

NOTE

Phosphorus fertilization and yerba mate (*Ilex paraguariensis* A. St.-Hil.) seed quality

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ABSTRACT: Yerba mate seedling production is inefficient and costly, as the seeds have slow and low germination (<20%) and are often embryoless. Because the plant is grown in low-fertility soils, particularly in terms of phosphorus, nutritional deficiencies in the mother trees likely contribute to poor seed quality. Therefore, this study investigates the impact of phosphorus fertilization on the seed quality of yerba mate (*Ilex paraguariensis* A. St.-Hil.). The experiment was conducted in a seed production area with 1,822 plants, using P₂O₅ doses of 0, 48, 96, 182, and 288 kg.ha⁻¹. Seed quality was assessed by the 1% tetrazolium test, thousand-seed weight (TSW), and the seed phosphorus content was determined. The results indicated no difference between treatments in seed phosphorus content or 1,000-seed weight, which was 8.0 g. However, seed viability increased with phosphorus application, with the highest quality observed in the 288 kg P₂O₅ ha⁻¹ treatment. Phosphorus-fertilized mother plants were 2.58 times more likely to produce seeds with embryos. The study demonstrated that phosphorus plays a fundamental role in seed quality, improving viability and embryo presence.

Index terms: Aquifoliaceae, germination, physiological quality, soil fertilization.

RESUMO: A produção de mudas de erva-mate é pouco eficiente pois as sementes apresentam germinação lenta e baixa (< 20%) e em muitos casos ausência de embrião. Por ser cultivada em solos de baixa fertilidade, principalmente em fósforo, a carência nutricional das árvores matrizes contribui para baixa qualidade das sementes. Este estudo investiga o impacto da adubação com fósforo na qualidade de sementes de erva-mate. O experimento foi realizado em área de produção de sementes com 1.822 plantas, sendo utilizadas doses de P₂O₅ de 0, 48, 96, 182 a 288 kg.ha⁻¹. A qualidade de sementes foi avaliada pelo teste de tetrazólio 1%, peso de mil sementes (PMS) e o teor de fósforo nas sementes foi determinado. Os resultados indicaram que não houve diferença entre os tratamentos para o teor de fósforo nas sementes e no PMS que foi de 8,0 g. A viabilidade das sementes aumentou com a aplicação de fósforo, sendo a maior qualidade observada no tratamento de 288 kg P₂O₅ ha⁻¹. Plantas matrizes adubadas com fósforo apresentaram 2,58 vezes mais chances de produzir sementes com embrião. O estudo demonstrou que o fósforo tem papel fundamental na qualidade da semente, melhorando a viabilidade e ocorrência de embrião.

Termos para indexação: Aquifoliaceae, germinação, qualidade fisiológica, fertilização do solo.

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INTRODUCTION

Yerba mate (*Ilex paraguariensis* A.St.-Hil.) is a crop of great economic, environmental and cultural importance for the southern region of South America, being produced and consumed in Brazil, Argentina, Paraguay and Uruguay. For Brazil, the crop is a source of one of the main non-timber forest products cultivated, which are the leaves, traditionally used for the production of mate tea, *chimarrão* and *tereré* (Magri et al., 2022). In addition, yerba mate has also been used for the production of soft drinks, beers, energy drinks, cosmetics, animal feed, and pharmaceuticals (Gonçalves and Valduga, 2023).

In Brazil, the area planted with yerba mate is 67,230 ha and the main producing states are Paraná, Rio Grande do Sul, Santa Catarina and Mato Grosso do Sul (IBGE, 2023).

Yerba mate is mainly propagated by seedlings obtained from seeds (Wendling et al., 2014). In the case of the species *Ilex paraguariensis*, the seeds have simple deep morphophysiological dormancy, which means that the embryo is immature at the point of fruit harvest and that its development is completed by the application of stratification at a temperature higher than 15 °C (Baskin and Baskin, 2004; Galindez et al., 2018). Embryo immaturity leads to low, slow and uneven germination, being a great challenge for seedling-producing nurseries. The stratification process consists of placing the seeds between layers of moist sand for a period of six months. However, after this long period of stratification, the percentage of germination is generally less than 20% (Duarte et al., 2023).

