

CRITICAL LEVELS AND DOSES OF MAXIMUM TECHNICAL EFFICIENCY OF PHOSPHORUS IN *Handroanthus heptaphyllus* SUBJECTED TO PHOSPHORUS FERTILIZATION

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Resumo

Níveis críticos e doses de máxima eficiência técnica de fósforo em *Handroanthus heptaphyllus* submetido a fertilização fosfatada. Nem sempre solos tropicais e subtropicais fornecem a quantidade necessária de fósforo (P) para suprir a demanda do nutriente de árvores nativas, como o *Handroanthus heptaphyllus* (Mart.) Mattos (Ipê-roxo). Por isso, torna-se necessário aplicar fertilizantes fosfatados. Porém, a tomada de decisão e doses a serem aplicadas serão mais assertivas caso sejam definidos os níveis críticos (NC) de P no solo e em folhas, e doses de máxima eficiência técnica (MET), para melhorar o sistema de recomendação de adubação às árvores nativas. O estudo objetivou propor NC e MET de P em Ipê-roxo submetidas a aplicação de P. O experimento foi conduzido em Argissolo Vermelho que foi submetido a aplicação de cinco doses de P (0, 20, 40, 80 e 160 kg ha⁻¹). Após 24 meses do plantio foram determinados o teor de P no solo e a sua concentração no tecido foliar, crescimento em altura (H) e diâmetro do coleto (DC), fluorescência da clorofila *a* e atividade da enzima fosfatase ácida. Os NC no solo e em folhas foram calculados. A adição da adubação fosfatada aumentou os teores de P no solo e em folhas, promovendo maior H e DC das plantas de Ipê-roxo. A maior disponibilidade de P reduziu as perdas de energia fotoquímica por fluorescência. O NC no solo para as variáveis de crescimento altura, diâmetro do coleto foi de 20,0 e 21,2 mg dm⁻³. O NC em folhas para os parâmetros de crescimento e fisiológicos foi em média 1,6 g kg⁻¹.

Palavras-chave: Ipê-roxo, Adubação fosfatada, APase, Fluorescência da clorofila *a*.

Abstract

Tropical and subtropical soils do not always provide the necessary amount of phosphorus (P) to supply the nutrient demand of native trees, such as *Handroanthus heptaphyllus* (Mart.) Mattos (Ipê-roxo). Therefore, it becomes necessary to apply phosphate fertilizers. However, decision-making and doses to be applied will be more assertive if P's critical levels (NC) in the soil and leaves and doses of maximum technical efficiency (MET) are defined to improve the fertilizer recommendation system for native trees. The study aimed to propose NC and MET of P in *H. heptaphyllus* submitted to P application. The experiment was conducted in Red Argisol submitted to the application of five doses of P (0, 20, 40, 80, and 160 kg ha⁻¹). After 24 months of planting, the P content in the soil and its concentration in the leaf tissue, height growth (H) and stem diameter (DC), chlorophyll A fluorescence, and acid phosphatase enzyme activity were determined. The NC in soil and leaves were calculated. The addition of phosphorus fertilization increased the P levels in the soil and leaves, promoting higher H and DC in the *H. heptaphyllus* plants. The greater availability of P reduced photochemical energy losses by fluorescence. The NC in the soil for growth variables height and stem diameter was 20.0 and 21.2 mg dm⁻³. The CN in leaves for growth and physiological parameters was, on average, 1.6 g kg⁻¹.

Keywords: Ipê-roxo, Adubação fosfatada, APase, Fluorescência da clorofila *a*.

INTRODUCTION

The soil does not always provide the phosphorus (P) required for tree species development. When this happens, it is necessary to apply phosphate fertilizers, particularly because P can stimulate the growth of the root system and aerial part of young tree species (BERGHETTI *et al.*, 2020). However, most of the P applied to tropical and subtropical soils is adsorbed by reactive soil particles such as Fe, Al, and Mn oxides, hydroxides, and clay minerals (FÄTH *et al.*, 2019). When this happens, a small part of the P will be available to the plants (KULMANN *et al.*, 2022). As a solution to understand the amount of P available in the soil, it is necessary to carry out long-term experiments with tree species to define the critical levels (NC) or sufficiency ranges (FS) of P in the vegetal tissue, soil, and even doses of maximum technical efficiency (MET). This information could improve the

recommendations proposed by liming and fertilization manuals for forest species at a regional level (BERGHETTI *et al.*, 2020, 2021).

Applying phosphate fertilizers increases P availability in forest soils (BERGHETTI *et al.*, 2020). The study reported that part of the fertilizer applied was available to the outer surface of the roots, enhancing the absorption, transport, and accumulation of P in reserve organs, such as roots and stems, and annual organs, such as leaves and branches (BERGHETTI *et al.*, 2021). Increased P in leaves can reduce damage to the photosystem II (PSII), reducing the synthesis of photosynthetic pigments and chlorophyll A fluorescence energy loss (BERGHETTI *et al.*, 2020). Thus, plants use light energy better, enhancing physiological processes such as ATP biosynthesis, ribulose-1,5-biphosphate carboxylation/regeneration, and cell growth (XU *et al.*, 2019).

The amounts of NC of P available to soil and leaves for native trees from "Mata Atlântica" (Brazilian biome), such as *H. heptaphyllus*, are unknown. This knowledge can ensure the growth and photosynthetic rate of the species. Thus, the definition of NC can be a strategy to ensure the sustainable management of fertilization in plantations of the species *H. heptaphyllus*, which is popularly known as "Ipê-roxo". A native species used in reforestation programs, recovery of degraded areas, and landscaping due to its silvicultural, ecological, and ornamental characteristics, respectively (BERGHETTI *et al.*, 2021).

