



# RESPONSE OF *CRATYLIA ARGENTEA* (DESVAUX) O. KUNTZE TO COATING SEEDS WITH TRIPLE SUPERPHOSPHATE

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**Abstract:** *Cratylia argentea* is a neotropical leguminous shrub with high phenotypic plasticity in response to abiotic factors; it is present in Brazil in the Amazon, Cerrado, and Caatinga biomes. It has potential for use as animal feed and green manure and stands out for its ability to grow in low fertility and tropical acidic soils and can be sown directly in the soil. However, in spite of these advantageous features, the species has slow initial development. In this context, the aim of this study was to evaluate the response of the species to coating seeds with triple superphosphate and the contribution of this technique to the initial stage of development. First, the physiological quality of seeds was evaluated in response to coating seeds with different amounts of triple superphosphate. In a second step, we carried out evaluation of initial development of the species in a *Latossolo Vermelho Amarelo* (Oxisol) under high and low phosphorus fertility conditions after coating seeds with triple superphosphate. This technique did not confer benefits to initial development of *C. argentea* and led to decreased seed germination and vigor.

**Keywords:** phosphate fertilizer, forage plant, green manure, initial development, tropical soils.

## 1. Introduction

*Cratylia* is a neotropical leguminous genus found mainly in Brazil, Peru, Bolivia, and the northeast of Argentina (Lascano et al., 2002). *Cratylia argentea* is the most widespread species (Queiroz, 1991; Lascano et al., 2002), which occurs in Brazil in different environments, such as the Cerrado (Brazilian tropical savanna), Amazon rainforest, and Caatinga (xeric shrubland) (Queiroz, 1991). *C. argentea* is a shrub that branches out at the base of the stem, reaching up to 3 meters height and has high capacity for producing new shoots, resulting from vigorous root growth (Lascano et al., 2002).

Although its use is not widespread in Brazil, the species shows potential for use in crop and livestock operations in the moist tropics, especially as sources of protein for animal feed (Argel et al., 2000; Sarria and Martens, 2013) and green manure. The nutritional value of *C. argentea* stands out among the leguminous shrubs adapted to acid soils (Cook et al., 2005). It is infected by nitrogen-fixing bacteria (Calazans et al., 2016; Mattar et al., 2018).

Knowing the nutritional demands of *C. argentea* is important for taking better advantage of the forage potential of this species (Xavier et al., 1997). Argel and Lascano (1999) and Maas (1995) highlighted the importance of studies in regard to the response of *C. argentea* to soil fertility, and Xavier et al. (1997) showed that growth of *C. argentea* responded to applications of phosphorus and limestone, and liming contributed to an increase in the efficiency of the P applied.

Though *C. argentea* exhibits greater vigor in relation to other species, it has slow initial growth (Argel and Lascano, 1999), and there are few studies regarding the growth of this species in response to fertilization, especially fertilization through coating of seeds, which has proven to be beneficial for species of economic value (Trigo et al., 1997; Peske et al., 2009; Vasconcellos et al., 2000 and Soares et al., 2014).

Since vegetative propagation of this species is not recommended (Pizarro et al., 1995), the technique of coating seeds with fertilizers may exhibit potential. The seeds are circular and flat and considered to be large in relation to other shrubs (Ramos et al., 2003), and they do not require scarification before sowing (Lascano et al., 2002; Sanabria et al., 2004). In addition, direct sowing in the soil may be carried out and has even been adopted on the property Las Cañas, located in the Vale do Cauca, Colombia. This planting method has also shown promising results in the Amazon (Aquino et al., 2020).



In this context and considering the low quantity of P available in tropical soils, the aim of this study was to evaluate the response of *C. argentea* to coating seeds with triple superphosphate (TSP), a phosphate fertilizer that contains 41% P<sub>2</sub>O<sub>5</sub> and 10% Ca (Brasil, 2007).

## 2. Materials and Methods

### 2.1. Seed coating

*C. argentea* seeds were coated with triple superphosphate (TSP) at the rates of 0, 1.25, 2.5, 5, 7.5, and 10 g of TSP per 100 seeds (approximately 25.98 g). These application rates were established in preliminary tests, considering an estimate of dry matter in 45 days after germination and the critical internal level of phosphorus for the species, based on the studies of Xavier et al. (1996 and 1997).

