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Growth and nutrient uptake by slash pine seedlings under phosphate fertilizer sources

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ABSTRACT

The effects of changes in substrate nutrient availability, as a function of doses and sources of phosphate and controlled release fertilizer were investigated in slash pine (*Pinus elliottii* Engel) seedlings. Pine seedlings were cultivated in substrate composed of 50% clay soil from the subsurface horizon of a Nitosol, and 50% pure sand, uniformly mixed. Fertilizer sources were: natural phosphate, soluble phosphate and a controlled release fertilizer, applied as half (0.18 kg P_2O_5 m^{-3}), full (0.36 kg P_2O_5 m^{-3}) and twice (0.72 kg P_2O_5 m^{-3}) the recommended dose, in a completely randomized design, with four replications. Assessments were made during the early seedling growth stage by evaluating morphological parameters, dry mass and nutrient accumulation at 330 days after germination. Results showed that controlled release fertilizer gave the best seedling growth, the largest stem diameter and the highest values for dry mass accumulation at 330 days after germination. Natural and soluble phosphates were not as effective to promote pine seedlings development as controlled release fertilizer, but the soluble phosphate was much better than natural phosphate.

Key words: controlled release fertilizer, forest nutrition, *Pinus elliottii*, seedlings quality

Crescimento e absorção de nutrientes por mudas de pinus sob fontes de fertilizantes fosfatados

RESUMO

Este trabalho objetivou avaliar o efeito das mudanças na disponibilidade de nutrientes do substrato, em função de doses e fontes de fósforo e fertilizante de liberação controlada em mudas de pinus (*Pinus elliottii* Engel). Mudas de pinus foram cultivadas em substrato composto por 50% de horizonte subsuperficial de um Nitossolo argiloso e 50% de areia pura, uniformemente misturado. Os tratamentos com fontes de fertilizante foram: fosfato natural, fosfato solúvel e um fertilizante de liberação controlada nas doses meia (0,18 kg P_2O_5 m^{-3}), total (0,36 kg P_2O_5 m^{-3}) e duas vezes (0,72 kg P_2O_5 m^{-3}) a recomendada em um delineamento experimental inteiramente casualizado, com quatro repetições. Foram avaliados o desenvolvimento das mudas, parâmetros morfológicos, massa seca e acúmulo de nutrientes aos 330 dias após a germinação. Os resultados mostram que o fertilizante de liberação controlada promove melhor crescimento das mudas, maior diâmetro do caule e maior acúmulo de massa seca aos 330 dias após a germinação. Fosfatos naturais e solúveis não são eficazes na promoção do desenvolvimento de mudas de pinus em comparação com fertilizante de libertação controlada mas o fosfato solúvel ainda é muito melhor do que o fosfato natural.

Palavras-chave: fertilizante de liberação controlada, nutrição florestal, *Pinus elliottii*, qualidade de mudas

Introduction

In Brazil, several pine genus are grown on approximately 2 million hectares, with an average annual yield of around 18 to 28 m^3 ha^{-1} $year^{-1}$. However, this yield could reach 40 m^3 ha^{-1} $year^{-1}$ at some specific sites and under certain management conditions (IPEF, 2010). Thus, yield may increase if some controlling factors are identified and optimized, such as genotype, plant nutrition, soil tillage, plant growth and soil management (IPEF, 2010). Seedling nutrition is a relevant issue which can be controlled at nursery stage by adjusting type of substrate and nutritional conditions (Watson & Tombleson, 2002). Nutrition of pine seedlings can affect in-field development after transplantation. The use of a balanced nutritional substrate will not only contribute for a better plant setting and initial growth in the field (Khasa et al., 2001) but also enhance plant resistance against pests, diseases and cold seasons (Gonçalves & Benedetti, 2000).

Nutrient uptake by plants is dependent on the composition and solubility of the fertilizer. Thus, the fertilizer source and application method can determine nutrient uptake. Brazilian soils, similar to most weathered soils in South America and Africa, are characterized by limited levels of available nutrients. *Pinus* species are known to have low nutritional requirements. Research studies have demonstrated that growth in high fertility soil requires significantly higher fertilizer requirements, although no symptoms of nutritional deficiency are visible under low soil fertility conditions (Gonçalves & Benedetti, 2000).