Factors that affect the physiological quality of the seeds include the mineral nutrition of the mother plant, which can be improved through fertilization. Yerba mate is recognized for occurring mainly in soils of low natural fertility, such as that of Atlantic Forests, being a rustic plant that in native stands is produced in nutrient-poor environments. In relation to nutrients, phosphorus is among the most important for the production of high-quality seeds, being present in the constitution of cell membranes that are rich in phospholipids and directly linked to their permeability and integrity, in addition to acting in the transfer of ATP, in respiration, besides being a source of inositol phosphate for the germination process (Marcos-Filho, 2016).

Phosphorus nutrition for plants grown in Brazilian soils may be compromised, given the low availability of this element (Pavinato et al., 2020). In addition, these soils have a high phosphorus fixation capacity resulting from the abundance of oxidic clays with high adsorption capacity or also due to the abundance of aluminum, which leads to the formation of low-solubility compounds (Barbosa et al., 2022). Both processes that lead to phosphorus fixation are aggravated in soils with low pH (Pavinato et al., 2020; Barbosa et al., 2022). However, it is under this condition of high phosphorus fixation capacity, acidic soils and high levels of toxic aluminum that yerba mate has its origin and has been produced in systems similar to extractivism or cultivated up to the present day (Barbosa et al., 2018; Motta et al., 2020; Magri et al., 2022). Despite these conditions, adequate growth can be obtained given the high capacity of resistance to aluminum (Benedetti et al., 2017).

The yerba mate crop responds positively to phosphorus fertilization, as reported in the literature for the growth of seedlings until the production of leaf biomass in adult plants of native and clonal stands (Santin et al., 2017; Walter et al., 2022; Clemente et al., 2024). On the other hand, the absence of response to phosphorus use has also been reported in the field (Arguello et al., 2025). However, no studies related to the effect of phosphorus fertilization on seed quality and improvement in germination are found in the literature.

The aim of the present study was to assess the effect of phosphorus fertilization on improving the quality of yerba mate seeds in acidic soils, in an area not fertilized for a long period.

MATERIAL AND METHODS

The experiment was carried out at the Vila Nova farm, in the yerba mate seed production area, of the company *Bitumirim Indústria e Comércio de Erva-mate*, located in Ivaí, Paraná, Brazil (25° 01' 54.6'' S, 50° 46' 00.75'' W).

According to Köppen's classification, the climate of the region is classified as Cfa, characterized as humid subtropical, with average temperatures of 18 °C, infrequent frosts and rainfall concentrated in the summer months, with no defined dry season. The soil of the experimental site is a *Latossolo Vermelho Distrófico* (Oxisol) according to the Soil Map of the State of Paraná (Bhering et al., 2007). The results of the chemical analysis of the soil before the experiment, at 0-20 cm depth, indicated phosphorus levels of 1.8 mg.kg⁻¹, which is considered very low according to the Manual of Fertilization and Liming of the Paraná state (Table 1). Physical analyses of this soil indicated 35% clay, 15% silt and 50% sand.

The experimental area contained 1,822 plants, 2/3 of which were female trees and 1/3 were male trees with plant spacing of 5 x 5 m. A randomized block design was used, with four blocks demarcated in the direction of the topography of the area and plots consisting of five plants (three female plants and two male plants).

The treatments consisted of zero (control), 48, 96, 182 and 288 kg of P₂O₅.ha⁻¹. The treatments were applied in a single dose in October 2022, in the flowering period, broadcast on the soil surface without incorporation, within a radius of 2 m around the plants that constituted the plots. The dose of 96 kg P₂O₅.ha⁻¹ is the recommended dose for the phosphorus content observed in the soil before the experiment, according to the Manual of Fertilization and Liming for the Paraná State (Pauletti and Motta, 2019). The phosphorus source used was single superphosphate, containing 18% P₂O₅. The history of the area indicated that no fertilization and liming interventions have been carried out in the last 10 years, which justifies the high acidity (Table 1).

The collection of seeds for analysis of physiological quality was carried out in February 2023. For the evaluations, ripe fruits were collected individually from the four azimuths of each female tree present in the plots. Then, the fruits of these trees were grouped to compose a single sample. After pulping, the seeds were washed with deionized water and dried, and the physiological quality was then evaluated before and after stratification.