Phosphate fertilization recommendations in southern Brazil are based on leaf and soil analyses (CQFS-RS/SC, 2016; SBRS-NEPAR, 2019). However, there are no recommendations for "Ipê-roxo" (*H. heptaphyllus*), which can compromise survival and growth when grown in soils with low P availability. Thus, defining the NC of P can guarantee the nutritional balance of the plants, making it possible to recommend the appropriate amount of phosphate fertilizers, reducing production costs and the potential for contamination of surface waters adjacent to the plantations. The hypothesis of this study is that the application of P promotes the absorption of inorganic phosphate by the roots, increasing the concentration of P in leaves. Additionally, enabling greater use of light energy in the plant photosynthetic processes, which could stimulate initial plant growth. Therefore, this study aimed to propose a critical level of phosphorus in leaves and soil and the efficiency of maximum technical doses in *H. heptaphyllus* (Ipê-roxo) subjected to P application.

MATERIAL E METHODS

Description of the experimental area

The study was conducted in the municipality of Santa Maria (29° 47' 30" S, 53° 39' 47" O), in the state of Rio Grande do Sul (RS), a region in the south of Brazil. The region's climate is classified according to Köppen as CFA (humid subtropical), characterized by a temperature of the coldest month between -3 and 18 °C, and the hottest month above 22 °C, with an average annual rainfall of 1,769 mm and well-distributed rainfall throughout the year (ALVARES *et al.*, 2013). The average air temperature and rainfall during the study period were 20.3 °C and 154.4 mm, respectively.

The soil in the experimental area is classified as "Argissolo Vermelho" (similar to Red Argisol) (EMBRAPA, 2013). When the experiment was set up, the soil had the following characteristics: 700 g kg⁻¹ of sand, 130 g kg⁻¹ of silt and 170 g kg⁻¹ of clay; 0.8 g kg⁻¹ of organic matter (Walkley-Black method); 6.0 pH in water (1:1 ratio); 10 and 48 mg. kg⁻¹ of available P and K, respectively (both extracted by Mehlich-1); 0.0, 1.2 and 0.6 cmol_c dm⁻³ of exchangeable Al, Ca and Mg, respectively (extracted by KCl 1 mol. L⁻¹); and 4.5 cmol_c dm⁻³ of cation exchange capacity at pH 7.0.

Experimental design

Seedlings with an average height and stem diameter of 31.5 cm and 4.5 mm, respectively, were used. The seedlings were grown in polypropylene tubs with a capacity of 180 cm³, filled with a commercial organic substrate made up of grape seed and pomace (*Vitis* sp.), ash, peat, charcoal, and carbonized rice husk. The base fertilizer used was 12 g. L⁻¹ of controlled-release fertilizer (NPK 15-09-12) was used as the base fertilizer, with a release time of six months.

The spontaneous vegetation was desiccated by applying 4.5 L ha⁻¹ of non-selective systemic herbicide [Glyphosate - N (phosphonomethyl) glycine], using a sprayer attached to a tractor (flow rate of 500 L. ha⁻¹) prior to planting. Three days later, holes were dug manually with a shovel; the hole's dimensions were 15 cm x 20 cm (diameter x depth), and they were spaced at 2 m x 2 m apart.

After planting, each seedling was irrigated with approximately two liters of water to eliminate the possible formation of air pockets between the roots and the soil and to keep the root system hydrated. All the seedlings were then fertilized with 90 kg N ha⁻¹ and 45 kg K₂O ha⁻¹, using urea (45% N) and potassium chloride (60% K₂O), respectively. The fertilizer was applied in four holes with depth of 10 cm each and 15 cm apart from the seedling. Phosphate fertilizer was applied on the same occasion, according to each treatment.

During the experiment maintenance, leaf-cutting ants of the genera *Atta* and *Acromyrmex* were controlled using granulated baits (based on Sulfluramid). Invasive plants were weeded manually (crowning), mowed, or

chemically weeded using the same herbicide described above, which was applied at a dosage of 4.5 L. ha⁻¹ with a knapsack sprayer (flow rate of 350 L ha⁻¹).

The experiment design was a randomized block, and the treatments were the application of five doses of P in the soil (0, 20, 40, 80, and 160 kg P ha⁻¹). Four repetitions were used per treatment, totaling 20 experimental units containing 24 plants each. Triple superphosphate (42 % P₂O₅) was used as the P source, and the application was carried out at a depth of between 10 and 15 cm in holes about 15 cm from the seedling. The nutritional, morphological, and physiological variables were assessed 24 months after planting the seedlings on the eight central plants of each experimental unit.

Sampling and analysis of P in soil and leaves

Soil samples were collected at eight points approximately 15 cm from the seedlings and distributed equidistantly in the 0-20 cm layer. The soil was air-dried, passed through a 2 mm mesh sieve, and subjected to extraction of available P (extracted with Mehlich-1: 0.05 mol L⁻¹ HCl + 0.0125 mol L⁻¹ H₂SO₄) according to Tedesco *et al.* (1995).

Fully expanded (fully developed) leaves from the upper third of the plants were collected from the eight central plants of each sampling unit, washed with distilled water, packed in paper bags, and dried in an air-conditioned oven ± 65 °C until constant weight. The tissue was dried and ground (20 mesh) in a Wiley mill. Sulphuric digestion was then carried out, and the total P content was determined (TEDESCO *et al.*, 1995). The concentration of P was determined by colorimetry, according to the methodology of Murphy and Riley (1962), in a spectrophotometer (SF325NM, Bel Engineering, Italy).

Measurement of dendrometric attributes and chlorophyll A fluorescence

The height of the plants was measured from ground level to the apical bud using a ruler graduated in centimeters, and the diameter of the stem was measured using a digital caliper (with a precision of 0.01 mm) at ground level.

The emission of chlorophyll A fluorescence was analyzed using a JUNIOR-PAM portable light-modulated fluorometer (Walz, Germany). The measurements were taken on sunny days, in the morning (8:00-11:00 am), using the first fully sprouted leaf. This leaf was previously adapted to the dark for 30 minutes to measure the initial fluorescence (F₀). Afterward, the sample leaf was subjected to a saturating light pulse (10000 mol m⁻² s⁻¹) for 0.6 s to assess maximum fluorescence (F_m). The maximum PSII quantum yield (F_v/F_m) was determined by the ratio between variable fluorescence (F_v = F_m - F₀) and F_m (BERGHETTI *et al.*, 2021).