The fertilizer was ground in a mortar and passed through a 60 mesh (0.250 mm) sieve. For coating the seeds with TSP, FTA glue (Voss and Benvegnú, 2008) was used at the proportion of 1 mL for 100 seeds (approximately 25.98 g). The mixture was placed in plastic containers and manually shaken for fertilizer to adhere to the seed. To determine the amount of material that adhered to the seeds, the containers and the seeds were weighed before and after the coating.

### 2.2. Physiological quality of the seeds coated with TSP

For evaluation of physiological quality, the seeds coated with 1.25, 2.5, 5, and 7.5 g of TSP per 100 seeds, and the control (seeds without coating with TSP) were used. For this step, germination, accelerated aging, and emergence in sand tests were used. The germination and accelerated aging tests were conducted with five replications of 20 seeds, and, in the emergence in sand test, 4 replications with 25 seeds, for a total of 100 seeds per treatment, the number of seeds recommended for studies with seeds of native species. A completely randomized experimental design was adopted for all the tests.

The germination test was conducted in the substrate of paper toweling in the form of a roll (Brasil, 2009). The rolls were placed in a germination chamber (Mangelsdorf type) at 25°C. The variables evaluated were germination percentage, germination speed index (GSI), and speed of germination (SG). Evaluations were performed daily up to stabilization of germination, and the percentages of normal seedlings, abnormal seedlings, dead seedlings, and hard seeds were determined. The fungicide Captan (0.2%) was sprinkled on the seeds weekly, and on the 15th day after setting up the test, the paper toweling was exchanged. The germination speed index and the speed of germination of the seedlings were calculated according to the formulas proposed by Maguire (1962) and Edmond and Drapala (1958), respectively.

For the accelerated aging test, the seeds were placed on suspended metal screens that were combined with plastic boxes (gerbox) containing 40 mL of water at the bottom. The boxes were covered with a lid and kept in a BOD type incubator regulated at 45°C for 24 h, according to the methodology described by Marcos Filho (2016). After this period, the seeds were subjected to the germination test according to the methodology already described, and the same variables were evaluated.

In the emergence test in sand, the seeds were individually distributed in the cells of polyethylene trays containing washed and autoclaved sand. Normal and abnormal seedlings were counted daily up to the 49th day after sowing, at which time there was stabilization in seedling emergence. The variables evaluated were emergence speed index (ESI), speed of emergence (SE), root growth, and dry matter.

The emergence speed index and the speed of emergence of the seedlings were calculated according to the formulas proposed by Maguire (1962) and Edmond and Drapala (1958), respectively. The length, area of projection, surface area, and volume of the roots of the seedlings were calculated using the roots of the seedlings collected at the end of the emergence test in sand through the WinRHIZO image analysis system. This system measured the total length of the root (cm of root / seedling), the area of root projection (cm<sup>2</sup> of root / seedling), the total area of the root surface (cm<sup>2</sup> of root / seedling), and the root volume (cm<sup>3</sup> of root / seedling), which were expressed based on images of the roots generated by a scanner. The height of the shoots and the length of the primary root were checked for each seedling with the use of a caliper rule. The results were expressed in cm / seedling. At the end, plant matter (shoots and roots) obtained in the emergence test in sand was placed in paper bags and dried in a thermoelectric laboratory oven, with forced air circulation, at a temperature of 65°C for 72 h. After cooling in a desiccator, 25 seedlings per replication were weighed.

### 2.3. Greenhouse test

The growth and the physiological characteristics of *C. argentea* were evaluated in a greenhouse test. Seeds were sown in plastic pots containing 5 dm<sup>3</sup> of soil classified as a *Latossolo Vermelho Amarelo* (Oxisol). The soil was amended and fertilized to raise the nutrient levels of N (100 mg/dm<sup>3</sup>), P (300 mg/dm<sup>3</sup>), K (150 mg/dm<sup>3</sup>), S (40 mg/dm<sup>3</sup>), B (0.81 mg/dm<sup>3</sup>), Cu (1.33 mg/dm<sup>3</sup>), Fe (1.55 mg/dm<sup>3</sup>), Mn (3.66 mg/dm<sup>3</sup>), Mo (0.15 mg/dm<sup>3</sup>), and Zn (4.0 mg/dm<sup>3</sup>), following the recommendation of Novais et al. (1991).