Many soil microorganisms, including fungi and bacteria, have the ability to solubilize soil phosphorus (P) in different ways, for example by exudation of organic acids. These organic acids compete with P for adsorption sites in the soil, resulting in more inorganic P in the soil solution (Guppy et al., 2005). *Pinus elliottii* has the ability to create colonies of mycorrhizal fungi in their root system, which can greatly increase both the volume of soil exploitable and organic acid exudation, and probably solubilize and take up more P and nitrogen (N) from the soil (Zhang et al., 2012).

Although some studies have investigated the effects of P fertilization on seedling production in Brazil (Tucci et al., 2007; Nicoloso et al., 2008), the effects of different sources of P on seedling production under nursery conditions remain unexplored. Information on P mobilization by crop root systems is important to understand the dynamics of this nutrient in the field. Moreover, controlled release fertilizers may be an alternative source to improve nutrient uptake by pine seedlings. Hence, this study aimed to evaluate the growth and nutrition of slash pine seedlings (*Pinus elliottii* Engel), and changes in substrate nutrient availability, as a function of doses and sources of phosphorus and controlled release fertilizer.

Material and Methods

The experiment was performed in 2009 at Nursery Forest of Federal Technological University of Paraná, Dois Vizinhos, Paraná, Brazil, located at 25º42'S latitude, 53º08'W longitude and about 561 m above sea level. *Pinus elliottii* seedlings were cultivated in black plastic bags (2 L capacity) filled with a substrate composed of 50% clay soil from a subsurface layer (68% clay) of a Nitosol Vermelho distroférrico típico (Embrapa, 2006) and 50% pure sand, mixed uniformly. The substrate presented: pH-$CaCl_2$ (1:2.5): 5.50; OM: 6.70 mg g^{-1}; P-resin: 1.96 mg dm^{-3}; and K: 0.28 $cmol_c$ dm^{-3}), Ca: 1.99 $cmol_c$ dm^{-3}), Mg: 0.53 $cmol_c$ dm^{-3} and Al: 0.0 $cmol_c$ dm^{-3}, with base saturation 56.1%. Germination date was January 20, 2009.

The trial incorporated a completely randomized design with four replications and treatments arranged in a 3 x 4 factorial, with three fertilizer sources and four doses. All plots with eight pine seedlings were distributed in a 10 x 1 m area of a nursery. Seedlings were protected against direct solar incidence with a 50% net shade in the initial phase. Manual irrigation was performed every day during entire growth cycle, maintaining at least 80% substrate water retention capacity.

The fertilizer sources used were: natural phosphate (Algeria natural phosphate - NF), soluble phosphate (single superphosphate - SPS) and a controlled release fertilizer (Basacote® - BAS). The fertilizer doses applied were based on the commercial recommendation of Basacote for *Pinus* (6.0 kg m^{-3}) equivalent to 0.36 kg P_2O_5 m^{-3}; being the half dose (0.18 kg P_2O_5 m^{-3}), full (0.36 kg P_2O_5 m^{-3}) and twice (0.72 kg P_2O_5 m^{-3}), and one control without P fertilizer. Basacote is a controlled release fertilizer, recommended for forest species under nursery conditions, composed of 13% N, 6% P_2O_5 and 16% K_2O. Natural phosphate (NF, 9% P_2O_5) and soluble phosphate (SPS, 17% P_2O_5) were applied at the same doses as for the Basacote treatments. Nitrogen and potassium (K) were balanced across treatments by using urea (46% N) and potassium chloride (60% K_2O).