Extraction and quantification of the acid phosphatase enzyme (APase)

Fractions were collected from the leaves used to analyze photosynthetic pigments to evaluate APase activity. The enzyme extract was obtained from 0.5 g of leaf tissue macerated with liquid N₂ and homogenized buffer solution with Tris/HCl (0.1 M), EDTA (0.001 M), and albumin (0.1 g) (pH 7.4). The samples were filtered with paper and centrifuged at 20,000 x g for 30 min. The resulting supernatant was used to determine the activity of the acid phosphatase enzyme (TABALDI *et al.*, 2007).

The inorganic phosphate (Pi) content was measured at 630 nm in a spectrophotometer (Celm E-205D), using malachite green as the colorimetric reagent and KH₂PO₄ as the reference for the calibration curve. Specific enzyme activities are measured in μmol Pi released min⁻¹ mg⁻¹ protein. Protein was determined using bovine serum albumin as a reference (BRADFORD, 1976).

Statistical analysis

The statistical analyses were conducted in the RStudio version 4.1.2 (R CORE TEAM, 2021). Initially, variance components analysis was carried out to quantify the percentage contribution of each source of variation (P doses, blocks, and residues) on the total variance of each response variable (plant height and diameter, F_v/F_m, F₀, APase, P concentration in leaves and P content in the soil). This statistical procedure was done using the "VCA" package (SCHUETZENMEISTER; DUFEY, 2020). Afterward, the results were submitted to analysis of variance (ANOVA), normality of residuals (Shapiro-Wilk test), and homoscedasticity (Bartlett's test) with a significance level of 5%. The means were compared using the Tukey test with a 95% confidence level. The statistical analysis was done using the "ExpDes.pt" package (FERREIRA; CAVALCANTI; NOGUEIRA, 2018).

Aiming to estimate the NC of P in the soil and leaves, the authors used the variables plant height and diameter, F_v/F_m, and APase, which were factored in. Before estimation, all variables were converted into relative yield (%). The estimation models were developed using Quantile Regression (KOENKER, 2017) with a threshold to quantify the relationship between the dependent variables (plant height and diameter, F_v/F_m and APase), with the concentration of P in leaves and P levels in the soil. The critical concentration (NC) was considered the point at which the adjusted line reaches the threshold, showing no further increase in the response variable as the concentration of P in the leaf or the soil increases. The regression was adjusted at the 95% percentile to ensure that the established model portrayed the relationship between the dependent variables (height and stem diameter, F_v/F_m,

and Apase) and the independent variables (concentration of P in leaves and soil). The regression line shows the maximum yield attainable at each independent variable level. The regression models were adjusted using the BFGS (Broyden-Fletcher-Goldfarb-Shanno) optimization method from the 'quantreg' package (KOENKER, 2017).

The doses of maximum technical efficiency (MET) in relation to the variables of interest were calculated using quadratic relationships with second-degree regression adjustment, in which the mathematical model $x = (-b)/2a$ was used to estimate the MET. The regressions were adjusted using the 'ggpmisc' package (APHALO, 2021).

Finally, the variables leaf P concentration, soil P content, P doses, height, diameter, F_v/F_m , F_0 , and Apase were subjected to principal component analysis (ACP) to explore the variance of the data, allowing the identification of more complex interactions between the variables, as well as verifying the similarity/dissimilarity between the P doses. Another calculation was the contribution of each variable in explaining the variance of the principal component data. The ACP was carried out using the 'FactoMineR' package (LÊ; JOSSE; HUSSON, 2008).

RESULTS

The doses of P were predominant in explaining the known variations in the data, especially in the activity of the acid phosphatase enzyme (APase), which accounted for approximately 95% of the variation (Figure 1). For the variables, P concentration in leaves, P in the soil, height, stem diameter, and F_v/F_m , the application of P accounted for 70%, 86%, 65%, 55%, and 81%, respectively, of the source of variation in the data (Figure 1).

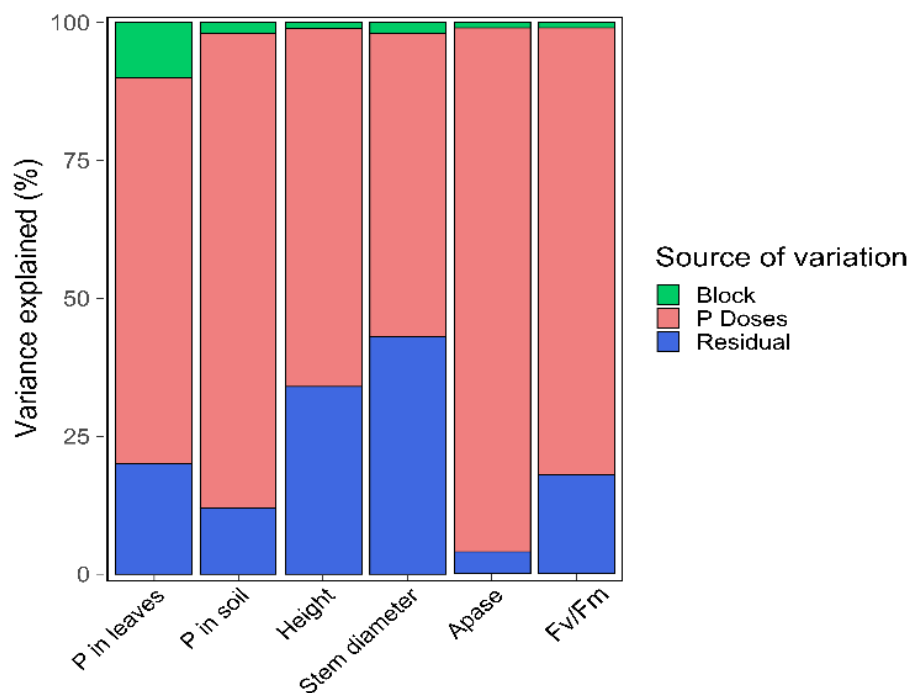


Figure 1. Components of variance, where the colors represent the source of variation (P dose, block and residual). Response variables are shown on the X-axis. The proportion of variance explained by each source of variation for each response variable is shown on the Y-axis.