The treatments were in a 2 × 2 factorial arrangement, consisting of two soil P conditions (with and without P fertilization) and two conditions of coating seeds with TSP (with and without coating, corresponding to 1.25 g of TSP per 100 seeds). The trial was conducted in a completely randomized experimental design, with 10 replications.

At 75 days after sowing, the height and number of nodes and leaves per plant were measured. Height was verified by a metric tape as of the point of attachment of the cotyledonary leaves to the stem. After that, the length, area of projection, surface area, and volume of the plant roots were determined according to the method described above.

Plants were harvested, separating the shoots and roots. The material was placed in paper bags and dried in a thermoelectric laboratory oven with forced air circulation at a temperature of 65°C for 72 h. After that, it was weighed on a precision balance.

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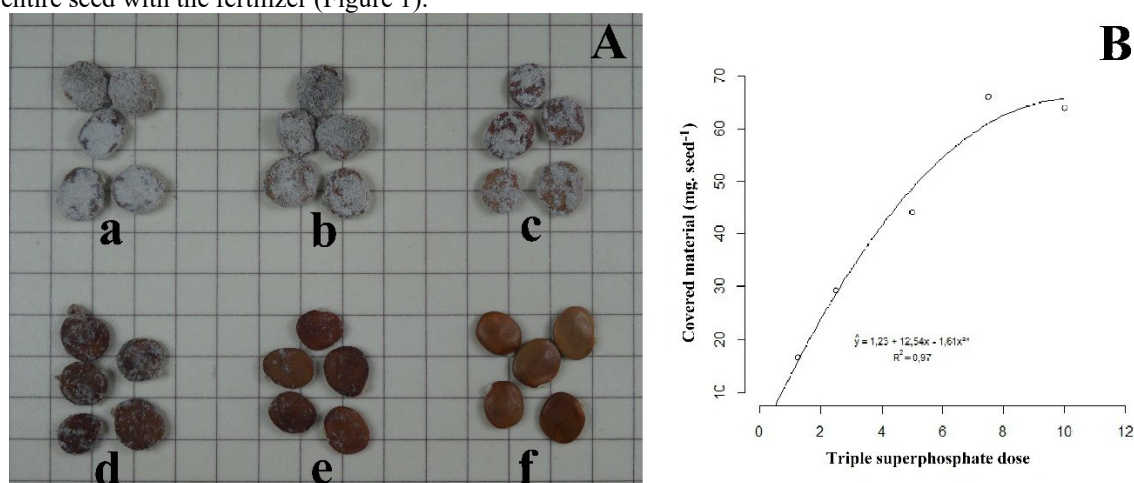
## 2.4. Statistical analysis

The data of all the variables evaluated were subjected to analysis of variance. The Tukey test was then applied at 5% probability for comparison of the mean values of the treatments. For the quantitative factors, regression analysis was carried out so as to evaluate the functional relationship between the independent variable and the response variable.

