, at 18-month intervals. The productivity of leaves, fine branches, and thick branches was evaluated. In the 2020 harvest, soil and leaf samples were collected to determine the critical level of P. The productivity of clone components increased with phosphate fertilization in all evaluated harvests. The critical levels of P for clones, Aupaba and F2, were 5.2 and 6.3 mg dm^{-3} in soil and 1.03 and 1.11 g kg^{-1} in leaves, respectively. Yerba mate is demanding of P, expressing maximum productivity in the planting phase, canopy formation 1 and 2, and production when doses of 5, 25, 40 g plant^{-1}, and 35 kg ha^{-1} of P_2O_5 are applied, respectively.
Key words: yerba mate fertilization, soil fertility, leaf content, macronutrients.

Doses e nível crítico de fósforo para erva-mate (*Ilex paraguariensis* St. Hil.) clonal

RESUMO: No Brasil, a dose de fósforo no cultivo de clones de erva-mate ainda não está definida. O objetivo do estudo foi verificar a produtividade de clones de erva-mate em resposta a aplicação de doses de P e determinar o nível crítico do nutriente no solo e folhas de erva-mate. O experimento foi implantado em Itaiópolis-SC, em Cambissolo Húmico, em 2013. Foram avaliadas cinco doses do nutriente em dois clones (BRS-BLD Aupaba e F2) de erva-mate. As adubações foram definidas como de plantio/pós-plantio (0, 2,3; 4,5; 6,8 e 9,0 g planta^{-1}), formação de copa 1 (0, 10, 20, 30, 40 e 50 g planta^{-1}), formação de copa 2 (0, 15, 30, 45 e 60 g planta^{-1}) e produção (0, 16,7; 33,3; 50,0; 66,7 kg ha^{-1}). As colheitas ocorreram entre julho de 2015 e janeiro de 2020, com intervalos de 18 meses. Avaliou-se produtividade de folhas, galhos finos e galhos grossos. Na safra de 2020, foram coletadas amostras de solo e folhas para determinação do nível crítico de P. A produtividade dos componentes dos clones aumentou com a adubação fosfatada em todas as safras avaliadas. Os níveis críticos de P para os clones Aupaba e F2 foram 5,2 e 6,3 mg dm^{-3} no solo e 1,03 e 1,11 g kg^{-1} nas folhas, respectivamente. A erva-mate é exigente em P, expressando produtividade máxima na fase de plantio, formação de copa 1 e 2 e produção, quando aplicado doses de P_2O_5 em torno de 5, 25, 40 g planta^{-1} e 35 kg ha^{-1}, respectivamente.
Palavras-chave: adubação de erva-mate, fertilidade do solo, teor foliar, macronutrientes.

INTRODUCTION

Yerba mate (*Ilex paraguariensis* St. Hil.) is a species native to the southern region of South America, where it plays important environmental and socioeconomic roles. Brazilian production reached 880,000 metric tons in 2019 and was concentrated in the south of the country, with the state of Paraná being the largest producer (IBGE, 2020). Leaves and branches are harvested for industrial processing, and the main form of consumption is infusion with hot (chimarrão) or cold water (tererê). Yerba mate is also consumed as a tea obtained from dry and toasted leaves, mostly in countries like Brazil, United States, Germany, and Syria. Recently, cosmetics, energy drinks, and food supplements have been prepared from yerba mate leaves (CARDOZO JUNIOR & MORAND, 2016). The interest in the species is due to its bioactive compounds, which give it antioxidant, anti-inflammatory, diuretic, and energetic properties

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(BERTÉ et al., 2011), in addition to its nutritional value (SOUZA et al., 2015; ULBRICH et al., 2022; MAGRI et al., 2022).

Yerba mate occurs naturally in soils with low fertility, low pH, and a high aluminum content (REISSMANN et al., 1985; WENDLING & SANTIN, 2015). Hence, the species has been considered nutritionally undemanding and fertilization has not been implemented by producers. However, recent studies have shown that yerba mate responds positively to the supplementation of nutrients (RIBEIRO et al., 2008; BARBOSA et al., 2018). SANTIN et al. (2013) concluded that the maximization of yerba mate growth depends on good availability of N, K, and Ca in the soil, as well as high levels of P. Although, clonal variations have been selected and recommended for yerba mate cultivation (WENDLING & SANTIN, 2015), little is known about nutrient requirements for contrasting yerba mate clones.

Phosphorus is fundamental in energy storage and transfer processes of plants (MARSCHNER, 2012). It is the fifth most absorbed nutrient by yerba mate (REISSMANN et al., 1985; SOUZA et al., 2008; OLIVA et al., 2014; BARBOSA et al., 2020), with the crop requiring low levels of P supplementation (REISSMANN et al., 1985; RADOMSKI et al., 1992). However, the plants, especially the seedlings, often respond to high supplementation. CECONI et al. (2007) and SANTIN et al. (2008) reported increased growth of yerba mate seedlings with phosphate fertilization. SANTIN et al. (2013) reported greater seedling growth with a high P content in the soil (18.5–28.6 mg dm^{-3}), indicating that yerba mate in the production phase could also respond positively to increased P content in the soil. However, information on P demand of yerba mate under field conditions is still scarce and, to some extent, contradictory. PANDOLFO et al. (2003) reported no response to P application in yerba mate in the production phase in soil with an availability of 4.2 mg dm^{-3} of the nutrient, while SANTIN et al. (2017) obtained increased productivity with the application of phosphate fertilizer with 1.32 mg dm^{-3} of P, indicating that the critical level of P in the soil may be a determining factor in the response to phosphate fertilization.