Figura 1. Componentes da variância, em que as cores representam a fonte de variação (dose de P, bloco e residual). As variáveis de resposta são apresentadas no eixo X e a proporção da variância explicada por cada fonte de variação para cada variável resposta está presente no eixo Y.

P in soil and leaves

The P levels extracted by Mehlich-1 in the soil increased with the application of higher doses of P (Figure 2a). The highest P levels in the soil (55.2 mg dm^{-3}) were observed at a dose of 160 kg P ha^{-1} , which was, on average, 15.1 times higher than in the control soil (Figure 2a).

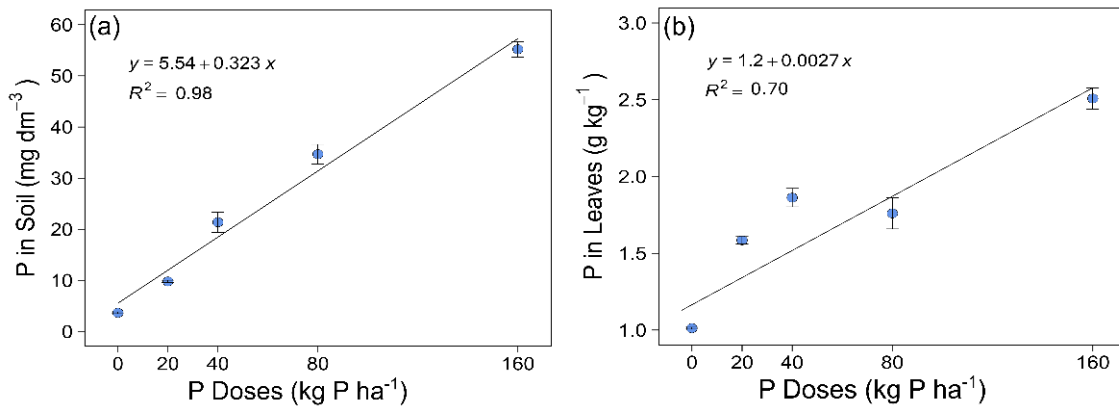


Figure 2. Relationship between P doses and P levels in soil and leaves of *Handroanthus heptaphyllus* submitted to phosphorus fertilization.

Figura 2. Relação entre as doses de P e os teores de P no solo e nas folhas de *Handroanthus heptaphyllus* submetidas à adubação fosfatada.

The P levels in the leaves of the plants increased linearly with the addition of P doses to the soil (Figure 2b). The highest concentrations were 2.0 g kg⁻¹ in plants grown with 160 kg P ha⁻¹. Under these conditions, P levels in the leaf tissue increased by 98.0% compared to that observed in plants grown without P (Figure 2b).

Growth and physiological parameters

P fertilization increased plant height, with the highest values (199.2 cm) being observed at the maximum technical efficiency (MET) dose of 106.4 kg P ha⁻¹. In this condition, the height was 91% higher when compared to the plants grown in the control treatment (Figure 3a).

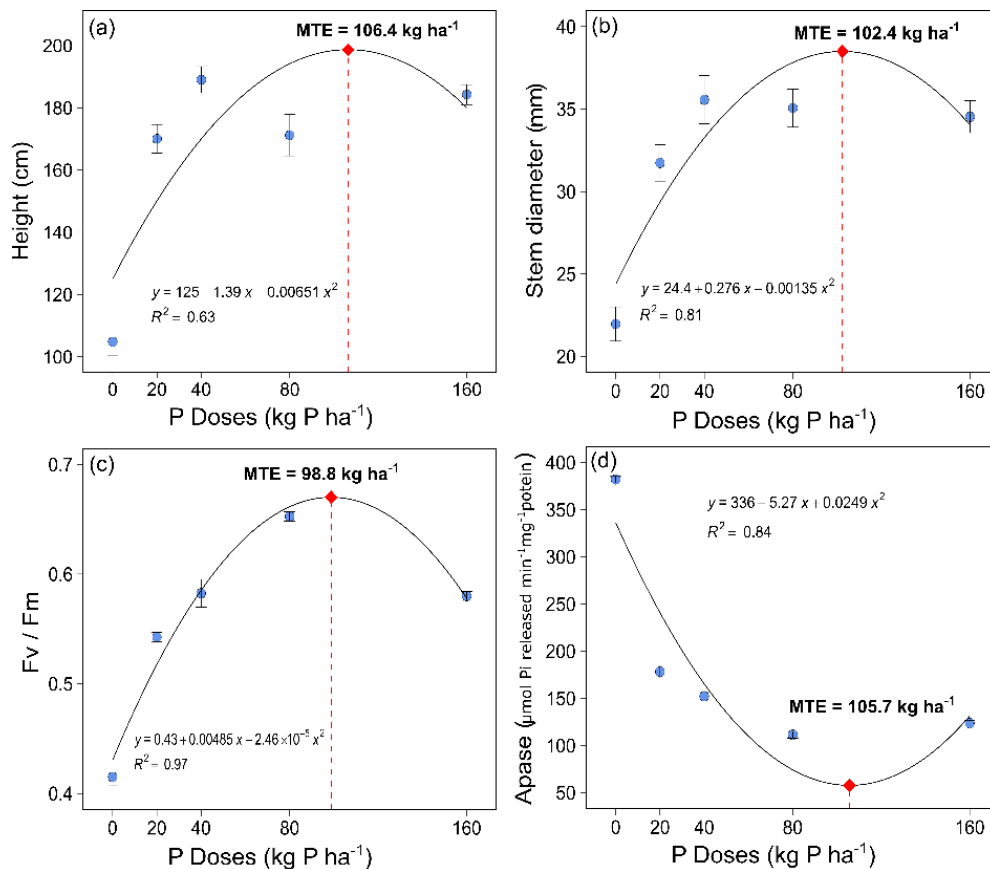


Figure 3. Maximum technical efficiency doses (MTE) in relation to the variables height (a), stem diameter (b), F_v/F_m (c) and Apase (d) in *Handroanthus heptaphyllus* plants submitted to phosphorus fertilization.