The variables studied to assess treatment effects on the physiological quality of the seeds were percentage of viable seeds and number of embryoless seeds, determined by the tetrazolium test, and 1,000-seed weight (TSW), according to the methodology of the Rules for Seed Testing (Brasil, 2025). Seed water content was determined using the recommended methodology (Brasil, 2025). The tests to evaluate the physiological quality of the seeds were carried out at the Seed Laboratory of Embrapa Forestry. For the tetrazolium test, 100 seeds of each treatment were stained in tetrazolium salt solution at a concentration of 1%, and the percentage of viable seeds was determined according to embryo color, as well as embryoless seeds. Phosphorus content in the seeds was evaluated according to the methodology proposed by Martins and Reissman (2007), and the analyses were carried out at the Plant Nutrition Laboratory of UFPR.

Stratification, a necessary practice for the development of the yerba mate seed embryo, was carried out between layers of moist sand (2 cm of sand), in gerbox boxes placed in a BOD growth chamber with a temperature variation of 15 and 20 °C and a photoperiod of 16 h of dark and 8 h of light for a period of five months. The sand was constantly moistened in order to maintain 60% field capacity. Four replications of 100 seeds per treatment were used.

Statistical analysis was performed using the generalized linear model with binomial distribution using the logit function to correct the standard errors of the estimated effects, for the data of seed viability and number of embryoless seeds. The model was subjected to analysis of variance using the chi-square test. The Gaussian linear model was used to analyze the data regarding phosphorus content in the seeds. When there was statistical difference, the means of all variables were compared by Tukey test at 5% probability level. The number of embryoless

Table 1. Chemical analysis of the soil in the experimental area, with sampling carried out at a depth of 0 – 20 cm.

pH	Al	(H+Al)	Ca	Mg	K	Sum of bases	CEC at pH 7.0	P Mehlich	Organic matter	V
CaCl ₂ 0.01M	-----mmol.c.dm ³⁻¹ -----							mg.dm ³⁻¹	g.dm ³⁻¹	%
3.99	48.5	141.9	6.0	2.1	1.41	9.51	151.49	1.8	24.74	6

seeds was subjected to contrast analysis, since there were two groups of data formed by seeds harvested from trees without and with fertilization. The probability of occurrence of seeds with embryos was calculated by the odds ratio, considering the existence of these two groups. All analyses were performed using R software, version 4.5.1 (R Core Team, 2025).

RESULTS AND DISCUSSION

The yerba mate seeds analyzed had an average water content of 13.9% after harvest, which is different from those reported in the literature by several authors. Duarte et al. (2023) observed a water content of 10.1% in yerba mate seeds of the same origin as those evaluated in the present study. Water contents of 5.7% to 10.4% were observed by Winhelmann et al. (2022) in freshly harvested seeds. Variation in the water content of seeds of the same species is expected, even though a standardized method is used to determine the percentage of water.

There was no significant difference in the phosphorus content of yerba mate seeds after fertilization with the different doses of P_2O_5 , with an average value of 2.7 g. There are no reports in the literature of phosphorus concentration in yerba mate seeds, and the only information available is on the amount of this element in leaves, which have an original content between 0.81 and 2.01 $g.kg^{-1}$, which is considered low when compared to the values of other species (Motta et al., 2020). According to the literature, phosphorus is the fifth most absorbed element by yerba mate plants and its absorption depends on the characteristics of the soil (Clemente et al., 2024).

Thousand-seed weight (TSW) is a characteristic that can be used as a quality indicator (Marcos-Filho, 2016). In the present study, there was no statistically significant difference in the weight of one thousand seeds harvested from mother plants that were not fertilized and fertilized with the evaluated P_2O_5 doses (Figure 1). The observed TSW was 8.0 g, and the literature reports a variation of 4.28 to 8.9 g for this characteristic in yerba mate seeds (Winhelmann et al., 2022). TSW has been evaluated in several studies about the effect of fertilization treatments on the quality of