Low P availability in the soil induces the plant to activate its own mechanisms, or use other organisms, to acquire this nutrient. One of these mechanisms is molecular exudation of the rhizosphere, which solubilizes less available forms of P and may be one of the factors responsible for the hyperaccumulation of Mn in the leaves (MAGRI et al., 2020; MOTTA et al., 2020). The P supply increases plant development and; consequently, the total root exudation of molecules (which also solubilizes other elements) may increase (VANCE et al., 2003; LIN et al., 2011). However, the uptake of P and other elements by plants will depend on plant genotype and soil type (BARBOSA et al., 2018). Few studies have determined the effect of phosphate fertilization on the nutritional composition of yerba mate, especially in crops in the production phase. SANTIN et al. (2013) and BARBOSA et al. (2018) studied seedlings and reported that P application positively influenced N, P, K, Ca, Mg, Zn, Mn, Cu, Ni, B, Fe, and Mo levels in leaves of yerba mate seedlings. Under field conditions, BARBOSA (2017) reported little influence of phosphate fertilization on P content in yerba mate leaves.

Most of the harvested product of yerba mate is composed of leaves and thin branches and the amount of nutrients removed from the cultivation area is substantial (REISSMANN et al., 1985; BISSO & SALET, 2000). This removal of nutrients by perennial species such as yerba mate varies according to time of harvest (CARON et al., 2014), origin (OLIVA et al., 2014), cultivation in full sun or in the shade (CARON et al., 2014), and type of pruning (SOUZA et al., 2008). Thus, the quantification of elements extracted and removed by yerba mate plants can assist in the management of soil fertility and the maintenance of yerba mate productivity and quality.

Therefore, this study determined the contribution of phosphate fertilization to the productivity and nutritional composition of two yerba mate clones, and determined the critical level of P in the soil and leaves to develop effective fertilization practices.

MATERIALS AND METHODS

The study was conducted as a field experiment in September, 2013, in Itaiópolis, Santa Catarina, Brazil (26°25′44 S and 49°55′45″ E; altitude 920 m). According to Santos et al. (2013), the soil in the experimental area was humic cambisol with a clay texture; the chemical properties and clay content before implementing the experiments are shown in table 1. According to the Koppen classification, the regional climate is Cfb, with an average annual temperature of 17.1 °C and average annual precipitation of 1,626 mm (ALVARES et al., 2013).

The treatments resulted from the combination of female F1-BRS BLD Aupaba

Table 1 - Chemical properties and clay content of the soil in the 0–20-cm layer before the experiment was initiated.

pH	Ca^{2+}	Mg^{2+}	Al^{3+}	$H+Al^{3+}$	CEC	V	m	Clay	OC	P	K	$S\text{-}SO_4^{2-}$
H_2O	----------------------cmol$_c$ dm^{-3}----------------------					---------%--------		g kg^{-1}	g dm^{-3}	-----------mg dm^{-3}----------		
4.9	2.39	0.51	4.71	13.7	17.12	20	57.9	510	23.8	8.2	204	7.95

Ca^{2+}, Mg^{2+}, Al^{3+} (extracted with KCl 1 mol L^{-1}); $S\text{-}SO_4^{2-}$ (extracted with monocalcium phosphate); H + Al^{3+} (extracted with Ca acetate 0.5 mol L^{-1}); organic carbon (OC) (potassium dichromate volumetric method); K^+ and P (Mehlich-1 extractor; soil: solution ratio 1:10); clay (densimeter method); CEC = cation exchange capacity at pH 7.0; m = Al^{3+} saturation.

(Aupaba) and F2-not yet registered (F2) clones from matrices in São Mateus do Sul, PR; with five doses of P; resulting in ten treatments. The fertilizers applied during the experiment were defined as planting/post-planting, canopy formation 1, canopy formation 2, and production fertilization, and were applied from planting until the first harvest, between the first and second harvests, second and third harvests, and third and fourth harvests, respectively. The doses in each treatment are described in table 2. In these phases, N was also applied at annual doses of 7.5, 20, and 40 g plant^{-1}, and 120 kg ha^{-1}; and K_2O at annual doses of 4.8, 10, 25 g plant^{-1}, and 60 kg ha^{-1}, respectively. Fertilization was applied twice: half of the annual dose in February and half in September. At planting, fertilizer was applied in the hole, incorporated into 15 dm^{-3} of soil. All subsequent doses were applied in the plant crown projection area. An amount of 3.5 t ha^{-1} of dolomitic limestone (CaO and MgO; 31.6 and 20.3%, respectively) was applied over the total surface area before planting to provide Ca and Mg to the plants. Urea (45% N), triple superphosphate (42% P_2O_5), and KCl (60% K_2 O) were used as sources of N, P, and K, respectively.

The treatments were arranged in a split-plot scheme, with nutrient doses in the plot and the yerba mate clones in the subplot. A randomized block design was used with four repetitions, totalizing 20 plots. The experimental unit consisted of 12 useful plants of each clone, plus two border lines composed of plants propagated by seed. Spacing was 2.0 m between plants and 2.5 m between rows.