Figura 3. Doses de máxima eficiência técnica (MTE) em relação as variáveis altura (a), diâmetro do coleto(b), F_v/F_m (c) e Apase (d) em plantas de *Handroanthus heptaphyllus* submetidas à adubação fosfatada.

Increasing the availability of P in the soil increased the diameter of the plant's stems. The highest value was 38.5 mm in the MET of 102.4 kg P ha⁻¹, 77.3% higher than in the control plants (Figure 3b). The F_v/F_m increased quadratically with the addition of P, with the highest value being 0.66, observed at the MET of 98.8 kg P ha⁻¹ (Figure 3c). Under these conditions, the F_v/F_m was 1.6 times higher than that observed in the control plants (Figure 3c).

APase activity in the leaves of the plants decreased as the doses of P applied to the soil increased. Plants grown without added P showed the highest activity of the enzyme (382.2 nmol Pi min⁻¹ mg⁻¹ protein), which was 6.7 times higher than the e estimated in the MET of 105.7 kg P ha⁻¹ (Figure 3 d).

Critical levels (NC) of P in soil and leaves

The NCs in the soil for the growth variables, height and stem diameter, were 20 and 21.2 mg P dm⁻³, respectively. For the physiological parameters F_v/F_m and APase, the NCs were 29.4 and 9.5 mg P dm⁻³, respectively (Figure 4).

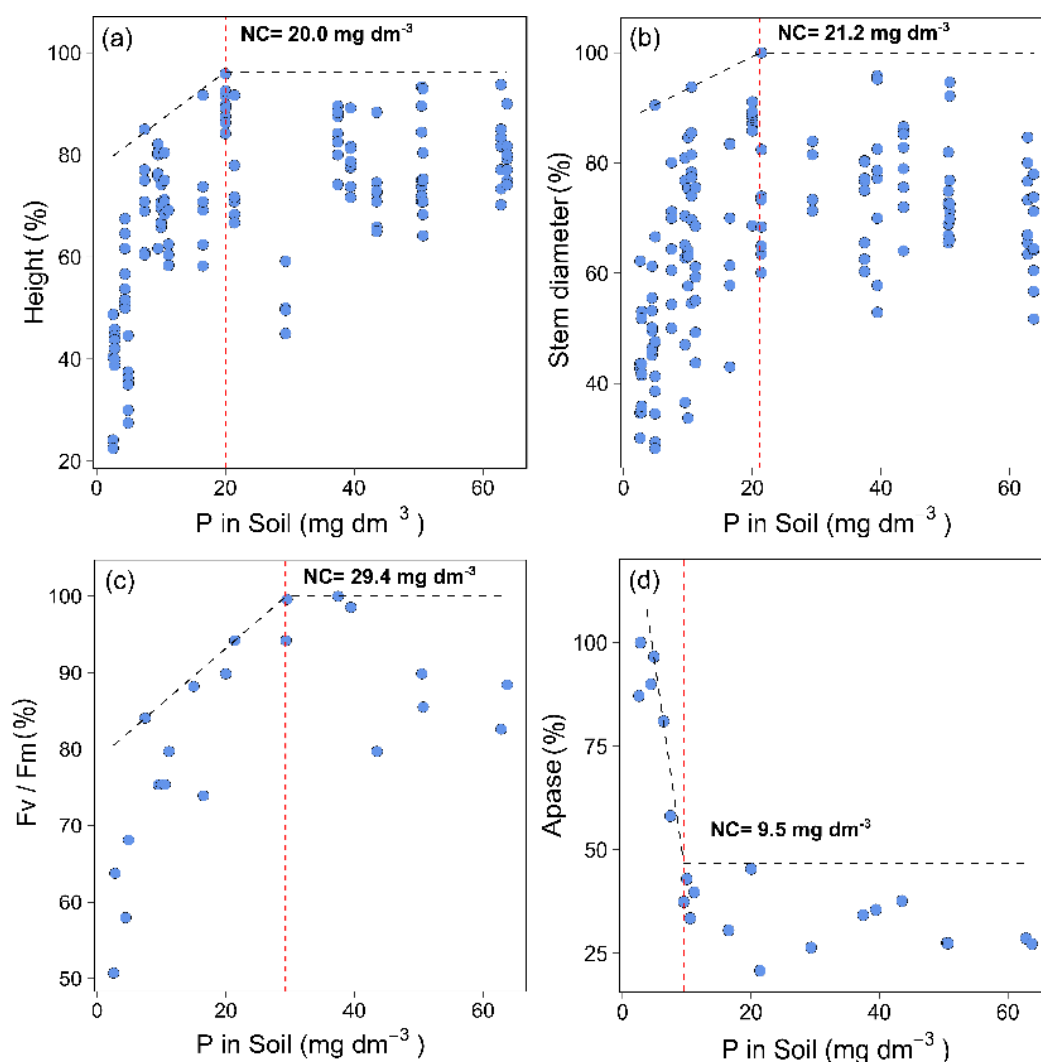


Figure 4. Critical levels (CL) of P in the soil in relation to height (a), diameter (b), F_v/F_m (c) and APase (d) of *Handroanthus heptaphyllus* plants submitted to phosphorus fertilization.

Figura 4. Níveis críticos (NC) de P no solo em relação à altura (a), diâmetro (b), F_v/F_m (c) e APase (d) de plantas de *Handroanthus heptaphyllus* submetidas à adubação fosfatada.

In leaves, the NCs were similar between the variables evaluated. The NC for height, stem diameter, F_v/F_m, and APase were 1.6, 1.7, 1.5, and 1.7 g P kg⁻¹, respectively (Figure 5). The NC for the growth and physiological parameters was, on average, 1.6 g P kg⁻¹, considering the response probability ranges adjusted for 95% relative yield.