Stem diameter and plant height were measured monthly up to 330 days. Whole-plant dry mass (DM) production and nutrient accumulation in the shoot and root system were determined at 330 days after germination (DAG). Shoot and root DM were determined by weighing the tissue after oven drying at 65 ºC for 72 h. Dried tissue was milled and sieved through 2 mm (Willey) prior to determination of nutrient content. Analysis of N, P and K were determined by destilation-titration, spectrometry and photometry, respectively, after acid digestion (Tedesco et al., 1995). Total nutrient content was estimated as the product of biomass and the nutrient concentrations in each plant. Chemical analysis was performed in substrate samples (330 DAG) according to the procedure of Tedesco et al. (1995).

Availability of P in soil was determined by anion exchange resin, since natural phosphate was applied in some treatments, and the conventional Mehlich-1 soil P test would overestimate available P by dissolving calcium phosphate in the fertilizer. Data were analysed by ANOVA and significant source effects were compared by t-test (LSD). Fertilizer dose effects were analysed by regression analysis (SAS Institute, 2001) and SigmaPlot 8.0 regression at 5% level of probability.

Results and Discussion

The main morphological parameters that define seedling quality are plant height, stem diameter, plant height/stem diameter ratio and dry mass production (Tucci et al., 2007). The use of controlled release fertilizer BAS enhanced plant growth by increasing plant height (Figure 1A and 1B). Seedlings were taller at all BAS doses, being statistically similar to soluble phosphate (SPS), but significantly higher than the control and natural phosphate source (Figure 1A). BAS and SPS resulted in taller plants from 60 DAG, regardless of the dose. Taller plants were obtained by using the highest P rate (BAS 0.72 kg P_2O_5 m^{-3}), followed by BAS at 0.36 kg P_2O_5 m^{-3} and SPS at 0.36 kg P_2O_5 m^{-3}. Natural phosphate (NF) was not effective in supplying P for these seedling plants and developed deficiency symptoms in the early growth stages. Plant growth was consequently lower using NF than using BAS or SPS. Natural phosphate is sufficiently reactive for use as a direct P fertilizer but is effective only under specific climatic and soil situations. However, numerous studies have been conducted to test ways to enhance dissolution and increase the P availability of this P source such as composting with organic manure, green manuring, partial acidification and chemical or bio-fertilizer solubilization (Kumari & Phogat, 2008).

The source BAS was most effective because it released nutrients gradually according to plant requirements. An organic membrane surrounding the BAS granules is protected by a resin that dissolves when substrate moisture or temperature changes allowing the fertilizer nutrients to be gradually released (Sgarbi et al., 1999). Thus, as humidity or temperature increases, the release of nutrients also increases.

Serrano et al. (2006), studying yellow passion fruit (*Passiflora edulis* F. flavicarpa) seedlings, concluded that controlled release fertilizer resulted in taller plants, greater stem diameter, more leaves, greater leaf area and shoot dry mass when compared to periodic foliar fertilization with NPK and soluble phosphate + KCl + urea. Also, in yellow passion fruit, Fey et al. (2010) observed a linear response in plant height and stem diameter to soluble phosphate, but the highest dose used by the authors was only 2.78 kg m^{-3} of SPS. Here, the