After the first harvest in July 2015, the experiment was evaluated at 18-month intervals until January 2020, totaling four harvests. The harvested biomass was fractionated into leaves (LV), thin branches (TnB) (Ø ≤ 7 mm), and thick branches (TkB) (Ø > 7 mm), the sum of LV + TnB being the commercial biomass (COM). The quantification of the total biomass (TT) produced resulted from the sum of COM and TkB. The productivity was obtained by multiplying the mean commercial biomass produced (green mass) per plant and the plant population per hectare. The LV/TnB ratio and the harvest index (HI) were calculated using the equation:

$$HI = (COM \times 100)/(LV + TnB + TkB) \quad (1)$$

In January 2020, the P content was determined using the index leaf, which was determined by randomly collecting 20 fully expanded leaves on branches containing current buds, in the middle third of the canopy (NEPAR, 2019) from five representative plants of each plot. Samples of leaves, thin branches, and thick branches were also collected from the harvested biomass of each treatment to

Table 2 - Phosphorus dose (p_2o_5) by treatment and growth stage of yerba mate plants.

Treatment	----------------------Crop growth phase----------------------			
	Planting/post-planting	Up to first harvest	From first to second harvest	Production
---------------------------------P_2O_5 dose---------------------------------				
	------------------g plant^{-1} year^{-1}------------------			kg ha^{-1} year^{-1}
T1	0.0	0	0	0.0
T2	2.3	10	15	16.7
T3	4.5	20	30	33.3
T4	6.8	30	45	50.0
T5	9.0	40	60	66.7

determine N, P, K, Ca, Mg, and S in the current harvest. The collected materials were dried in an oven at 60 °C until reaching a constant weight, ground in a coffee grinder, and passed through a 0.5 mm sieve. Nitrogen content was determined by dry combustion of 20 mg of sample in a non-metallic, elemental analyzer (Elementar, VarioEL III model, Hanau, Germany). Acetanilide (Acros Organics; Geel, Belgium) was used for instrument calibration. The contents of the other nutrients were obtained by acid digestion (MARTINS & REISSMANN, 2007) of 0.2 g of dry sample in a closed system using a microwave oven with a nitric acid solution (2 mL of HNO_3, 1 mL of hydrogen peroxide, and 1 mL of ultrapure water). The concentration of each nutrient in the extract was determined using inductively coupled plasma optical emission spectrometry (ICP-OES; axial mode; Varian 720-ES model, Mulgrave, Victoria, Australia). For ICP-OES calibration, solutions containing increasing concentrations of analyzed elements were prepared from a multi-elemental standard solution (QuimLab, Curitiba, Brazil).

The soil was collected with a soil sampling auger from the 0–10- and 10–20-cm layers after the January 2020 harvest, in ten places per plot, from the crown projection area of the plants. The soil collected was then homogenized to compose one sample per plot. Samples were dried in an oven at 40 °C and passed through a 2-mm sieve to obtain the available P content, according to the method of Tedesco et al. (1995), with extraction using Mehlich-1 and nutrient concentration analysis using ICP-OES.

The data obtained were subjected to the Shapiro–Wilk normality test, analysis of variance (ANOVA), and, in case the case of a significant effect of dose, regression analysis. The doses providing the maximum COM yield and critical P level in leaves and soil were obtained, respectively, by regression equation derivation and by relating the content of the nutrient in the leaves and soil to 90% of the maximum COM yield obtained. The data from the January 2020 crop were used to determine the critical level when the plants were already in the production phase.

RESULTS AND DISCUSSION

COM and TT productivity increased as a function of P fertilization in all crop seasons evaluated (Figure 1). The clones responded differently to P application only in the 2020 harvest (Figures 1 g, h). In this harvest, without P application, clone F1 produced less LV, TkB, and COM compared to clone F2. Conversely, F1 was more productive at the maximum productivity dose, and can be considered more responsive to P fertilization than F2. The clones differed in TkB productivity in the 2015 harvest; TnB, LV, and COM in 2017; and TkB in the 2018 harvest (Table 3). The highest COM productivity was obtained with an application of 5.2, 24.5, and 39.9 g plant^{-1} of P_2O_5 in the 2015, 2017, and 2018 harvests, respectively; and 34.90 and 35.77 kg ha^{-1} of P_2O_5 in clones Aupaba and F2, respectively, in the 2020 harvest. These doses resulted in a productivity of 0.97, 3.92, 7.88, 14.12, and 15.78 Mg ha^{-1} of yerba mate COM. The productivity obtained in the production phase (2020 harvest) was double that of the Brazilian average of 7.7 Mg ha^{-1} (IBGE, 2020).

Although, yerba mate has a low demand on soil fertility (REISSMANN et al., 1985; RADOMSKI et al., 1992), the present study proves that the species responds to P application from planting to production, with increases of 30–95%, compared to no P application. Similar results were observed by CECONI et al. (2007) and SANTIN et al. (2017). However, the current study is the first reporting that yerba mate clones respond to fertilization under field conditions. BARBOSA et al. (2018) reported a response of yerba mate clones to P application under greenhouse conditions.

The increased productivity due to phosphate fertilization (Figure 1) did not affect the percentage of LV in COM, indicating that LV and TkB productivity increased proportionally. In the 2015, 2017, 2018, and 2020 harvests, the mean percentage of LV in COM was 77.6, 78.6, 75.6, and 72.7 %, respectively. This is a parameter of interest to the industry, considering that most chemical compounds found in the species are concentrated in the leaves (SOUZA et al., 2015). However, phosphate fertilization does not appear to influence the percentage of LV in COM, since SANTIN et al. (2017) also reported no influence of this nutrient in this relationship.