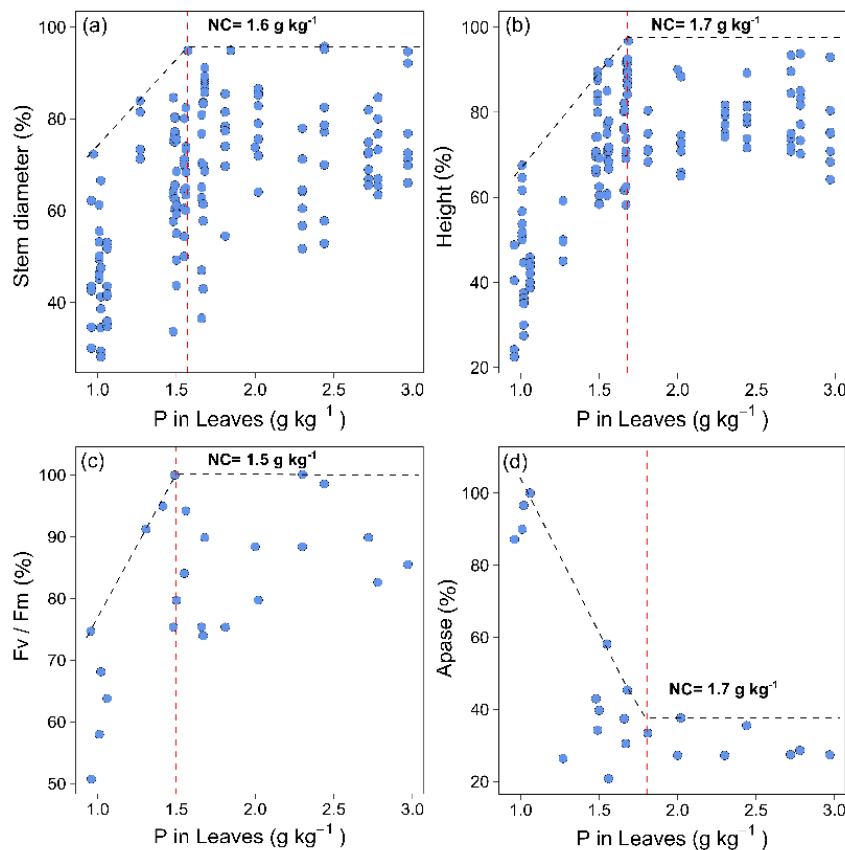


Figure 5. Critical levels (NC) of leaf P in relation to height (a), diameter (b), solids F_v/F_m (c) and Apase (d) of *Handroanthus heptaphyllus* plants submitted to phosphorus fertilization.

Figura 5. Níveis críticos (NC) de P em folha em relação à altura (a), diâmetro (b), sólidos F_v/F_m (c) e Apase (d) de plantas de *Handroanthus heptaphyllus* submetidas à adubação fosfatada.

Principal components analysis

Principal components analysis (ACP) was carried out by extracting only the first two components, which together explained approximately 78.4% of the original variability in the data (Figure 6). Principal component 1 (PC1) explained 63.8% of the variability, and principal component 2 (PC2) explained approximately 14.6% of the variation in the data.

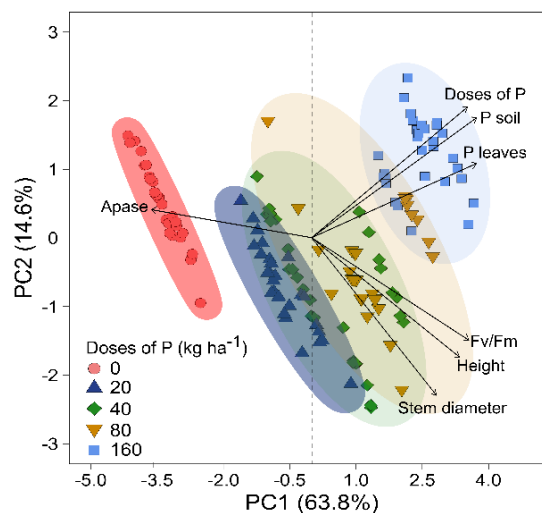


Figure 6. Relationship between principal component 1 (PC1) and principal component 2 (PC2) for P concentration in leaves, P content in soil, P doses, height, stem diameter, F_v/F_m , and Apase in *Handroanthus heptaphyllus* plants submitted to phosphorus fertilization.

Figura 6. Relação entre o componente principal 1 (PC1) e o componente principal 2 (PC2) para a concentração de P em folhas, teor de P no solo, doses de P, altura, diâmetro do coleto, F_v/F_m e Apase em plantas de *Handroanthus heptaphyllus* submetidas à adubação fosfatada.

The results showed that higher doses of P significantly influence P levels in the soil and leaves, especially in plants grown with 160 kg P ha⁻¹ (light blue ellipse). The doses of P showed a positive correlation with height, stem diameter, and F_v/F_m . On the other hand, the activity of the enzyme Apase showed a negative correlation with P doses and increased P availability in the soil. The results show that the PCA efficiently grouped the plants grown without fertilization (pink ellipse) with the highest Apase enzyme activity (Figure 6).

DISCUSSION

One of the main characteristics observed in this study was the increase in available P in the soil due to the application of doses of phosphate fertilizer. The available P in the soil resulted in higher absorption of the element by the plant, supported by an increase in the concentration of P in the leaves. In addition, there was a strong correlation with growth in plant height and diameter, demonstrating the importance of P for the initial start-up of plants in the field. Thus, the P fertilization strategy is essential for tree species grown in soils with low natural fertility, such as the one used in this study, where the initial P content of the soil was considered low (10.0 mg P kg⁻¹ in the soil), associated with 130 g kg⁻¹ of clay (CQFS-RS/SC, 2016).

The plants grown with the P addition had the highest P concentrations in the leaves. This may be related to the contact of the roots with forms of available P in the soil, increasing the possibility of absorption and transport via the xylem, which reflects in more accumulation of P in the leaves (KULMANN *et al.*, 2022). The high concentrations of P in the leaves observed in the higher doses can be explained by the fact that plants grown in soils with a high P content absorb quantities of P above their metabolic needs, causing this nutrient to be allocated to the vacuole of cells in excessive amounts, which can characterize luxury consumption (NOACK *et al.*, 2014).

