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Growth and nutritional efficiency of acacia seedlings in response to phosphate fertilization

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ABSTRACT: *Acacia mangium* Willd cultivation is a viable option to meet the growing demand for wood in the forest market. However, there is little information related to its nutritional requirements in the seedling growth phase. The objective of this study was to evaluate the growth and nutritional efficiency of acacia seedlings in response to phosphate fertilization. The experimental design was completely randomized with five treatments, and with five replications each. The treatments consisted of five phosphorus (P) doses: 0, 50, 100, 150 and 200 mg dm^{-3}, and triple superphosphate (46% P_2O_5) was used as the mineral source. The plant height, stem diameter, leaf number, shoot, root and total dry matter, P content in leaves, stem and roots, and P efficiency of uptake, translocation and use were subsequently evaluated 120 days post-transplant. The rate of 150 mg dm^{-3} of P showed the best growth and efficiency results for P use. The increases observed in acacia seedlings due to the increase in P rates show the importance of adequate phosphate nutrition in the development of this species. Thus, 150 mg dm^{-3} of P is the recommended rate for *Acacia mangium* seedling cultivation.

Key words: forest nutrition; macronutrient; phosphorus

Crescimento e eficiência nutricional de mudas de acácia em resposta à adubação fosfatada

RESUMO: O cultivo de *Acacia mangium* Willd é uma opção viável para suprir a crescente demanda de madeiras no mercado florestal. No entanto, existem poucas informações relacionadas às suas exigências nutricionais em fase de crescimento de mudas. Objetivou-se avaliar o crescimento e a eficiência nutricional de mudas de acácia em resposta à adubação fosfatada. O delineamento experimental utilizado foi inteiramente casualizado, com cinco tratamentos, com cinco repetições cada. Os tratamentos foram constituídos de cinco doses de fósforo (P): 0, 50, 100, 150 e 200 mg dm^{-3}, sendo a fonte mineral utilizada o superfosfato triplo (46% de P_2O_5). Após 120 dias do transplantio, foram avaliadas a altura da planta, diâmetro do coleto, número de folhas, matérias secas da parte aérea, raiz e total, conteúdos de P nas folhas, caule e raízes, e eficiências de absorção, translocação e uso. A dose de 150 mg dm^{-3} de P apresentou os melhores resultados de crescimento e de eficiência de uso de P. Os incrementos observados nas mudas de acácia em razão do aumento das doses de P evidenciam a importância da adequada nutrição fosfatada no desenvolvimento desta espécie. Assim, recomenda-se a dose 150 mg dm$_{-3}$ de P para o cultivo de mudas de *Acacia mangium*.

Palavras-chave: nutrição florestal; macronutriente; fósforo

Introduction

The Brazilian forest sector is in growing demand for raw forest materials, especially planted forests, and Brazil currently has about 7.8 million hectares of planted area (Ibá, 2018). However, these plantations are not sufficient to supply the demand, which makes it extremely important to develop practices and studies to increase the productive potential of forest species (Ibá, 2018).

In this scenario, the cultivation of *Acacia mangium* Willd is an option to meet the growing demand for wood and may positively contribute to developing the Brazilian forest sector (Lombardi, 2013). This species has high adaptability to Brazilian edaphoclimatic conditions, fast growth, good biomass production, and above all is capable of symbiotic association with atmospheric nitrogen-fixing bacteria (Duarte et al., 2011).

The species is native to the rainforests of northeastern Australia, New Guinea and Indonesia, belongs to the *Acacia* genus, *Leguminoseae* family (Krisnawati et al., 2011). *A. mangium* Willd has great potential for use, being widely implemented in recovering degraded areas, in wood production for coal, energy, cellulose, wood panels and for ornamental purposes, as well as being used as windbreakers in agroforestry systems.

The areas intended for cultivation are usually soils with low fertility, and few soil management practices are performed in most cases, thus making it impossible to express their productive potential. Although the species presents good adaptability in soils with low nutrient availability (Pardos et al., 2005), it is necessary to apply fertilizers and correctives in order to increase the productivity (Schumacher et al., 2013).