## 3. Results

### 3.1. Application rates and coating

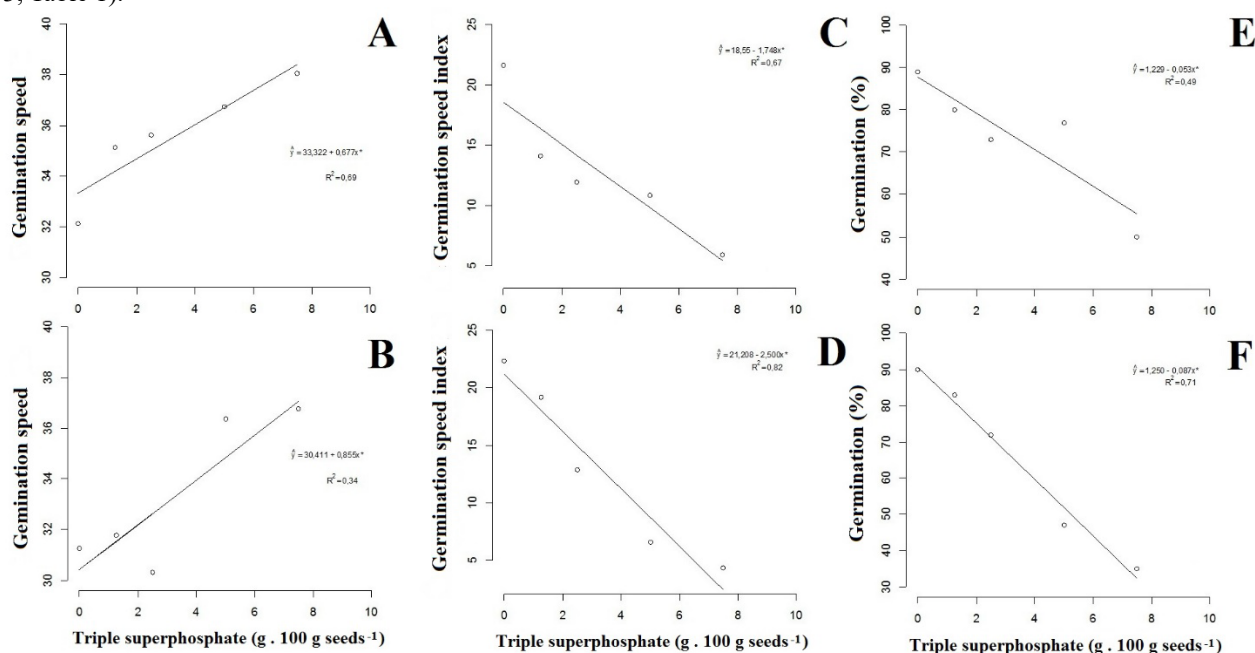
The straight line of the regression shows a tendency of greater accumulation of material that adheres in relation to an increase in the application rate used and suggests that as of the application rate of 7.5 g of triple superphosphate per 100 seeds, there is stabilization in adhesion of the material. It should be noted that the rate of 7.5 g of triple superphosphate per 100 seeds was sufficient to coat the entire seed with the fertilizer (Figure 1).



**Figure 1** – A. Seeds with different coating conditions. (a) Application rate of 10 g per 100 seeds, (b) Application rate of 7.5 g per 100 seeds, (c) Application rate of 5 g per 100 seeds, (d) Application rate of 2.5 g per 100 seeds, (e) Application rate of 1.25 g per 100 seeds, and (f) without coating. B. Ratio between material adhering to *C. argentea* seeds and application rates of triple superphosphate.

### 3.2. Seed germination and vigor as a function of coating with TSP

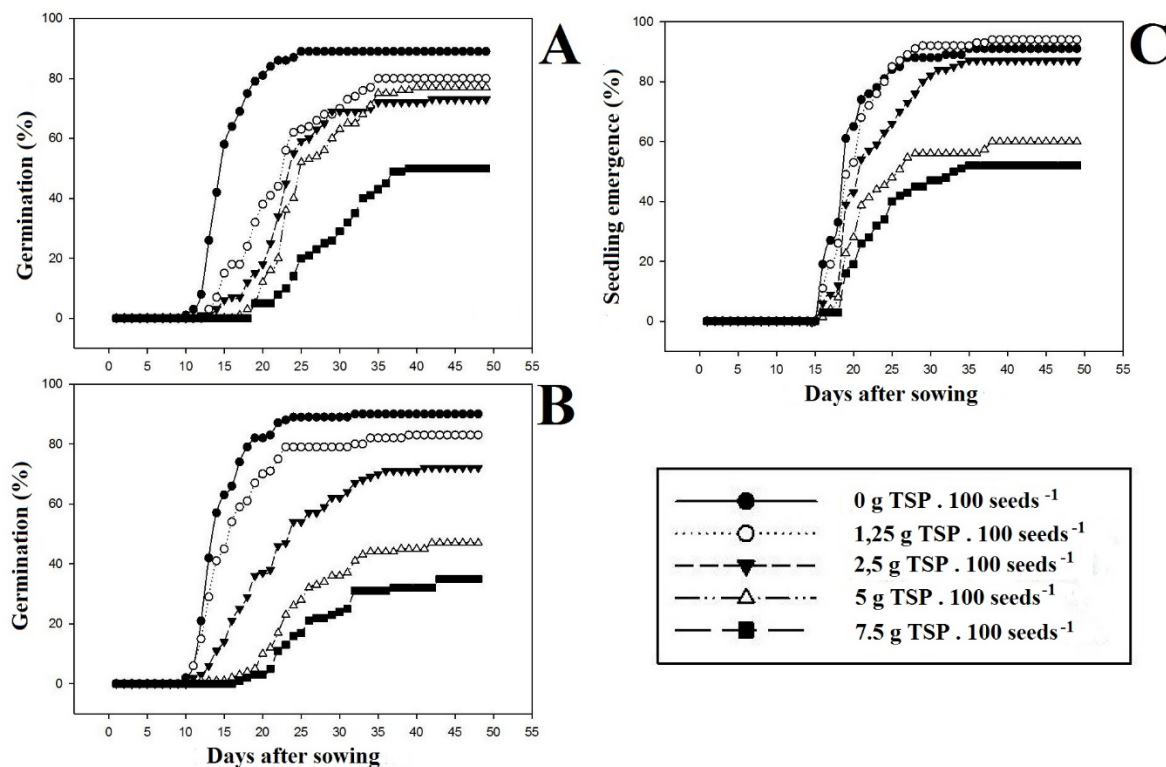
The percentages of germination in the germination and accelerated aging tests were influenced by the use of the coating technique, and both were more affected in accordance with an increase in the amount of material that adhered to the seeds (Figures 2 and 3, Table 1).