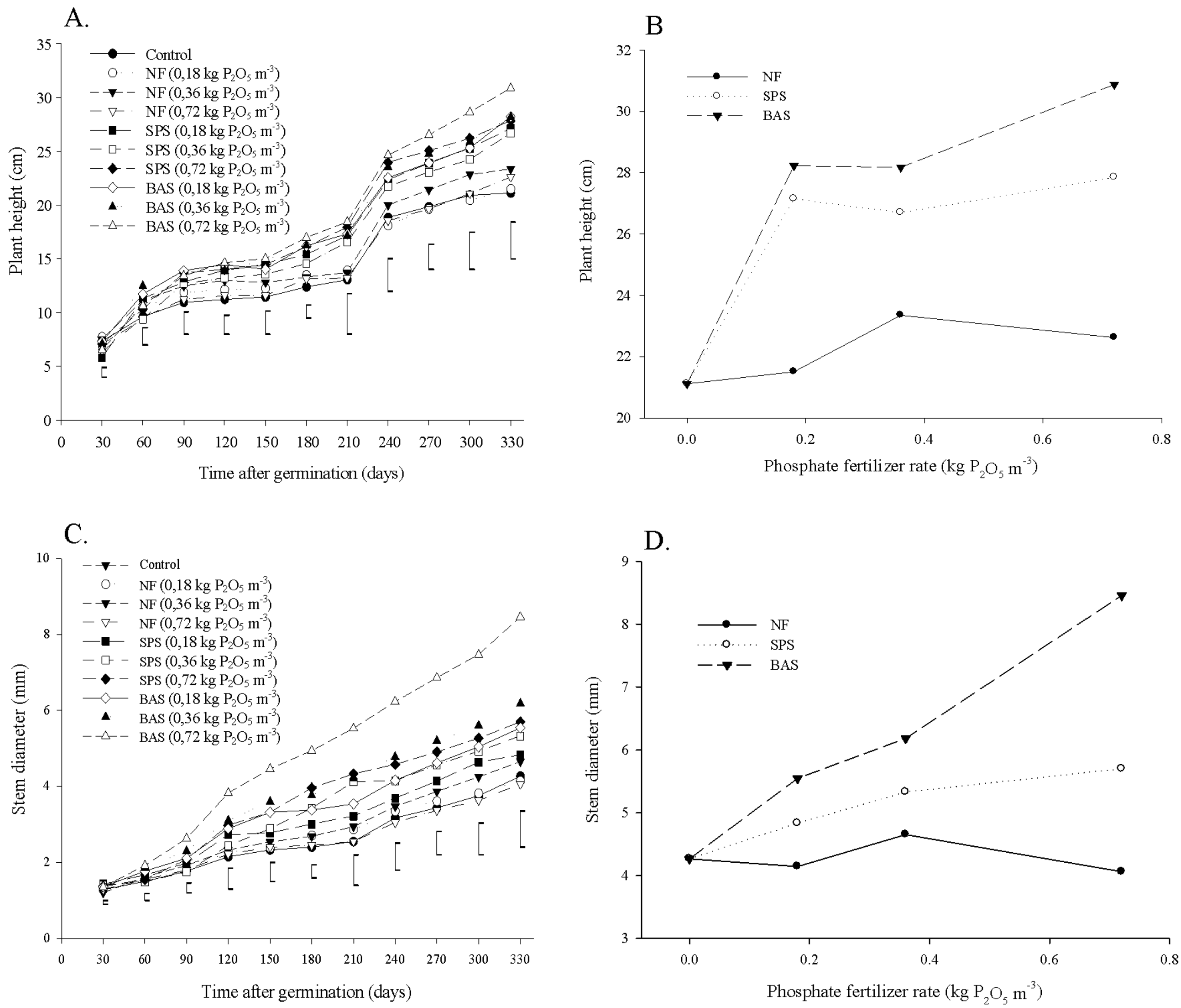

Figure 1. Plant height and stem diameter of *Pinus elliottii*, from germination until 330 days after germination (A and C), and at the end of the experiment (B and D) under doses and sources of phosphate fertilizers. NF: natural phosphate; SPS: simple superphosphate; BAS: Basacote (Vertical bars: LSD by t test at 5%)

highest SPS dose was 4.22 kg m^{-3}. In this study, pine seedlings fertilized with BAS grew faster implying ealier transplanting time to the field than other fertilizer sources. Moraes Neto et al. (2003), working with five native tree species from Brazil, also observed increased growth under controlled release fertilizer (NPK 19-06-10; 4.8 kg m^{-3}) relative to other fertilizer sources.

Stem diameter was also superior under BAS and SPS sources, increasing according to the P dose (Figure 1C and 1D). Applying BAS at 0.72 kg P_2O_5 m^{-3}, increased stem diameter after 90 DAG compared to other sources, with vigorous and stronger seedlings. The effect of BAS in P availability on *Peltophorum dubium* seedlings was tested by Venturin et al. (1999) using the missing element technique. They observed a reduction in stem diameter and plant height without P fertilization. Moraes Neto et al. (2003) reported that the greatest stem diameter of five native trees occurred when 4.8 kg m^{-3} of controlled release fertilizer,or with 1.6 kg m^{-3} of controlled release fertilizer + 1,5 kg m^{-3} of powder fertilizer + 2 g L^{-1} of NPK 19-06-20 was aerially applied weekly.