Nutrient maintenance at adequate levels for plant development is achieved through mineral or organic fertilization, an essential practice to achieve the desired growth (Bernardino & Garcia, 2009). The issue becomes even more important when it comes to crops in Cerrado soils which have low fertility and high acidity (Tavares et al., 2016). Therefore, the application of correctives and fertilizers in adequate quantities for the crop requirements is fundamental for the crops' efficiency (Vieira et al., 2014).

Plant growth depends on the availability of macronutrients (N, P, K, Ca, Mg, and S) and micronutrients (B, Cl, Cu, Fe, Mn, Mo, Ni, and Zn), among other factors (Faquin, 2005). Of these, phosphorus (P) is one of the most required nutrients by forest species (Schumacher et al., 2003). In addition, P participates in vital metabolic processes for plant development such as energy transfer, nucleic acid and glucose synthesis, respiration, membrane synthesis and stability, enzyme activation and deactivation (Taiz & Zeiger, 2013).

There are still few studies related to the nutritional demand of P in seedling production and its importance for forest species in Cerrado soils. However, some studies show the importance of P in the initial development of plants, as observed by Cardoso et al. (2015), who assessed the initial development of mahogany (*Swietenia macrophylla* King.), verifying the species' requirement for adequate doses of P close to 41.6 mg dm^{-3}. In turn, Vasconcelos et al. (2017) found the requirement of 240 mg dm^{-3} P for African mahogany (*Khaya senegalensis* A. Juss), as well as Moro et al. (2014) who obtained the required dose of 140 mg dm^{-3} of P for *Pinus taeda*.

Due to the importance of P for plant growth, it is necessary to carry out specific studies on phosphate fertilization in the seedling growth of forest species such as *Acacia mangium* Willd in order to obtain the appropriate dose and requirement of this nutrient. Thus, the objective of this study was to evaluate the growth and nutritional efficiency of acacia seedlings in response to phosphate fertilization in a greenhouse.

Materials and Methods

Study area

The experiment was conducted in a greenhouse in an experimental area of Goiás State University, Campus Ipameri (geographic coordinates 17º43'19" latitude S and 48º09'35" longitude W and an altitude of 764 m). The greenhouse characteristics are: 3.5 m in height, 30.0 m in length, 7.0 m in width, closed at the sides with 50% black shade and cover with transparent plastic of 150 microns. The temperature inside the greenhouse during the experimental period ranged from 18 °C (minimum) to 30 °C (maximum), with an average of 23 °C.

Experimental design and treatments

The experimental design was completely randomized with five treatments and five replications each, totaling 25 experimental units. The treatments consisted of five doses of P: 0, 50, 100, 150 and 200 mg dm^{-3}, with triple superphosphate being the implemented fertilization source (46% of P_2O_5).

Seedling production

The species used was *Acacia mangium* Willd, with its seedlings being produced from seeds from the municipality of Ipameri. Seed dormancy was broken by immersion in hot water at 100 °C for one minute to favor germination (Dutra et al., 2013). Then the seeds were sown in 53 cm^3 tubes filled with commercial Carolina Soil® substrate [pH in water = 5.53; organic matter = 350 g kg^{-1}; total nitrogen = 1.1 g kg^{-1}; Ca^{2+} = 0.7 cmol$_c$ dm^{-3}; Mg^{2+} = 5.18 cmol dm^{-3}; K^+ = 7.6 x 10^{-4} cmol$_c$ dm^{-3}; available P (Mehlich^{-1}) = 0.06 mg dm^{-3}], where they remained until the transplantation date (60 days after sowing). The plants were not fertilized during this period.

Soil, container and nutrient application

The soil used was a dystrophic Latossolo Vermelho-Amarelo (Embrapa, 2018), collected in the subsurface layer (0.2-0.4 m). The soil was passed through a 4 mm mesh sieve after collection, and homogenized for physicochemical characterization (Table 1).