The mean harvest indices were 72.7, 74.7, 69.5, and 68.9 in the 2015, 2017, 2018, and 2020 harvests, respectively. These results contrast to those of SANTIN et al. (2017), in which the HI decreased with increasing P dosage. The clones differed from one another only in the 2015 harvest, when the Aupaba clone had a mean of 71.2, and the F2 clone, of 74.3. This result showed that the Aupaba clone uses a greater amount of energy in TkB production than F2 before the first harvest to sustain COM production. This indicated that the HI of yerba mate can be affected by plant age and genetics, and there may be ways in which the HI is influenced by nutrient application. However, higher HI values are more

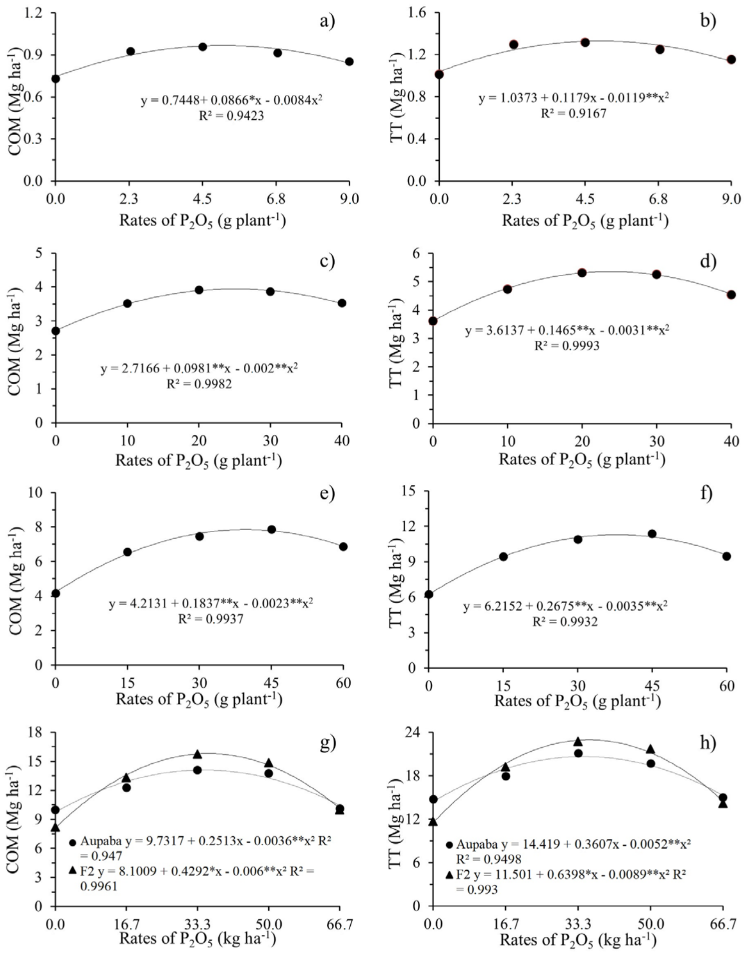

Figure 1 - Commercial green yerba mate mass (com) and total biomass (tt) productivity as a function of phosphate fertilization dose in the 2015 (a, b), 2017 (c, d), 2018 (e, f) and 2020 (g, h) harvests, respectively.

** and *; significant at 1 and 5% probability, respectively.

interesting to the producer, since a higher HI means a higher percentage of the total harvest available for commercial use.

Phosphate fertilization increased P availability in the soil in the 0–20-cm layer (Figure 2), with the highest content being 41.9 mg dm^{-3}. The highest increase occurred in the 0–10-cm layer (Figure 2), with a P content of 75.8 mg dm^{-3}; however, there was also an increased availability in the 10–20-cm layer with the highest dose of P_2O_5 applied (Figure 2). The specific adsorption that occurs between P and Fe and Al oxides and hydroxides in the soil reduces nutrient mobility in the profile (NOVAIS & MELLO, 2007), justifying the greater accumulation of the nutrient in the 0–10-cm layer, since the application was on the soil surface. Thus, in systems where the soil is left undisturbed, the expected P content increase in the 10–20-cm layer is less substantial. The results are corroborated by GALETTO (2016), who also reported increased P content in this layer with P application in a direct drilling system. Importantly, there is no soil disturbance in this type of system or in forest species plantation, including yerba mate. Successive fertilization with high doses of P saturates the higher energy adsorption sites, so that the fraction of the nutrient bound with a lower degree of energy to the soil can be displaced into the soil solution more easily (ERNANI, 2016). Thus, the increased P

Table 3 - Mean leaf (lv), thin branch (tnb), thick branch (tkb), and commercial yerba mate productivity (com; mg ha^{-1}) of yerba mate clones, aupaba and f2, as a function of phosphate fertilization.