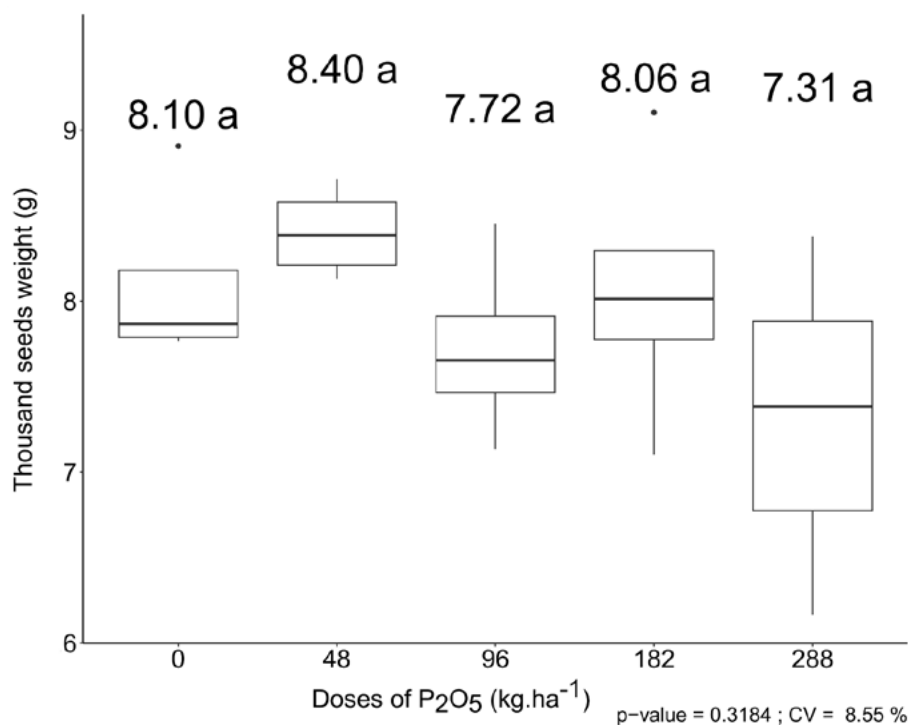


Figure 1. Thousand-seed weight of yerba mate seeds harvested from mother plants not fertilized and fertilized with P_2O_5 at doses of 48, 96, 182 and 288 $kg.ha^{-1}$.

seeds of tree species such as conifers, and contradictory results have been reported. *Pinus sylvestris* seeds harvested in orchards that received only phosphorus fertilization or phosphorus combined with nitrogen and potassium showed no increase in TSW as a result of fertilization (Saarsalmi et al., 1994). On the other hand, Qianchun et al. (2024) reported that phosphorus fertilization led to an increase in TSW in *Larix kaempferi* (Japanese larch).

Regarding the physiological quality of the seeds, evaluated by the tetrazolium test, the phosphorus fertilization treatments contributed to the production of yerba mate seeds with higher quality, which showed higher viability values (Figure 2).

The viability of freshly harvested seeds and seeds after stratification for five months was evaluated separately; however, there was no statistical difference between these two evaluation periods (Table 2).

In the present study, the quality of the seeds, indicated by the vigor evaluated by the tetrazolium test, increased proportionally to the amount of phosphorus applied to the soil, and the dose of 288 kg P₂O₅ ha⁻¹ resulted in a greater number of viable seeds. Although the acidity of the soil in the experimental area (pH 3.99) and the high percentage