Due to the physicochemical analysis of the collected soil, dolomitic limestone (PRNT = 92%) was applied to the soil before beginning the experiment, aiming to increase the base

Table 1. Physicochemical characteristics of a Latossolo Vermelho-Amarelo used in the experiment.

pH	Available P	P_{REM}	K^+	Ca^{2+}	Mg^{2+}	H+Al	CEC	SOM	V
($CaCl_2$)	(mg dm^{-3})	(mg L^{-1})	($cmol_c$ dm^{-3})					(g dm^{-3})	(%)
5.4	0.8	11.1	0.04	0.2	0.1	1.8	2.14	7.0	16.09

B	Cu	Fe	Mn	Zn	Sand	Silt	Clay	Bulk density
(mg dm^{-3})					(g kg^{-1})			(g cm^{-3})
0.28	0.3	34	10.1	0.3	610.0	80.0	310.0	1.21

Extractors used: P and K = Mehlich-1; Ca^{2+}, Mg^{2+} and Al^{3+} = 1 mol/L KCl; H+Al = 0.5 mol/L calcium acetate at pH 7.0. B = saturation extract in hot water; Cu, Fe; Mn and Mn = DTPA/$CaCl_2$ (CAT). P_{REM} = remaining phosphorus (Alvarez et al., 2000). SOM = soil organic matter (Walkley & Black, 1934). V = base saturation. H+Al = potential acidity. CEC = cation exchange capacity.

saturation (V) to 60%. The soil was kept moist at 60% of the soil water retention capacity (17 kPa) during the 40-day period to favor the limestone reaction.

The samples were placed in 5 dm^3 black polyethylene pots at the end of the soil incubation period, which received the following P doses: 0, 50, 100, 150 and 200 mg dm^{-3}. The triple superphosphate doses (46% P_2O_5) were applied by incorporation and homogenization into the soil.

In addition, 150 mg dm^{-3} N and 150 mg dm^{-3} K in the form of urea and potassium chloride, respectively, were also added. Moreover, the following micronutrients were applied: B (0.5 mg dm^{-3}), Mn (1.5 mg dm^{-3}), Zn (0.5 mg dm^{-3}), Cu (0.5 mg dm^{-3}) mg dm^{-3}) and Mo (0.1 mg dm^{-3}), having boric acid, manganese chloride, zinc chloride, copper sulfate and ammonium molybdate as sources, respectively. Nutrients were individually applied in each pot via nutrient solution, divided into 4 applications with an interval of 30 days each, with the first dose applied 60 days after sowing. The nutrient doses were adapted according to the work of Araújo et al. (2017).

The seedlings were transplanted to the pots at 60 days after sowing. Soil moisture was maintained throughout the experimental period at approximately 60% of the maximum soil water retention capacity. The volume of evapotranspirated water was replaced daily by weighing the vessels. The positions of the pots with the plants were randomly changed every seven days in all treatments in aiming toward homogenization and randomization of uncontrolled factors.

Phytotechnical characteristics evaluated

The following growth characteristics were evaluated 120 days after transplantation: plant height (H), stem diameter (SD) and number of leaves per plant (NL). Then the plants were separated into leaves, stem and roots to determine dry matter. The plant parts were then washed with distilled water and then placed in a forced air oven for 72 hours at 70 °C. After drying, they were weighed in analytical scale to determine shoot dry matter (SDM = shoot dry matter [leaf dry matter + stem dry matter]), and root dry matter (RDM), and these values were summed to obtain total dry matter (TDM).

The plant material was subsequently milled in a Wiley type stainless steel mill with a 20 mesh sieve, and the P, leaf and stem contents were analyzed. The molybdenum blue spectrophotometry method was used to determine P levels, according to the methodology described by Miyazawa et al. (2009). The P content in the leaves (LPC), stem (SPC) and root (RPC) were then calculated using the obtained data.

P nutritional efficiency

From the plant dry matter and P content, the phosphorus absorption (PAE) (Swiader et al., 1994), translocation (PTE) (Li et al., 1991) and usage (PUE) efficiencies were calculated (Siddiqi & Glass, 1981) according to Equations 1, 2 and 3.

$$\mathrm{PAE}\left(\mathrm{mg\ g^{-1}}\right) = \frac{\left(\mathrm{LPC}+\mathrm{SPC}+\mathrm{RPC}\right)}{\mathrm{RDM}} \tag{1}$$

$$\mathrm{PTE}\left(\%\right) = \frac{\left(\mathrm{LPC}+\mathrm{SPC}\right)}{\left(\mathrm{LPC}+\mathrm{SPC}+\mathrm{RPC}\right)} \times 100 \tag{2}$$

$$\mathrm{PUE}\left(\mathrm{g^2\ mg^{-1}}\right) = \frac{\left(\mathrm{SDM}+\mathrm{RDM}\right)^2}{\left(\mathrm{LPC}+\mathrm{SPC}+\mathrm{RPC}\right)} \tag{3}$$

in which: LPC = leaf phosphorus content; SPC = stem phosphorus content; RPC = root phosphorus content; RDM = root dry matter; SDM = shoot dry matter (leaf dry matter + stem dry matter).