**Figure 2** – Speed of germination in (A) Germination test and (B) Accelerated aging test; germination speed index in the (C) Germination test and (D) Accelerated aging test; and germination in the (E) Germination test and (F) Accelerated aging test, all in reference to *C. argentea* seeds in relation to application rates of triple superphosphate.

In the two tests, with an increase in the application rate of triple superphosphate, the germination speed index (GSI) and the speed of germination (SG) underwent reduction and increase, respectively (Figures 2 and 3; Table 1).



**Figure 3** – Curves of formation of normal seedlings in response to the different application rates of triple superphosphate used in coating of *C. argentea* seeds in the germination (A) and accelerated aging (B) tests. Curve of emergence of normal seedlings in sand in response to the different application rates of triple superphosphate used in coating *C. argentea* seeds (C).

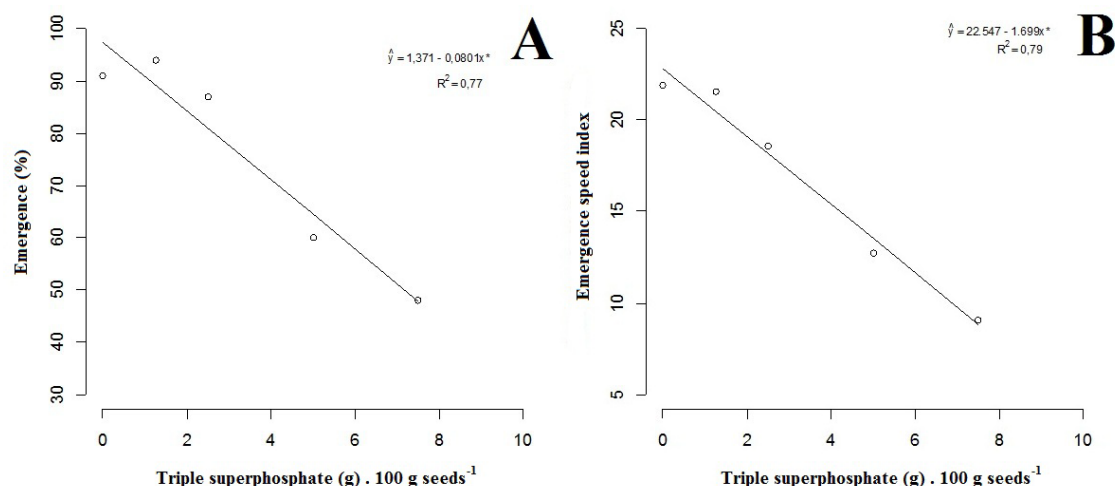
**Table 1** – Mean squares obtained in analysis of variance, experimental coefficients of variation (CV %), and overall mean for the 3 variables evaluated in the germination and accelerated aging tests. Germination percentage (G), germination speed index (GSI), and speed of germination (SG).

SV	GL	Mean Square					
		Germination			Accelerate daging		
		G	GSI	SG	G	GSI	SG
RATE	4	0.155*	164.85*	24.39*	0.35*	302.46*	46.07*
RESIDUE	20	0.02	8.22	1.14	0.02	8.261	11.13
CV %		13.5	22.27	3	17.04	22.02	10.02
OVERALL MEAN		73.8	12.87	35.52	62.83	12.17	33.28

\*significant at 5% probability by the F test.