Component	Clone						Mean/Eq.	R^2
2015 harvest								
		P_2O_5 dose(g $plant^{-1}$)						
		0	2.3	4.5	6.8	9.0		
LV	Aupaba	0.54	0.71	0.74	0.69	0.64	0.66	
	F2	0.59	0.73	0.77	0.72	0.69	0.70	
	Mean	0.57	0.72	0.75	0.70	0.66	Q*	0.92
TnB	Aupaba	0.16	0.21	0.21	0.20	0.18	0.19	
	F2	0.17	0.21	0.20	0.22	0.20	0.20	
	Mean	0.16	0.21	0.21	0.21	0.19	Q**	0.92
TkB	Aupaba	0.28	0.40	0.39	0.35	0.31	0.35 a	
	F2	0.28	0.34	0.32	0.31	0.30	0.31b	
	Mean	0.28	0.37	0.36	0.33	0.30	Q**	0.83
2017 harvest								
		P_2O_5 dose (g $plant^{-1}$)						
		0	10	20	30	40		
LV	Aupaba	1.97	2.80	2.84	2.98	2.49	2.62b	
	F2	2.34	2.78	3.27	3.06	3.03	2.90 a	
	Mean	2.15	2.79	3.06	3.02	2.76	Q**	0.99
TnB	Aupaba	0.52	0.74	0.81	0.79	0.67	0.71b	
	F2	0.59	0.71	0.90	0.90	0.86	0.79 a	
	Mean	0.56	0.72	0.86	0.85	0.77	Q**	0.99
TkB	Aupaba	0.80	1.26	1.34	1.41	0.88	1.14	
	F2	1.04	1.20	1.47	1.38	1.15	1.25	
	Mean	0.92	1.23	1.41	1.39	1.02	Q*	0.97
2018 harvest								
		P_2O_5 dose (g $plant^{-1}$)						
		0	15	30	45	60		
LV	Aupaba	3.05	4.80	5.30	5.82	5.43	4.88	
	F2	3.17	5.00	6.13	6.09	5.07	5.09	
	Mean	3.11	4.90	5.71	5.95	5.25	Q**	0.99
TnB	Aupaba	1.00	1.56	1.86	1.90	1.64	1.59	
	F2	1.13	1.79	1.67	1.95	1.61	1.63	
	Mean	1.07	1.68	1.76	1.92	1.63	Q**	0.96
TkB	Aupaba	2.02	2.73	2.96	3.16	2.66	1.70b	
	F2	2.12	3.01	3.83	3.79	2.53	3.06 a	
	Mean	2.07	2.87	3.40	3.48	2.60	Q**	0.96
2020 harvest								
		P_2O_5 dose (kg ha^{-1})						
		0	16.7	33.3	50	66.7		
LV	Aupaba	7.21 a	8.96 a	10.53 a	10.02 a	7.22 a	Q**	0.95
	F2	6.12b	9.81 a	11.23 a	10.94 a	7.13 a	Q**	0.98
	Mean	-	-	-	-	-		
TnB	Aupaba	2.81	3.36	3.61	3.78	2.94	3.30	
	F2	2.11	3.52	4.54	3.91	2.87	3.39	
	Mean	2.46	3.44	4.08	3.85	2.90	Q**	0.98
TkB	Aupaba	4.81 a	5.67 a	6.99 a	5.96 a	4.88 a	Q**	0.87
	F2	3.50b	5.94 a	7.02 a	6.92 a	4.24 a	Q**	0.98
	Mean	-	-	-	-	-		

Eq = equation; Q = quadratic equation; ** and *; significant at 1 and 5% probability, respectively; means with different letters differ between clones (ANOVA; P < 0.05).

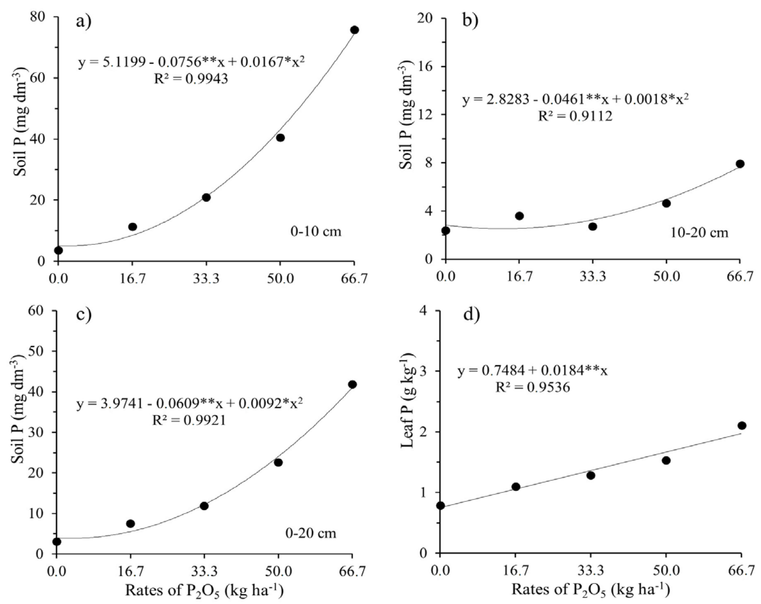

Figure 2 - Soil p content in the 0–10-cm soil layer (a), 10–20 cm (b), and 0–20 cm layers (c) and in leaves (d) as a function of posphate fertilization.

** and *; significant at 1 and 5% probability, respectively.

content in the 10–20-cm layer observed in the current study may have occurred due to P detachment caused by saturation of the adsorption sites in the 0–10-cm layer. The treatment without nutrient application showed a P content in the 0–20-cm layer of the soil of 3.9 mg dm^{-3}, which was lower than the content before the implementation of the experiment, of 8.2 mg dm^{-3}, indicating the removal of nutrients by harvesting.

The critical P level in the 0–20-cm layer, extracted using Melich-1, was 5.2 and 6.3 mg dm^{-3} for the Aupaba and F2 clones, respectively (Figure 3). MOTTA et al. (2020) evaluated soil fertility of 30 native yerba mate sites and reported low and very low P levels (NEPAR, 2019) in more than 90% of the evaluated locations, with a large part of the samples presenting contents below 2 mg dm^{-3}. Considering that this type of plant is quite representative and even preferred by the yerba mate industry, it is assumed that, among other factors, very low soil P content is one of the factors contributing to the low Brazilian average productivity of yerba mate of 7.7 Mg ha^{-1} (IBGE, 2020). In the current study, increasing the soil-P content to 5.2 and 6.3 mg dm^{-3} improved productivity to 12.7 and 14.2 Mg ha^{-1}, respectively, for the clones, Aupaba and F2. These results suggested that high P contents for forest species described in NEPAR (2019) are adequate for obtaining high yerba mate yields at the production stage.