Statistical analyses

The data were subjected to analysis of variance (ANOVA) and simple regression analysis after verifying the assumptions of normality and homogeneity of residual variances of the data to evaluate the influence of P doses on each variable. Statistical analyzes were conducted using the SISVAR 5.4 software program (Ferreira, 2011).

Results and Discussion

According to the results obtained in this work there was a significant effect of P doses on all studied variables, except on translocation efficiency, which demonstrates the importance of P in the initial growth of acacia seedlings. Visually, the 150 mg dm^{-3} P dose provided the highest plant growth (Figure 1). P influences the development of seedlings, as it participates in plant metabolism, is part of the energy transfer process (ATP formation), the structure of various organic molecules, composing membranes and esters of carbohydrates, being fundamental for cellular and photosynthetic activities (Marschner, 2012; Taiz & Zeiger, 2013). Thus, the availability of P is essential in all acacia development phases, and its deficiency can slow down the shoot and root growth.

Figure 1. *A. mangium* Willd seedlings submitted to increasing doses of P at 120 days after transplantation.

The H, SD and NL variables presented significant adjustment ($p<0.01$) to the quadratic regression model, showing increases in response to the initial doses and decreasing in the highest P dose. The H of plants obtained with the estimated dose of 152 mg dm^{-3} of P was 70.3 cm, corresponding to the maximum height reached by the plants, whose value was 143% higher than the plants grown at the P dose of 0 mg dm^{-3} (Figure 2A). An increase in the SD variable was observed up to the estimated dose of 127 mg dm^{-3} of P, which corresponded to 8.4 mm, constituting a value of 68% higher than plants submitted to the 0 mg dm^{-3} dose of P (Figure 2B). SD and H are seedling quality indicator variables which are greatly influenced by the addition of P (Caldeira et al., 2014). This is important because vigorous seedlings provide greater post-planting survival, increased growth and root formation (Araújo, 2018).

Phosphate fertilization was also important for leaf production. The highest NL was obtained at the estimated dose of 147 mg dm^{-3}, corresponding to 58 leaves per plant^{-1} (Figure 2C). There was a 544% reduction in NL in the absence of P (0 mg dm^{-3}) in relation to the estimated maximum yield. Freiberger et al. (2014) stated that plants with P deficiency present reduced leaf number and reduced leaf area, which corroborates the results found in the present work.

Similar results to those obtained in this study were observed by Leite et al. (2014), who found a significant increase for H, SD and NL due to the increase in P doses in mulungu (*Erythrina velutina* Willd) seedlings up to 200 mg dm^{-3}. In *Eucalyptus benthamii* and *Eucalyptus dunnii* seedlings, Dias et al. (2015) also observed a positive response of the analyzed variables (dry matter production and accumulated P) to different P sources, showing the importance of P in the development of forest species, since the plants showed a positive response independent of the source applied. In *Cassia grandis* L. seedlings, Freitas et al. (2017) found that phosphate fertilization at a dose of 600 mg dm^{-3} positively influenced seedling growth and quality.

When analyzing plant biomass, a significant adjustment ($p<0.01$) was also observed for the quadratic regression model