The increase in height and stem diameter values is associated with higher P levels in the soil, which increases nutrient absorption by the roots and accumulation in the leaves (BERGHETTI *et al.*, 2020). It is assumed that the P in the leaf tissue entered the metabolic compartment of the plants, stimulating photophosphorylation and ATPase activity and, consequently, ATP biosynthesis, ensuring more efficient use of light, photosynthetic rates, and cell division and expansion (CROUS; ÓSVALDSSON; ELLSWORTH, 2015). This increased plant growth and biomass production (NIELSEN *et al.*, 2015), as seen in this study. Some studies on tree species grown with increased P have shown a similar effect on plant growth (BERGHETTI *et al.*, 2020; CROUS; ÓSVALDSSON; ELLSWORTH, 2015).

The increase in P availability positively influenced the F_v/F_m of the plants. This response may be related to increased phosphate absorption by the roots and, consequently, a higher concentration of P in the leaf tissue, resulting in greater use of light energy by PSII and a lower rate of energy loss through fluorescence (BERGHETTI *et al.*, 2020). As a result, *H. heptaphyllus* plants showed increased height and stem diameter.

The principal component analysis corroborates this statement since there is a positive correlation between the concentration of P in leaves and the F_v/F_m , indicating that with an increase in the concentration of P, there is a more efficient use of light energy. In addition, plants with a higher F_v/F_m show increased photosynthetic activity, carbon assimilation, and growth (XU *et al.*, 2019), since there is a reduction in the damage caused by excess energy in the PSII reaction center (BERGHETTI *et al.*, 2020).

The *H. heptaphyllus* plants grown without P showed higher activity of the acid phosphatase enzyme (Apase) in the leaves. The increase in enzyme activity was probably due to the lower concentration of P in the soil, as indicated by the principal component analysis. With the lower availability of P in the soil, a common strategy of forest species for absorbing P is the production and secretion of Apase (HINSINGER *et al.*, 2011). These non-specific enzymes are vital physiological mechanisms that promote organic conversion to inorganic P, thus improving P acquisition and regulating P nutrition within plants (YE *et al.*, 2015).

In this study, the NC of P in the 0-20 cm soil layer was, on average, 20.6 mg dm⁻³ for growth parameters height and stem diameter (Figure 4a and b). This value is slightly higher than the official values recommended for forest species (15.0 mg dm⁻³) in sandy soils in southern Brazil (CQFS-RS/SC, 2016). The difference from the official recommendation can be explained by the lack of specificity in NC calculation, with the NC being derived from forest species and medicinal, aromatic, spice, root, sugar cane, and tobacco crops (CQFS-RS/SC, 2016). Furthermore, the response probability ranges in this study were adjusted to 95% relative yield, while the official recommendation established 75-90% relative yield (CQFS-RS/SC, 2016).

The NC in the leaves showed a slight variation (± 0.2 g kg⁻¹) between the growth and physiological parameters evaluated, with an average value of 1.6 g kg⁻¹. According to the classification proposed by Mellert and Göttlein (2012), where: deficient = P < 1.1 (g kg⁻¹ dry matter); normal = P 1.1-1.9 (g kg⁻¹ dry matter); excessive = P > 2.0 (g kg⁻¹ dry matter), then the proposed NC can be classified as normal.

Finally, the proposed NC for P in the soil and leaves is an essential diagnostic tool for managing phosphate fertilization in commercial plantations and for projects to recover degraded areas with “Ipê-roxo” (*H. heptaphyllus*). Despite the importance of this information, the literature has not presented these values for *H. heptaphyllus*, a species with many purposes in Brazil, such as reforestation, recovering degraded areas, and ornamental purposes. Thus, the proposed NCs have contributed to the proper management of soil fertility and nutrition of *H. heptaphyllus* plants, reducing implementation costs and the potential for contamination of adjacent surface waters.

CONCLUSIONS

- Phosphate fertilization increases the P content in the soil and in the leaves of *H. heptaphyllus*, reducing photochemical energy losses through fluorescence.
- Using 103 kg of P ha⁻¹ promotes higher plant height and stem diameter growth among the tested P additions.
- The NC in the soil for the growth variables height and stem diameter was 20.0 and 21.2 mg P dm⁻³.
- The average leaf NC for growth and physiological parameters was 1.6 g P kg⁻¹.

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REFERENCES

- ALVARES, C. A.; STAPE, J. L.; SENTELHAS, P. C.; DE MORAES G., J. L.; SPAROVEK, G. Köppen’s climate classification map for Brazil. *Meteorologische Zeitschrift*, v. 22, n. 6, p. 711–728, 2013. Available in: <https://www.scopus.com/inward/record.uri?eid=2-s2.0-84897100402&doi=10.1127%2F0941-2948%2F2013%2F0507&partnerID=40&md5=0f37f18be7e8900bbb829d3ea64aad3b>.
- APHALO, P. J. **Package ‘ggpmisc’**. R package, 2021. <https://CRAN.R-project.org/package=ggpmisc>
- BERGHETTI, Á. L. P.; ARAUJO, M.M.; TABALDI, L.A.; TURCHETTO, F.; AIMI, S.C.; RORATO, D.G.; MARCHEZAN, C.; GRIEBELER, A.M.; BARBOSA, F.M.; BRUNETTO, G. Effects of nitrogen fertilization on the growth and on photochemical efficiency in plants of *Handroanthus heptaphyllus*. *Journal of Plant Nutrition*, p. 1–12, 2021. Available in: <https://doi.org/10.1080/01904167.2021.1899216>.
- BERGHETTI, Á. L. P.; ARAUJO, M. M.; TABALDI, L. A.; TURCHETTO, F.; TASSINARI, A.; BERNARDY, D.; GRIEBELER, A. M.; BARBOSA, F. .; AIMI, S. .; BRUNETTO, G. Morphological, physiological and biochemical traits of *Cordia trichotoma* under phosphorous application and a water-retaining polymer. *Journal of Forestry Research*, v. 32, n. 2, p. 855–865, 2020. Available in: <http://link.springer.com/10.1007/s11676-020-01132-8>.
- BRADFORD, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, v. 72, n. 1, p. 248–254, 1976. Available in: <http://www.sciencedirect.com/science/article/pii/0003269776905273>.
- CQFS-RS/SC. **Manual de Calagem e Adubação para os estados do Rio Grande do Sul e Santa Catarina**. 11. ed. Frederico Westphalen, 2016. v. 11 ed.
- CROUS, K. Y.; ÓSVALDSSON, A.; ELLSWORTH, D. S. Is phosphorus limiting in a mature *Eucalyptus* woodland? Phosphorus fertilisation stimulates stem growth. *Plant and Soil*, v. 391, n. 1, p. 293–305, 2015. Available in: <https://doi.org/10.1007/s11104-015-2426-4>.
- EMBRAPA, E. B. D. P. A. **Sistema brasileiro de classificação de solos**. 3. ed. Brasília: Embrapa, 2013, 353 p..
- FÄTH, J.; KOHLPAINTNER, M.; BLUM, U.; GÖTTLEIN, A.; MELLERT, K. H. Assessing phosphorus nutrition of the main European tree species by simple soil extraction methods. *Forest Ecology and Management*, 2019.
- FERREIRA, E. B.; CAVALCANTI, P. P.; NOGUEIRA, D. A. **ExpDes: An R Package for ANOVA and Experimental Designs**. Versão 1.2.0. : R package, 2018. <https://CRAN.R-project.org/package=ExpDes>.