Shoot and root dry mass were significantly affected by fertilizer source and solubility (Figure 2). NF was not able to supply enough P for the plants, resulting in low dry mass accumulation at 330 DAG, similar to the control. SPS promoted a linear increase in root system dry mass at 330 DAG (Figure 2B) which was not observed in the shoot system under higher doses (SPS 0.36 and 0.72 kg P_2O_5 m^{-3}) (Figure 2A), possibly due to the plant nutritional imbalance (Table 1). These results are in agreement with Vogel et al. (2005), who reported a quadratic response from *Pinus taeda* to soluble P in the first two years of development under field conditions, however, in contrast to the present work, available P was considered critical for plant development at the start of that experiment. Sardans et al. (2004), working with allepo pine in calcareous and silicate soils, showed that seedlings total biomass was increased when soluble P fertilizers were used. Furthermore, Ceconi et al. (2006) reported a response in dry mass production of *Luehea divaricata* seedlings to increasing doses of P applied up to 360 mg P kg^{-1}, a higher rate than the highest rate used in this study (0.72 kg P_2O_5 m^{-3}).

Table 1. Nutrients in tissues of pine seedlings (shoot plus root) at 330 DAG, under doses and sources of phosphate fertilizers

Source**	Nutrient in plant tissue N	P	K
	mg pl^{-1}		
Shoots			
Control	18.51	2.20	20.76
NF 0.18 kg m^{-3}	15.23	3.90	23.59
NF 0.36 kg m^{-3}	15.19	3.06	26.51
NF 0.72 kg m^{-3}	16.08	2.76	23.25
SPS 0.18 kg m^{-3}	14.63	2.79	23.28
SPS 0.36 kg m^{-3}	21.47	5.39	28.00
SPS 0.72 kg m^{-3}	11.53	11.23	39.13
BAS 0.18 kg m^{-3}	19.95	6.69	60.40
BAS 0.36 kg m^{-3}	59.52	9.72	89.34
BAS 0.72 kg m^{-3}	56.05	15.67	87.08
Mean	24.82	6.34	42.13
LSD (t test)	29.73	2.61	20.16
Source	*	*	*
Dose	ns	*	*
Source X Dose	ns	*	*
Roots			
Control	19.75	2.32	16.50
NF 0.18 kg m^{-3}	18.88	5.92	20.73
NF 0.36 kg m^{-3}	24.27	5.88	25.26
NF 0.72 kg m^{-3}	24.96	3.16	29.57
SPS 0.18 kg m^{-3}	24.60	3.77	28.86
SPS 0.36 kg m^{-3}	28.48	6.93	29.25
SPS 0.72 kg m^{-3}	49.05	13.31	34.37
BAS 0.18 kg m^{-3}	41.95	4.08	45.67
BAS 0.36 kg m^{-3}	68.48	8.76	56.96
BAS 0.72 kg m^{-3}	82.92	23.22	76.35
Mean	38.33	7.74	36.35
LSD (t test)	24.22	3.53	17.27
Source	*	*	*
Dose	*	*	*
Source X Dose	*	*	*

* Significative at 5% by ANOVA test. ns: not significant
** NF: natural phosphate; SPS: simple superphosphate; BAS: basacote

Controlled release fertilizer was the most efficient source of nutrients to promote dry mass production. Root dry mass increased linearly (Figure 2B) and shoot dry mass exponentially (Figure 2A). Wilsen Neto & Botrel (2009) also used a controlled release fertilizer (Osmocote) and reported in *Pinus taeda* that 2.54 kg m^{-3} would be the ideal fertilizer dose for seedling shoot dry mass accumulation. Sardans et al.