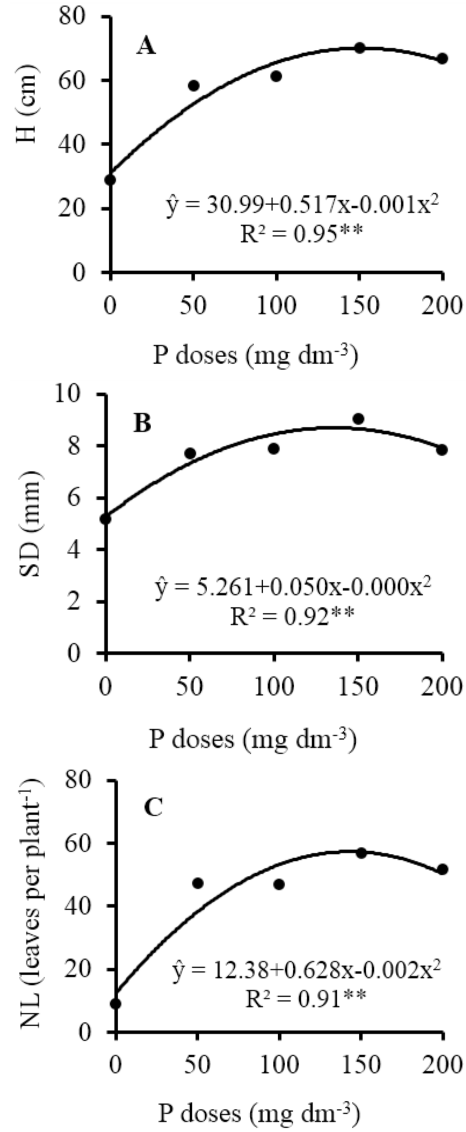

Figure 2. Equations adjusted for plant height (A), stem diameter (B), and number of leaves per plant (C) of acacia as a function of P. * and ** = significant at 5 and 1% significance, respectively.

in the SDM, RDM and TDM variables (Figure 3). The addition of P in highly weathered soils (with low available P content) promotes significant increases in plant biomass (Rodrigues et al., 2014; Cabral et al., 2016), as occurred in the soil used in this work, whose initial content P was low (0.8 mg dm^{-3}) (Table 1).

SDM showed an estimated increase of 27.8 g plant^{-1} when grown at the estimated P dose of 136 mg dm^{-3}, being 8 times higher than the control (0 mg dm^{-3} of P). In turn, plants grown at the maximum P dose (200 mg dm^{-3}) showed a 24% reduction in relation to the maximum SDM production (Figure 3A). These results corroborate Araújo et al. (2018), who reported that high P doses may reduce SDM production, and therefore it is essential to ensure the adequate availability of this nutrient.

There was higher RDM production at the estimated P dose of 153 mg dm^{-3}, corresponding to 22.4 g plant^{-1} (Figure 3B).

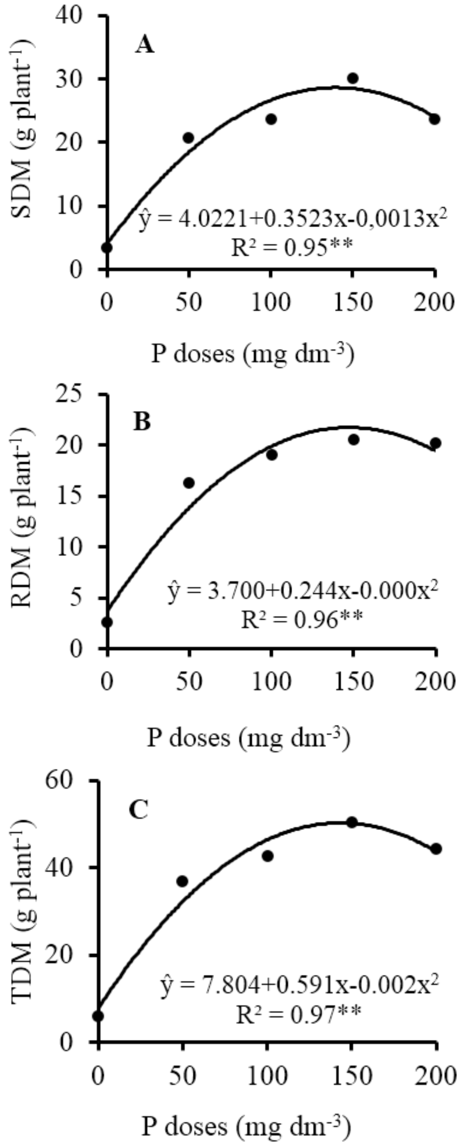

Figure 3. Equations adjusted for shoot dry matter (A), root dry matter (B), and total dry matter (C) of acacia plants as a function of P. * and ** = significant at 5 and 1% significance, respectively.