HINSINGER, P.; BRAUMAN, A.; DEVAU, N.; GÉRARD, F.; JOURDAN, C.; LACLAU, J-P.; LE CADRE, E.; JAILLARD, B.; PLASSARD, C. Acquisition of phosphorus and other poorly mobile nutrients by roots. Where do plant nutrition models fail?. **Plant and Soil**, v. 348, n. 1, p. 29, 2011. Available in: <https://doi.org/10.1007/s11104-011-0903-y>.

KOENKER, R. **Handbook of Quantile Regression**. Cambridge: Chapman and Hall/CRC, 2017. *E-book*. Available in <https://www.cambridge.org/core/books/quantile-regression/C18AE7BCF3EC43C16937390D44A328B1>.

KULMANN, M. S. DE S.; ARRUDA, W. S.; VITTO, B. B.; DE SOUZA, R. O. S.; BERGHETTI, Á. L. P.; TAROUÇO, C. P.; ARAUJO, M. M.; NICOLOSO, F. T.; SCHUMACHER, M. V.; BRUNETTO, G. Morphological and physiological parameters influence the use efficiency of nitrogen and phosphorus by *Eucalyptus* seedlings. **New Forests**, v. 53, n. 3, p. 431–448, 2022. Available in: <https://doi.org/10.1007/s11056-021-09864-z>.

LE, S.; JOSSE, J.; HUSSON, F. FactoMineR: An R Package for Multivariate Analysis. **Journal of Statistical Software; Vol 1, Issue 1 (2008)**, 2008. Available in: <https://www.jstatsoft.org/v025/i01>.

MELLERT, K. H.; GÖTTLEIN, A. Comparison of new foliar nutrient thresholds derived from van den Burg's literature compilation with established central European references. **European Journal of Forest Research**, v. 131, n. 5, p. 1461–1472, 2012. Available in: <http://link.springer.com/10.1007/s10342-012-0615-8>.

MURPHY, J.; RILEY, J. P. A modified single solution method for the determination of phosphate in natural waters. **Analytica Chimica Acta**, v. 27, p. 31–36, 1962. Available in: <http://www.sciencedirect.com/science/article/pii/S0003267000884445>.

NIELSEN, U. N.; PRIOR, S.; DELROY, B.; WALKER, J. K. M.; ELLSWORTH, D. S.; POWELL, J. R. Response of belowground communities to short-term phosphorus addition in a phosphorus-limited woodland. **Plant and Soil**, v. 391, n. 1, p. 321–331, 2015. Available in: <https://doi.org/10.1007/s11104-015-2432-6>.

NOACK, S. R.; MCLAUGHLIN, M. J.; SMERNIK, R. J.; MCBEATH, T. M.; ARMSTRONG, R. D. Phosphorus speciation in mature wheat and canola plants as affected by phosphorus supply. **Plant and Soil**, v. 378, n. 1, p. 125–137, 2014. Available in: <https://doi.org/10.1007/s11104-013-2015-3>.

R CORE TEAM. **R: A Language and Environment for Statistical Computing**. Vienna, Austria: R Foundation for Statistical Computing, 2021.

SBCS-NEPAR. **Manual de Adubação e Calagem para o Estado do Paraná**. 2. ed.ed. Curitiba: Núcleo Estadual Paraná da Sociedade Brasileira de Ciência do Solo, 2019.

SCHUETZENMEISTER, A.; DUFEY, F. VCA: Variance Component. Analysis. R package version 1.4.3. 2020. <https://CRAN.R-project.org/package=VCATABALDI> L, A.; RUPPENTHAL, R.; CARGNELUTTI, D.; MORSCH, V. M.; PEREIRA, L. B.; SCHETINGER, M. R. C. Effects of metal elements on acid phosphatase activity in cucumber (*Cucumis sativus* L.) seedlings. **Environmental and Experimental Botany**, v. 59, n. 1, p. 43–48, 2007. Available in: <http://www.sciencedirect.com/science/article/pii/S009884720500184X>.

TEDESCO, M. J.; GIANELLO, C.; BISSANI, C. A.; BOHNEN, H.; VOLKWEISS, S. J.. **Análise de solo, plantas e outros materiais**. Porto Alegre: Universidade Federal do Rio Grande do Sul, 1995.

XU, L.; ZHAO, H.; WAN, R.; LIU, Y.; XU, Z.; TIAN, W.; RUAN, W.; WANG, F.; DENG, M.; WANG, J.; DOLAN, L.; LUAN, S.; XUE, S.; YI, K. Identification of vacuolar phosphate efflux transporters in land plants. **Nature Plants**, v. 5, n. 1, p. 84–94, 2019. Available in: <https://doi.org/10.1038/s41477-018-0334-3>.

YE, D.; LI, T.; LIU, D.; ZHANG, X.; ZHENG, Z. P accumulation and physiological responses to different high P regimes in *Polygonum hydropiper* for understanding a P-phytoremediation strategy. **Scientific Reports**, v. 5, n. 1, p. 17835, 2015. Available in: <http://www.nature.com/articles/srep17835>.