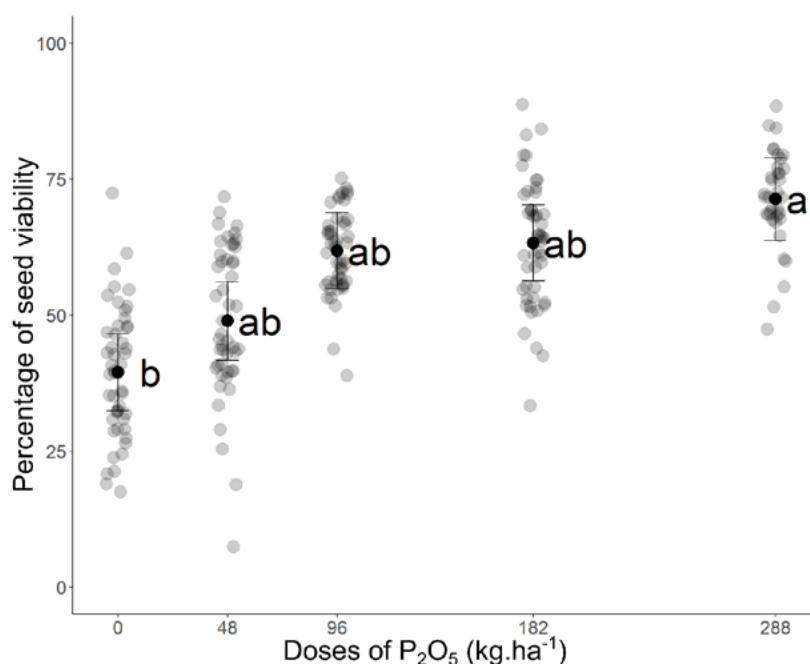


Figure 2. Viability of yerba mate seeds harvested from mother plants not fertilized and fertilized with P₂O₅ at doses of 48, 96, 182 and 288 kg.ha⁻¹, obtained by the 1% tetrazolium test.

Table 2. Percentage of viable yerba mate seeds, obtained from mother plants not fertilized and fertilized with P₂O₅ at doses of 48, 96, 182 and 288 kg.ha⁻¹, freshly harvested and after five months of stratification in moist sand.

P ₂ O ₅ doses (kg.ha ⁻¹)	% Viability	
	Freshly harvested	After stratification
0	37 b	43 b
48	46 ab	57 ab
96	58 ab	65 ab
182	58 ab	64 ab
288	72 a	70 a

Means followed by different letters (lowercase in the rows) differ from each other by Tukey test ($p < 0.05$).

of aluminum saturation (m equal to 83% calculated from the Al, Ca, K and Mg contents) lead to the fixation of phosphorus in the soil and there is no significant difference in its content in the chemical composition of seeds harvested from fertilized and unfertilized mother plants, there was an improvement in seed quality. Studies related to the production of leaf biomass in yerba mate report that the application of doses higher than 200 kg P₂O₅ ha⁻¹ led to increased yield even under conditions of high soil acidity (pH in water of 3.92), high value of exchangeable Al (Al – 44.7 mmol_c dm⁻³ and Al saturation or m – 71%) and very low P availability (1.31 mg dm⁻³) (Santin et al., 2017). Liming aimed at reducing phosphorus fixation should be evaluated in future studies, which may contribute to a decrease in the dose of phosphorus to be applied.

The results obtained confirm that the nutritional condition of the mother plant is essential for the production of seeds with high physiological quality (Marcos-Filho 2016), and yerba mate is traditionally cultivated in poor soils. The element phosphorus is an essential component of cell membranes, which are rich in phospholipids, playing a crucial role in the permeability and integrity of these structures. In addition, it actively participates in ATP transfer, cellular respiration, and photosynthesis. In seeds, phosphorus is found in the form of phytic acid, participating in the constitution of nucleic acids and phosphates that are essential for embryo development and consequent seedling growth. During the formation and maturation of fruits in yerba mate plants, there is a reduction of this nutrient in the leaves due to its migration to the fruits, where phytic acid is synthesized (Taliman et al., 2019). Probably the greater availability of phosphorus in the soil, despite the chemical conditions that lead to the fixation of this element, must have increased its reserves in the seeds, which contributed to the viability of the embryo.

The low germination and viability of yerba mate seeds is an inherent problem of the *Ilex paraguariensis* species, and several studies have been carried out in an attempt to improve germination (Cuquel et al., 1994; Sansberro et al. 1998; Souza et al., 2019; Souza et al., 2022; Duarte et al., 2023). However, few advances have been achieved, especially with seeds harvested from non-selected mother plants, and treatments applied in the stratification process result in a small increase in seed viability. Reinke et al. (2024) reported viability percentages of 22% in non-stratified seeds and 15.4% after stratification. Duarte et al. (2023) reported that stratification carried out at different temperatures (5 and 15 °C) and in different substrates (sand and paper) had no positive effect on seed viability, when compared to the viability of non-stratified seeds and seeds stratified by the traditional method.