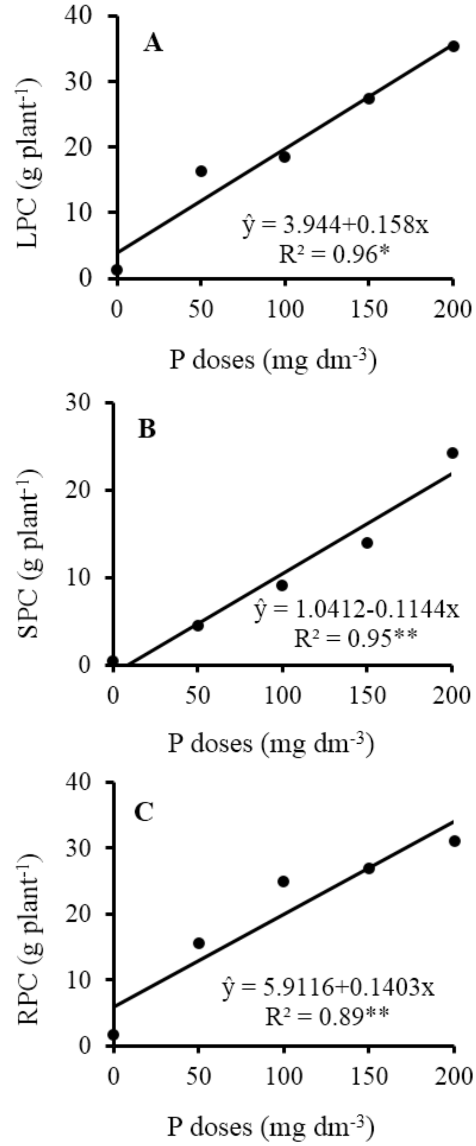

Figure 4. Regressions adjusted for P content in leaves (A), stem (B) and root (C) of acacia plants as a function of P. * and ** = significant at 5 and 1% significance, respectively.

These results are in agreement with the main function of P in the induction of root system growth (Corrêa et al., 2004), since P is essential for its formation due to its role as energy carrier, in addition to other functions in the plant (Silva et al., 2015).

TDM yields increased to the estimated P dose of 141 mg dm^{-3}, which resulted in a weight of 49.5 g $plant^{-1}$ (Figure 3C). It is important to highlight that the plants reduced biomass production by applying P doses above 141 mg dm^{-3}. However, the absence of phosphate fertilization (dose 0 mg dm^{-3}) promoted higher biomass losses than an excess of P; this behavior was also observed by Miranda et al. (2016).

The LPC, SPC, and RPC variables presented significant adjustment to the linear regression model, which showed the highest values by applying the maximum P dose (200 mg dm^{-3}) (Figure 4).

Although the maximum P dose (200 mg dm^{-3}) provided the highest P content values in the plant, it was the P dose of 150 mg dm^{-3} which resulted in the highest biomass production. Therefore, the plants had a "luxury intake" at the maximum P dose, resulting in higher nutrient levels which usually do not result in increases in dry matter yield (Taiz & Zeiger, 2013). Although this "luxury consumption" of P occurs in the early stages of seedling development, this available P reserve will contribute to meeting plant needs in future field conditions (Gonçalves et al., 2000).

Santos et al. (2008) observed an increase of P content in mahogany (*Swietenia macrophylla* King) seedlings grown in dystrophic Yellow Latosol under greenhouse conditions as a function of increasing P rates in the soil, showing a linear response in P content up to a dose of 800 mg dm^{-3}. In guapuruvu plants (*Schizolobium parahyba* (Vell.) S.F.Blake), Araújo et al. (2018) also observed that leaf P concentrations were positively influenced by increasing P doses, showing increasing linear behavior as a function of applied doses.

The plant LPC obtained at the P dose of 200 mg dm^{-3} was 35.5 g plant^{-1}, being 35 times higher than that found (4.1 g plant^{-1}) for the lowest P dose (0 mg dm^{-3}) (Figure 4A). The higher P dose (200 mg dm^{-3}) provided a 24-fold increase in SPC and a 15-fold increase in plant RPC when compared to the lowest dose (0 mg dm^{-3}) (Figures 4B and C).

When analyzing the nutritional efficiency of the acacia seedlings, a significant effect (p<0.01) was observed for PAE and PUE as a function of increasing P doses, adjusting to the linear and quadratic regression model, respectively (Figure 5A and B). However, there was no significant adjustment of regression models as a function of P doses for the PTE variable (Figure 5B).