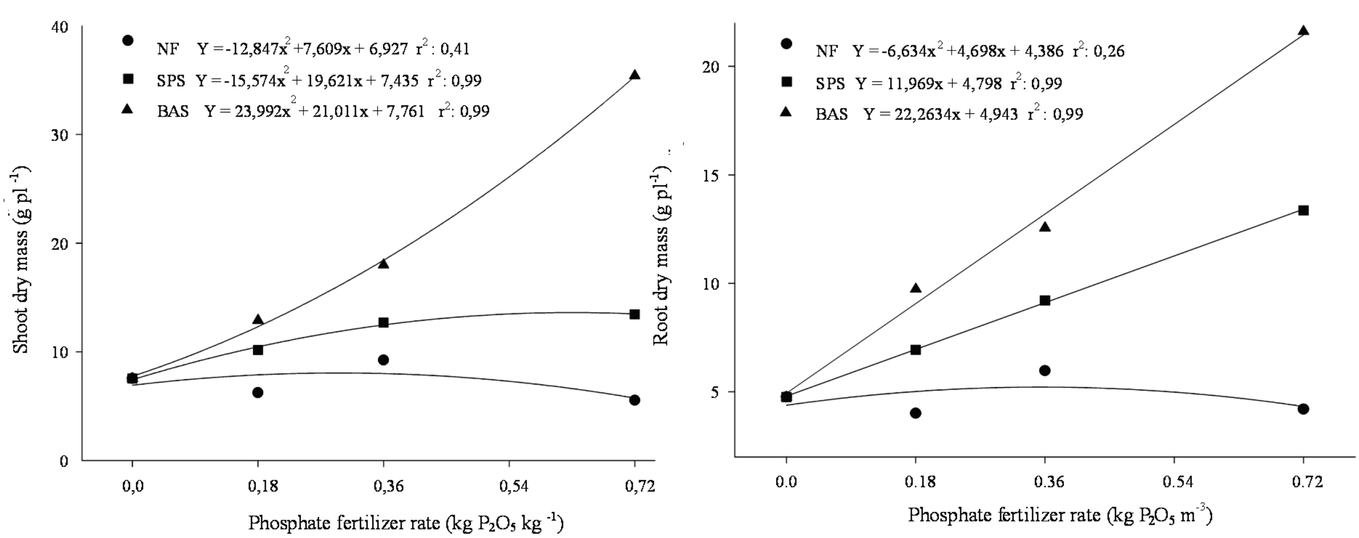

Figure 2. Shoot (A) and root (B) dry mass of *Pinus elliottii* seedlings at 330 days after germination, under doses and sources of phosphate fertilizers. NF; natural phosphate; SPS: simple superphosphate; BAS: basacote

(2004) reported that potted pine seedlings presented an easy plasticity for P tissue allocation, depending on soil availability, increasing the allocation of biomass to roots as P availability increases, especially on silicate soil. The addition of P supply in pine increases significantly the allocation to fine roots, particularly the absorbent root structures, which can also be observed in the present experiment when SPS was used.

Accumulation of nutrients in plant tissue was strongly influenced by fertilizer source and dose (Table 1). Nitrogen accumulation increased with BAS compared to other fertilizers. Shoot N accumulation was significantly affected only by fertilizer source, whereas root system N accumulation increased according to sources and doses. Phosphorus accumulation in plant tissue, both in shoot and root, increased with increasing SPS and BAS doses, whereas NF did not produce any positive effect. Higashi et al. (2003), in *Pinus caribaea* seedlings at 150 DAG, presented reference values of N and P content of 17.49 and 4.97 mg plant^{-1}, respectively.