Phosphate fertilization linearly increased the P content of yerba mate leaves (Figure 2); the contents ranged between 0.75 and 1.97 g kg^{-1}. SANTIN et al. (2017) also observed this effect, with contents of 1.26–1.47 g kg^{-1}. BARBOSA et al. (2018) reported an increase from 0.6 to 1.2 g kg^{-1} of P in yerba mate leaves with P fertilization. The first studies on the nutritional composition of the species reported contents of 1.1 g kg^{-1} (REISSMANN et al., 1983) and 1.4 g kg^{-1} (REISSMANN et al., 1985), which was considered low by the authors when compared to the content in other species.

The critical P content in yerba mate leaves for the clones Aupaba and F2 was 1.03 and 1.11 g kg^{-1}, respectively (Figure 3). In a survey conducted in 30 locations, MOTTA et al. (2020) reported a P content of yerba mate leaves of 0.81–2.01 g kg^{-1}, with the highest content observed when the soil nutrient content was 134 mg dm^{-3}, demonstrating that this species is naturally low in this nutrient. MOTTA et al. (2020) reported that 19 of the 30 locations had levels below the critical level obtained in the current study, a fact attributed to the low P availability in the soils of the locations evaluated. These data indicated that

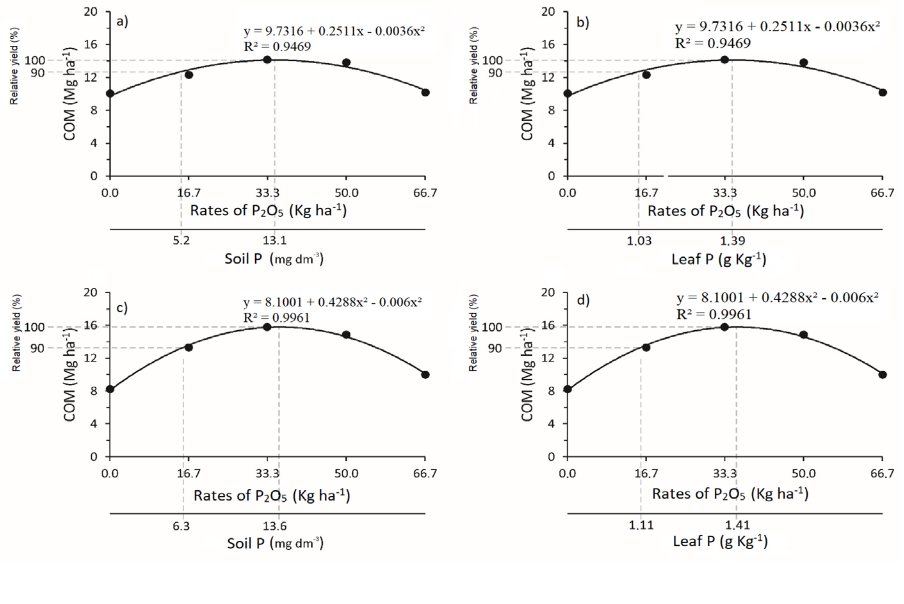

Figure 3 - Critical p level in soil and leaf for aupaba (a, b) and f2 (c, d) clones, respectively, for commercial yerba mate productivity (com).

high yerba mate yields depend on good P availability in the soil and, consequently, on adequate P content in the leaf.

There was no interaction between harvested yerba mate biomass components on nutrient content**.** However, phosphate fertilization increased P levels in LV, TnB, and TkB; and K and S in TnB (Table 4). Increased P levels may be explained by increased P availability in the soil (Figure 2). The clones differed only in P levels in LV. This difference between clones corroborates the results obtained by OLIVA et al. (2014), which indicated a different nutritional composition in different genetic materials. Besides P, SANTIN et al. (2013) and BARBOSA et al. (2018) reported that phosphate fertilization influenced N, K, Ca, and Mg contents in yerba mate seedling leaves, contradicting the results of the current study. This difference may have occurred due to the age of the plant, since the plants evaluated by those authors were 120 and 210 days old, respectively, while the ones evaluated in the present study were seven years old. In addition, genetic materials respond differently to phosphate fertilization, and there may or may not be changes in the nutritional composition of plant leaves (BARBOSA et al., 2018). RIBEIRO et al. (2018) testing N doses in yerba mate and found a change in the nutrient content of leaves only in the winter, with no influence of nutrient application on this parameter in summer. Thus, the authors suggested that measurements in winter are more accurate in demonstrating the effects of fertilization. In the current study, biomass components for the evaluation of nutrient composition of yerba mate were collected in the summer. The fact that dolomitic limestone (CaO and MgO at 31.6% and 20.3%, respectively) was applied before the experiment, in addition to N and K fertilization, may have contributed to the lack of response in N, K, Ca, and Mg to P doses.

The content of all nutrients in COM and TT increased with phosphate fertilization (Table 5). This effect was due to the increased productivity caused by P application. The highest TT dry mass yield of the yerba mate clones, Aupaba and F2, was 7.7 and 8.5 Mg ha^{-1}, respectively; N, P, K, Ca, Mg, and S contents were 115.6, 12.3, 100.5, 39.9, 34.8, 6.3; and 125.7, 12.8, 112.7, 41.4, 37.7, 6.9 kg ha^{-1}, respectively. The maximum dry mass yields of COM were 4.6 and 5.1 Mg ha^{-1} for the yerba mate clones, Aupaba and F2, respectively; N, P, K, Ca, Mg, and S contents were 97.4, 9.1, 75.6, 28.0, 28.3, 5.1; and 106.7, 9.2, 83.3, 30.0, 31.0, 5.5 kg ha^{-1}. The decreasing order of macronutrient quantity absorbed by yerba mate was N>K>Ca>Mg>P>S, while the order of nutrient removal in COM was N>K>Mg>Ca>P>S. These data also indicate that N, P, K, Ca, Mg, and S removal was 59.8, 84.3, 74.0, 75.2, 70.2, 81.2;

Table 4 - Macronutrient content (g kg^{-1}) of yerba mate leaves, thin branches, and thick branches in response to phosphate fertilization.