In the emergence test in sand, the emergence curves of normal seedlings as a function of time were similar between the treatment containing the lower application rate (1.25 g.100 seeds<sup>-1</sup>) and the treatment containing the seeds without coating (Figure 3). The emergence percentage and the emergence speed index (ESI) decreased with an increase in the application rates used in the coating technique (Figure 4).





**Figure 4** – Percentage of emergence (A) and emergence speed index (B) of *C. argentea* seedlings as a function of the application rates used.

The shoot dry matter, root dry matter, speed of emergence (SE), shoot height, root depth, total root length, area of root projection, root surface area, and total root volume did not show significant differences with an increase in the application rates used in coating (Table 2).

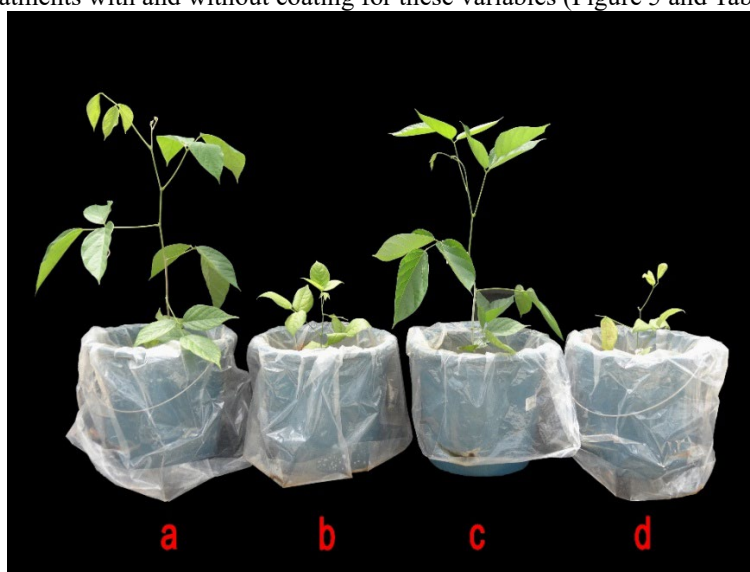
**Table 2** – Mean squares obtained in analysis of variance, experimental coefficients of variation (CV %) and overall mean for the 11 variables evaluated in the seedling emergence test in sand. Percentage of seedling emergence (PSE), primary root length (PRL), shoot length (SL), shoot dry matter (SDM), root dry matter (RDM), emergence speed index (ESI), speed of emergence (SE), total root length (TRL), area of root projection (ARP), root surface area (RSA), and root volume (RV). PRL (cm / seedling), SL (cm / seedling), SDM (mg / seedling), RDM (mg / seedling), TRL (cm of root / seedling), ARP (cm<sup>2</sup> of root / seedling), RSA (cm<sup>2</sup> of root / seedling) and RV (cm<sup>3</sup> of root / seedling).

SV	GL	Mean Square										
		PSE	PRL	SL	SDM	RDM	ESI	SE	TRL	ARP	RSA	RV
RATE	4	0.24*	0.05 <sup>ns</sup>	0.11 <sup>ns</sup>	1572.32 <sup>ns</sup>	80.71 <sup>ns</sup>	108.01*	2.51 <sup>ns</sup>	458.81 <sup>ns</sup>	4.22 <sup>ns</sup>	41.68 <sup>ns</sup>	0.001 <sup>ns</sup>
RESIDUE	15	0.016	0.45	0.5	672.39	84.97	5.8	1.21	1805.4	4.31	42.56	0
CV%		10.89	8.14	13.85	23.08	22.21	14.13	3.14	28.96	28.82	28.82	29.08
OVERALL MEAN		79.2	8.25	5.14	112.31	41.49	17.04	35.12	146.7	7.2	22.63	0.03

\*significant at 5% probability by the F test; <sup>ns</sup>not significant at 5% probability by the F test.

### 3.3. Plant development with and without seed coating with TSP

Under the condition of soil without phosphate fertilization, the variables of number of nodes, shoot height, shoot and root ratio, shoot dry matter, and root dry matter did not show differences between the treatments with and without seed coating. However, the number of leaves was greater in the treatment with seed coating. But under the soil condition with phosphate fertilization, there was no difference between the treatments with and without coating for these variables (Figure 5 and Table 3).