Furthermore, studies in *Eucalyptus grandis* at 150 DAG, observed values of N from 5.49 to 27.32 mg plant^{-1}, and P from 0.87 to 4.54 mg plant^{-1}, according to the substrate used and irrigation plan as found by Lopes et al. (2007). Values of dry mass of shoot and N accumulation in this study were lower than those mentioned by Higashi et al. (2003), but the seedlings were older in this study, (330 DAG against 150 DAG) and a dilution effect could have occurred in older leaves regardless of the pine species. In this study, pine seedlings accumulated significantly more P using BAS as the nutrient source, irrespective of the dose applied, and at a SPS rate of 0.72 kg P_2O_5 m^{-3}. In both cases, the amounts of P accumulated were much greater than was observed by Lopes et al. (2007) in *Eucaliptus*, whose values were from 0.87 to 4.93 mg plant^{-1}.

Potassium (K) accumulation in shoot and root system was higher using BAS than using other sources, with a significant increase related to dose rate (Table 1). Tucci et al. (2007), working with *Swietenia macrophylla*, found highest accumulation of N,

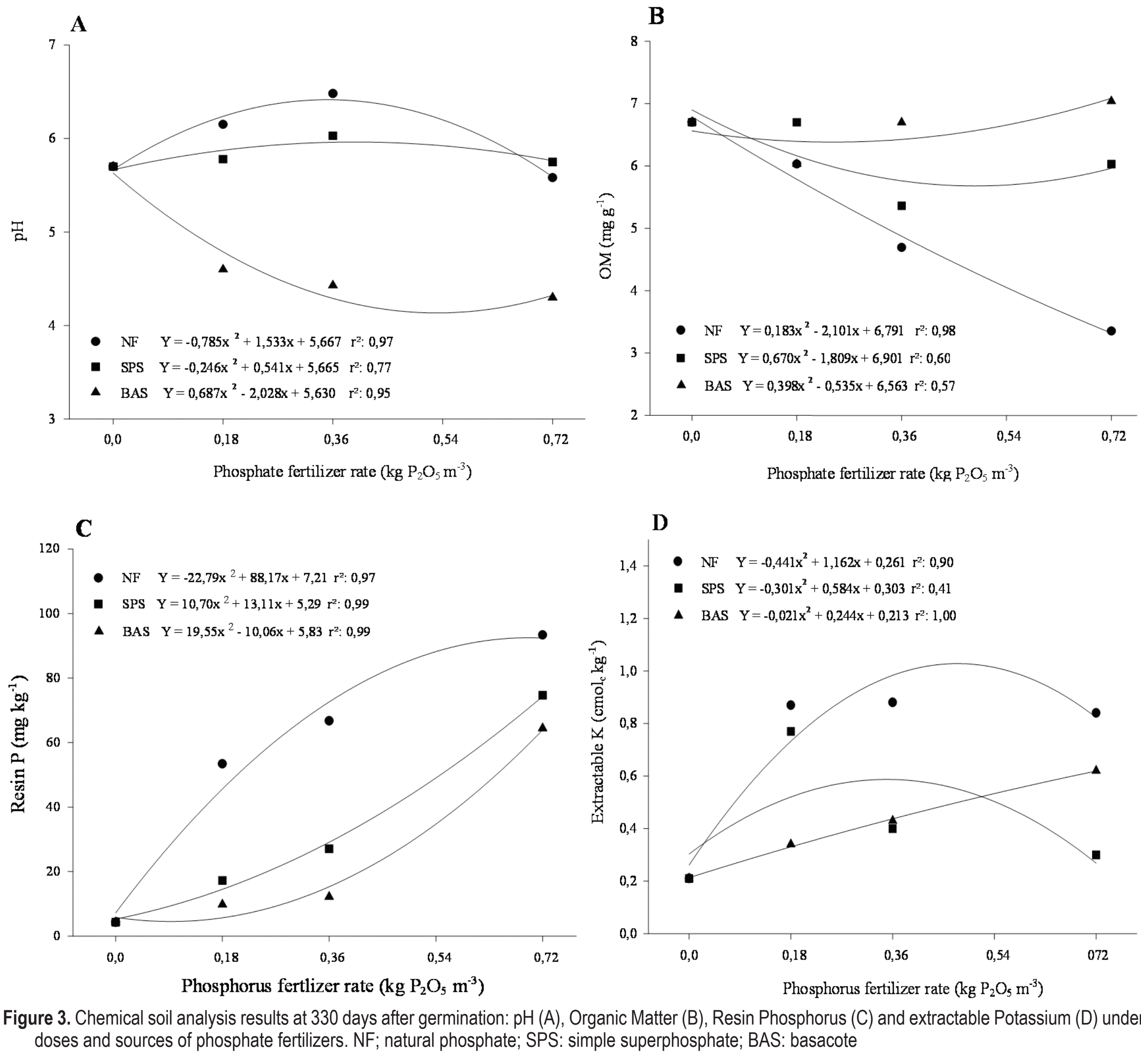

Figure 3. Chemical soil analysis results at 330 days after germination: pH (A), Organic Matter (B), Resin Phosphorus (C) and extractable Potassium (D) under doses and sources of phosphate fertilizers. NF; natural phosphate; SPS: simple superphosphate; BAS: basacote

P and K in plants at 90 DAG when phosphate was applied at nursery stage. Thus, the adequate supply of a particular nutrient (in this case, P) can promote better absorption of other nutrients by plants. Schiavo et al. (2009) observed K accumulation from 56.0 to 68.86 mg K plant^{-1} at 150 DAG in shoots of *Eucalyptus*. Similar values were observed here for slash pine at 330 DAG but only with BAS.

In relation with soil chemical characteristics, soil pH was significantly altered by fertilizer sources (Figure 3A). A slight increase was observed with lower doses of NF and SPS, whereas a considerable decrease was observed as BAS doses increased. Organic matter (OM) in soil/substrate showed a tendency to decrease with increasing SPS and NF doses, however, it was unaffected by BAS doses (Figure 3B). Absolute OM values were very low.