Nutrient	Clone	------P_2O_5 dose (kg ha^{-1})------ 0	16.7	33.3	50	66.7	Mean/eq.	R^2
		------leaves------						
N	Aupaba	24.42	22.22	23.17	24.30	20.61	22.94	
	F2	22.84	21.37	23.16	23.61	21.98	22.59	
	Mean	23.64	21.80	23.16	23.96	21.30	22.67^{ns}	-
P	Aupaba	0.98	1.38	1.73	2.08	2.33	1.70 a	
	F2	0.96	1.61	1.4	1.64	1.98	1.52b	
	Mean	0.97	1.50	1.57	1.86	2.16	L^{**}	0.93
K	Aupaba	15.06	13.83	16.83	15.04	15.17	15.19	
	F2	14.82	16.26	15.52	14.23	14.69	15.10	
	Mean	14.92	15.04	16.17	14.63	14.93	15.14^{ns}	-
Ca	Aupaba	5.87	5.78	6.73	6.24	5.53	6.03	
	F2	5.24	5.63	5.42	6.65	5.84	5.76	
	Mean	5.55	5.7	6.07	6.44	5.68	5.89^{ns}	-
Mg	Aupaba	5.96	6.38	6.36	6.69	6.29	6.34	
	F2	6.18	5.98	6.08	6.8	6.32	6.27	
	Mean	6.07	6.18	6.22	6.75	6.3	6.30^{ns}	-
S	Aupaba	1.05	0.98	1.11	1.15	0.96	1.05	
	F2	1.03	1.01	1.11	1.07	1.03	1.05	
	Mean	1.04	1.00	1.11	1.11	1.00	1.05^{ns}	-
------Thin branches------								
N	Aupaba	8.75	9.08	8.56	9.23	8.18	8.76	
	F2	8.86	8.06	9.82	8.74	8.82	8.86	
	Mean	8.81	8.57	9.19	8.98	8.50	8.81^{ns}	-
P	Aupaba	0.99	1.77	1.93	2.17	1.99	1.77	
	F2	1.18	1.74	1.81	2.1	1.75	1.72	
	Mean	1.09	1.76	1.87	2.14	1.87	Q^{**}	0.92
K	Aupaba	13.14	13.06	15.16	14.36	12.97	13.74	
	F2	12.95	13.79	13.55	14.32	13.42	13.61	
	Mean	13.05	13.43	14.36	14.34	13.20	Q^{*}	0.96
Ca	Aupaba	4.19	3.7	3.6	4.36	3.94	3.958	
	F2	4.14	3.73	4.11	4.52	4.59	4.218	
	Mean	4.17	3.72	3.86	4.44	4.27	4.09^{ns}	-
Mg	Aupaba	3.71	3.8	3.13	4.15	4.1	3.79	
	F2	3.58	3.28	4.02	3.79	4.03	3.74	
	Mean	3.65	3.54	3.58	3.97	4.07	3.76^{ns}	-
S	Aupaba	0.73	0.8	0.88	0.83	0.69	0.79	
	F2	0.72	0.74	0.8	0.83	0.66	0.75	
	Mean	0.73	0.77	0.84	0.83	0.68	Q^{*}	0.88
------Thick branches------								
N	Aupaba	6.49	6.31	6.40	6.39	5.88	6.29	
	F2	6.66	6.69	6.27	6.48	7.49	6.72	
	Mean	6.57	6.50	6.34	6.43	6.68	6.51^{ns}	-
P	Aupaba	0.61	1.1	1.18	1.2	1.21	1.06	
	F2	0.62	1.14	1.21	1.27	1.27	1.10	
	Mean	0.62	1.12	1.20	1.24	1.24	Q^{**}	0.87
K	Aupaba	9.46	9.05	9.36	9.14	9.09	9.22	
	F2	9.44	9.79	10.23	9.35	9.74	9.71	
	Mean	9.45	9.42	9.80	9.25	9.42	9.47^{ns}	-
Ca	Aupaba	4.19	3.95	4.24	4.24	3.11	3.95	
	F2	3.84	3.39	3.95	4.04	4.72	3.99	
	Mean	4.02	3.67	4.10	4.14	3.92	3.97^{ns}	-
Mg	Aupaba	2.21	2.66	2.12	2.31	1.93	2.25	
	F2	2.41	2.19	2.17	2.33	2.57	2.33	
	Mean	2.31	2.43	2.15	2.32	2.25	2.29^{ns}	-
S	Aupaba	0.39	0.44	0.42	0.4	0.4	0.41	
	F2	0.39	0.45	0.48	0.46	0.46	0.45	
	Mean	0.39	0.45	0.45	0.43	0.43	0.43^{ns}	-

Eq = equation; L = linear equation; Q = quadratic equation; ns = regression not significant; ** and *, significant at 1 and 5% probability, respectively; means with different letters differ between clones (ANOVA, P < 0.05).

Table 5 - Macronutrient content (g kg^{-1}) in commercial yerba mate (com) and total biomass (tt) of yerba mate clones, aupaba and f2, in response to phosphate fertilization.