Regarding translocation efficiency, which is characterized as the process of transferring a nutrient from one organ or absorption region to another (Faquin, 2005), it was verified that they showed similar behavior regarding nutrient translocation, although the acacia seedlings were subjected to different P concentrations in the soil. Thus, the plants had proportionally the same PTE under excess P conditions (200 mg dm^{-3}) and P deficiency (0 mg dm^{-3}) (Figure 5B).

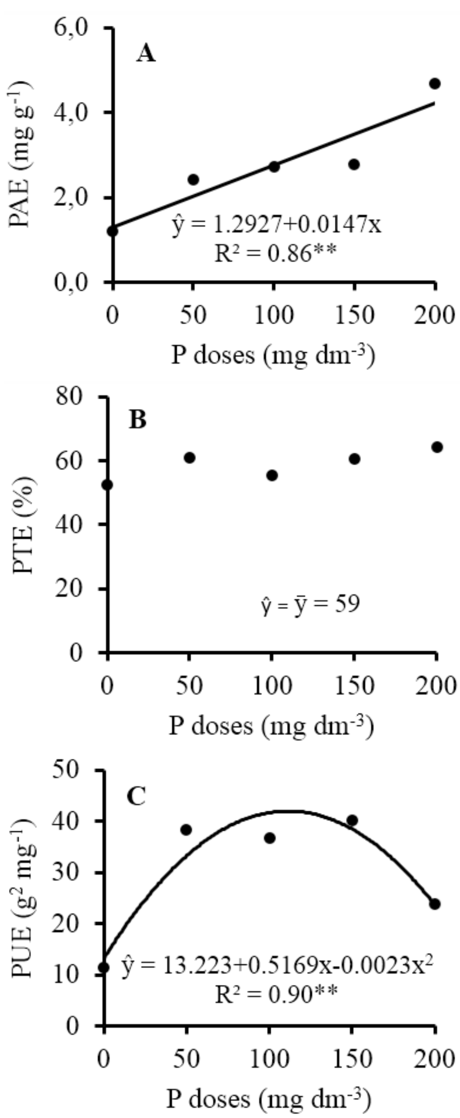

Figure 5. Adjusted equations for P (A) absorption, P (B) translocation and P (C) usage efficiencies of acacia plants as a function of P. * and ** = significant doses at 5 and 1% of significance, respectively.

Acacia seedlings showed higher PAE (4.7 mg g^{-1}) when grown at the maximum P dose (200 mg dm^{-3}) (Figure 5A). This value was 291% higher than plants grown in the absence of phosphate fertilization (0 mg dm^{-3} of P). This behavior was similar to that observed in the study by Stahl et al. (2013), in which *Eucalyptus dunnii* obtained increasing linear gains in PAE as the P supply to the soil increased.

Although seedlings absorbed more P when applied at the maximum dose, the highest PUE was observed when plants were subjected to a P dose of 150 mg dm^{-3} (Figure 5C). Therefore, the more P was made available in the soil, the higher the PAE by the plants. However, the higher PAE was not accompanied by higher PUE or higher biomass production. Corroborating with the present study, the decrease of PUE with the increase in the P dose has been observed in the literature, as shown by Stahl et al. (2013) and Fageria et al. (2011).

The PUE was reduced by 66% at the maximum P dose (200 mg dm^{-3}) compared to the 150 mg dm^{-3} dose. Thus, a P dose application of 150 mg dm^{-3} is recommended for the studied edaphic conditions in order to avoid waste of phosphate fertilizer, because the seedlings expressed their maximum development potential in H, SD, NL and biomass production (SDM and RDM) in this condition.

Finally, it is noteworthy that acacia seedlings grown at the P dose of 0 mg dm^{-3} showed visual symptoms of P deficiency (Figure 1), as characterized by reduced leaf area and low growth (Ferreira, 2012). Visual deficiency symptoms are usually the last stage of several irreversible metabolic problems, and a subsequent P supply to adequate levels will not compensate for the damage which has already occurred (Barroso et al., 2005). The results obtained in this work showed that adequate P supply is essential in the early stages of forest seedling growth.

Conclusions

Acacia mangium Willd is responsive to phosphate fertilization during its early growth.

The approximate P dose of 150 mg dm^{-3} showed the best results for growth and nutritional efficiency of P use. Therefore, this P dose is recommended for *Acacia mangium* Willd seedling cultivation under similar edaphic conditions.

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