Substrate P availability was highly influenced by fertilizer sources and doses (Figure 3C), explaining the accumulation of this nutrient in plant tissue (Table 1). Natural Phosphate increased P availability (extractable resin) to 90 mg kg^{-1}, however, it was not correlated to plant growth, as mentioned previously, thus promoting an accumulation of available P forms in the substrate. SPS also promoted an increase in substrate available P in proportion to the applied doses. BAS was the most efficient source to provide nutrients, especially P, for pine seedlings, thus available P in soil was almost all used by the plant under lower doses (0.18 kg m^{-3} and 0.36 kg m^{-3}), and only started to accumulate at the 0.72 kg m^{-3} rate. Lower available soil P levels, such as 15 mg P kg^{-1} of soil or less (van Raij et al., 1997) are considered critical for plant development, but this was not the case in this study for NF and SPS sources, because these sources were not able to promote plant development.

The amount of extractable K in the substrate was also altered by fertilizer sources (Figure 3D). The source NF increased the amount of extractable K probably as a consequence of the low plant development and lack of utilization of this source. The source SPS also increased the amount of extractable K in the substrate when the lowest dose was applied, however a reduction in this nutrient was observed under higher doses. The BAS source presented a linear increment in extractable K according to the applied doses, an expected behavior since this fertilizer contains a high concentration of this nutrient. Most of the K accumulation in plant tissue discussed previously (Table 1) is directly related to the extractable K in the substrate, as expected.

Other soil chemical characteristics, like Al saturation, were also significantly affected by Basacote. Extractable Al was not detected in control, NF and SPS sources, but BAS promoted a significant pH decrease (Figure 3A), responsible for 20.1 and 34.1% of Al saturation under BAS (0.36 kg m^{-3} and 0.72 kg m^{-3}) treatments, respectively (data not presented, as this was only observed for the BAS doses). These changes did not affect plant growth at nursery stage, until 330 DAG, as can be observed in Figures 1-2.

Conclusions

Results from this short-term study clearly demonstrated that fertilization with controlled release fertilizer promotes better seedling growth, with larger stem diameter and higher dry mass accumulation at 330 days after germination.

Natural and soluble phosphates are not as effective to promote pine seedling development as controlled release fertilizer, but the soluble phosphate is much better than natural phosphate.

A further study is required to examine the effect of controlled release fertilizer on pines development in the field after transplantation.

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