**Figure 5** – *C. argentea* plants at 75 days after sowing. (a) with coating and with phosphate fertilization – WCWP; (b) with coating and without phosphate fertilization – WCNP; (c) without coating and with phosphate fertilization - NCWP; (d) without coating and without phosphate fertilization -NCNP.



**Table 3** – Mean of the variables: shoot and root ratio (SRR), shoot dry matter (SDM), root dry matter (RDM), total root length (TRL), area of root projection (ARP), total root surface area (RSA), total root volume (RV), number of nodes (NN), number of leaves (NL), and height (H) after 75 days after sowing, in 4 treatments: with seed coating by triple superphosphate and with phosphate fertilization (WCWP), without seed coating by triple superphosphate and with phosphate fertilization (NCWP), with seed coating by triple superphosphate and without phosphate fertilization (WCNP), and without seed coating by triple superphosphate and without phosphate fertilization (NCNP). SDM (g), RDM (g), TRL (cm), ARP (cm<sup>2</sup>), RSA (cm<sup>2</sup>), RV (cm<sup>3</sup>), and height (cm).

Treatment	SRR	SDM	RDM	TRL	ARP	RSA	RV	NN	NL	H
WC WP	6.24 a	4.90 a	0.89 a	1429.6 a	75.00 a	235.62 a	0.306 a	7.7 a	7.7 a	37.58 a
NC WP	6.74 a	4.59 a	0.74 a	1105.1 ab	59.91 ab	188.21 ab	0.256 ab	7.7 a	7.7 a	33.82 a
WC SP	3.58 b	1.07 b	0.32 b	985.1 ab	47.70 ab	149.87ab	0.178 ab	5.0 b	4.6 b	15.05 b
SR SP	2.80 b	0.72 b	0.30 b	692.5 b	30.92 b	97.14 b	0.106 b	3.9 b	3.2 c	11.67 b
CV (%)	35.81	30.75	46.62	26.89	36.88	36.88	49.23	18.4	19.1	29.8

Mean values followed by the same letter in the column do not differ statistically by the Tukey test ( $p < 0.05$ ).

In regard to root development, there were no significant differences between the treatments with and without coating of seeds with triple superphosphate under the two soil conditions analyzed. The variables of total root length, area of root projection, area of the root surface, and total root volume (Table 3) increased with the use of the coating technique, especially with the use of soil fertilized with P. Nevertheless, the treatments with and without coating did not exhibit significant differences under the two soil conditions.

#### 4. Discussion

In the germination and accelerated aging tests, both conducted in paper substrate, the seedling formation curves exhibit sharper losses in germination and vigor of coated seeds when compared to the results of the emergence test. In these first two tests, not even the treatment containing the lowest application rate (1.25 g.100 seeds<sup>-1</sup>) exhibited a tendency similar to the treatments with the uncoated seeds. This may be associated with a greater accumulation of salts in the paper substrate in relation to that of sand.

The seed germination rates of the control in the germination and accelerated aging tests did not exhibit significant differences and were similar to those found by Fava (2008), Bárcenas et al. (2009), and Ramos et al (2003). Such a condition indicates that the time of accelerated aging used was insufficient to promote deterioration in the seeds and/or that the seed lot proved to be quite vigorous.

The use of seed coating with triple superphosphate had a negative influence on seed germination and vigor in the species *C. argentea*. In the germination test, reduction in the GSI and increase in SG (Figure 4 and Table 1) as a function of the increase in the application rate of TSP show reduction in the vigor of seeds coated with phosphate fertilizer. This condition is also shown in the emergence test in sand through reduction in emergence percentage and in the ESI. For *Glycine max* (L.) Merr., the use of coating with monobasic sodium phosphate at the rate of 0.6 to 0.8 g per 100 g of seeds resulted in an increase in germination and vigor, with improvement in emergence percentage and ESI (Soares, 2009). In *Pennisetum glaucum* (L.) R. Br, the coating of seeds with simple superphosphate and sodium triphosphate at the rate of 0.5 mg P per seed reduced emergence by 50% and there was no emergence at the application rate of 5.0 mg P per seed (Rebafka et al., 1993). The use of triple superphosphate negatively affected the performance of *C. argentea* seeds.

#### 5. Conclusion

Results indicate that the technique of coating seed with triple superphosphate is not recommended for the species *C. argentea* since it did not result in significant benefits for plant development in the two soil conditions. The technique impaired seed germination and vigor.

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