Yerba mate mother plants that did not receive fertilization and those that received the lowest dose of P₂O₅ produced a higher number of embryoless seeds, as can be seen in Figure 3. By calculating the odds ratio, it was shown that yerba mate mother plants fertilized with phosphorus are 2.58 times more likely to produce seeds with embryos. The absence of embryos in *Ilex paraguariensis* seeds is reported by several authors and was also observed in the present study (Winhelmann et al., 2022; Reinke et al. 2024). There are several reasons for the occurrence of embryoless seeds such as zygote degeneration, embryo death, mutations that lead to embryo non-development, egg degeneration, and insect attack (Baskin and Baskin, 2004).

The present study is a pioneer in investigating the relationship between phosphorus fertilization and the quality of yerba mate (*Ilex paraguariensis*) seeds, demonstrating that this silvicultural practice is of great relevance to the crop, as it contributes significantly to the improvement of seed viability. It is worth pointing out that the production of high-quality seeds begins in the field, through the proper management of soil fertility.

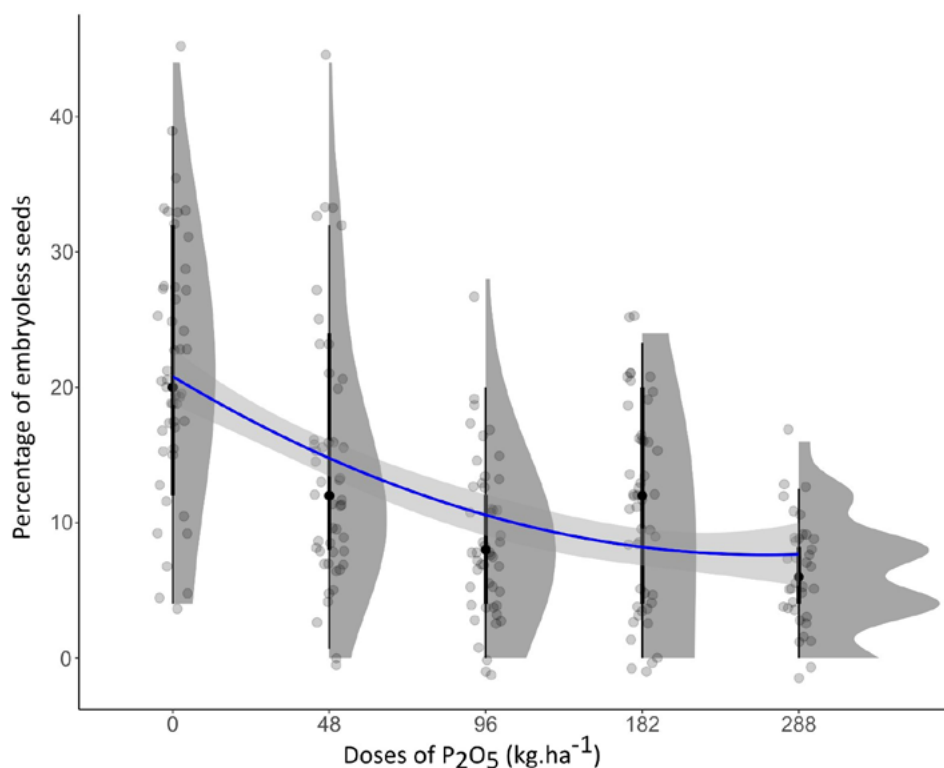


Figure 3. Percentage of embryoless seeds, harvested from mother plants not fertilized and fertilized with P₂O₅ at doses of 48, 96, 182 and 288 kg.ha⁻¹.

CONCLUSIONS

The maximum dose of P₂O₅ tested (288 kg.ha⁻¹) contributed to improving the quality of yerba mate seeds, which showed greater viability and embryo presence.

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AUTHORS' CONTRIBUTION

Conceptual idea: Vieira, E.S.N.; Motta, A.C.V.; Methodology design: Vieira, E.S.N.; Motta, A.C.V.; Data collection: Novakoski, D.; Stremel, J.S.; Data analysis and interpretation: Michelon, T.B.; Vieira, E.S.N.; Writing and editing: Vieira, E.S.N.; Motta, A.C.V.

DATA AVAILABILITY

Additional data will be made available by the authors upon reasonable request.

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