Nutrient	Clone	P_2O_5 dose (kg ha^{-1}) 0	16.7	33.3	50	66.7	Mean/Eq.	R^2
	COM							
N	Aupaba	71.59 a	86.42 a	93.09b	100.10 a	62.24 a	Q^{**}	0.91
	F2	58.72b	83.92 a	109.36 a	102.32 a	63.76 a	Q^{**}	0.91
	Mean	-	-	-	-	-	-	-
P	Aupaba	3.46	6.81	8.34	10.24	8.16	7.40	-
	F2	3.06	7.70	8.51	9.01	6.54	6.96	-
	Mean	3.26	7.26	8.42	9.63	7.35	L^{**}	0.77
K	Aupaba	51.54 a	60.91 a	77.75b	72.44 a	53.05 a	Q^{**}	0.98
	F2	43.36b	72.88 a	84.86 a	73.04 a	49.17 a	Q^{**}	0.96
	Mean	-	-	-	-	-	-	-
Ca	Aupaba	19.16	23.56	28.14	27.81	18.40	23.41	-
	F2	14.94	23.98	28.30	31.57	18.88	23.53	-
	Mean	17.05	23.77	28.22	29.69	18.64	Q^{**}	0.99
Mg	Aupaba	19.05 a	25.44 a	26.30b	29.36 a	20.40 a	Q^{**}	0.92
	F2	16.65 a	24.73 a	30.86 a	31.15 a	19.68 a	Q^{**}	0.93
	Mean	-	-	-	-	-	-	-
S	Aupaba	3.42 a	4.26 a	4.97b	5.22 a	3.23 a	Q^{**}	0.93
	F2	2.89b	4.38 a	5.76 a	5.18 a	3.22 a	Q^{**}	0.94
	Medium	-	-	-	-	-	-	-
	TT							
N	Aupaba	84.37	100.93	112.50	116.92	74.82	97.91	-
	F2	68.55	99.83	127.83	121.36	77.33	98.98	-
	Mean	76.46	100.38	120.46	119.14	76.08	Q^{**}	0.92
P	Aupaba	4.63	9.38	11.82	13.30	10.78	9.98	-
	F2	4.00	10.43	12.11	12.74	8.84	9.62	-
	Mean	4.32	9.91	11.97	13.02	9.81	L^{**}	0.79
K	Aupaba	70.35 a	81.94b	105.97 a	96.29 a	72.53 a	Q^{**}	0.96
	F2	57.24 a	96.34 a	114.79 a	100.56 a	66.54b	Q^{**}	0.97
	Mean	-	-	-	-	-	-	-
Ca	Aupaba	27.52	32.69	40.97	38.69	25.04	32.98	-
	F2	20.59	32.03	40.00	43.38	27.35	32.67	-
	Mean	24.06	32.36	40.49	41.04	26.19	Q^{**}	0.99
Mg	Aupaba	23.45 a	31.49 a	32.65b	35.50 a	24.52 a	Q^{**}	0.92
	F2	20.17 a	29.91 a	37.26 a	37.94 a	24.32 a	Q^{**}	0.93
	Mean	-	-	-	-	-	-	-
S	Aupaba	4.20 a	5.28 a	6.25b	6.30 a	4.10 a	Q^{*}	0.91
	F2	3.49b	5.47 a	7.20 a	6.53 a	4.07 a	Q^{**}	0.96
	Mean	-	-	-	-	-	-	-

Eq = equation; L = linear equation; Q = quadratic equation; ns = regression not significant; ** and *, significant at 1 and 5% probability, respectively; means with different letters differ between clones (ANOVA, P < 0.05).

and 60.2, 84.9, 71.5, 73.9, 72.5, 82.2% in clones, Aupaba and F2, respectively, in relation to the total extracted by the plant. Although the quantity of Mg absorbed was lower than that of Ca, the quantity removed was greater. This is due to the content of Mg in LV, which was higher than the Ca content (Table 4). MOTTA et al. (2020) found that yerba mate has a high capacity for Mg uptake, even in soils with low Mg availability. Those authors reported Mg contents of 3.9–9.2 g kg^{-1} in yerba mate leaves, while in the present study, the mean was 6.30 g kg^{-1}. These values are higher than those found in tea (*Camellia sinensis*), a species with characteristics and use similar to yerba mate, with a Mg content in the leaves lower than 3 g kg^{-1} (FUNG et al., 2008). The fact that Mg is removed in larger quantities than Ca necessitates strategic Mg supply to the plants; this nutrient is commonly applied through liming and the choice of calcitic limestone (MgO < 5%) over dolomitic limestone (MgO > 12%), may induce a Mg deficiency in yerba mate.

CONCLUSION

Yerba mate is P-demanding and expressed its maximum productivity at planting, canopy formation 1, canopy formation 2, and production stages with annual doses of P_2O_5 around 5, 25, and 40 g $plant^{-1}$, and 36 kg ha^{-1}, respectively.

The critical level of P in the soil in the 0–20-cm layer extracted by Mehlich-1 was 5.2 and 6.3 mg dm^{-3}; and in the leaf, 1.03 and 1.11 g kg^{-1}, for the clones, Aupaba and F2, respectively.

Phosphate fertilization affects P content in LV, TnB, and TkB; and K and S content in TnB, and increases the content of all macronutrients in yerba mate COM and TT. The decreasing order of macronutrient quantity removed by yerba mate was N>K>Mg>Ca>P>S.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The funding sponsors had no role in the study design; data collection, analysis, or interpretation; manuscript writing; or in the decision to publish the results.

AUTHOR'S CONTRIBUTIONS

All authors contributed equally to the conception and writing of the manuscript. All authors critically reviewed the manuscript and approved the final version.

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