Organomineral Fertilizer in Pineapple Cultivars in the Acclimatization Stage of Seedlings Produced "In vitro"

ABSTRACT

This study aimed at evaluating the effect of organomineral fertilizer doses on the growth and nutrition of seedlings of pineapple cultivars during the acclimatization stage. The trial was carried out in the University Federal Rural do Semi-Árido under greenhouse conditions, at Mossoró – Rio Grande do Norte, from July 2013 to April 2014. The trial was carried out in randomized blocks design in a 2x5 factorial scheme, with 2 pineapple cultivars (Imperial and Vitoria) and 5 doses of organomineral fertilizer (0; 2.5; 5; 7.5 and 10 g), amounting 10 treatments with 4 replications and 5 seedlings per plot. The qualitative data means were compared by Scott-Knott test at 5% of probability, and the quantitative data were submitted to regression analysis and the mathematical models were adjusted with a probability for the t-test of $p<0.05$ for the equation parameters. The cultivars reached appropriate growth for transferring to the field after 210 days. The doses of organomineral fertilizer that increased the biometrical characteristics were 10 g and 5 g for both 'Vitoria' and 'Imperial' cultivars, respectively

Keywords: Ananas comosus L., fruits, phosphorus, propagation.

1. INTRODUCTION

The 'Imperial' [1] and 'Vitoria' cultivars were introduced in the agriculture, to aiming at reducing significant losses with fusariosis [2]. These in vitro seedlings cultivars are produced by biofactories due to the micropropagated seedlings advantages [3]. However, the reduction of air humidity and the availability of nutrients during acclimatization stage tend to become a bottleneck for pineapple seedlings, increasing their susceptibility to drastic environmental variations, even the acidic photosynthetic metabolism of Crassulaceae favoring them [4].

Comment [H1]: Was contradictory.

In addition, the long periods required for acclimatization of pineapple seedlings influence the in vitro production. Studies aiming at maintaining the micropropagated seedlings performance in the field, and at reducing the period of acclimatization stage of the pineapple seedlings have been carried out. The phosphate fertilization enhances the formation and the growth of premature roots, improves the water use efficiency, and keeps the phosphate uptake by the seedlings whether in high levels in the soil, according to [5].

In the production stage of seedlings of other fruit species, some studies indicate that the application of phosphorus increases the development of cultivated plants, [6] (castor bean plants); [7] (mangaba plants); [8] (Graviola plants). Notwithstanding, studies of the action of phosphate on the acclimatization of pineapple seedlings are still incipient.

This work aimed at evaluating the influence of organomineral fertilizer on the growth and nutrition of pineapple cultivars during the acclimation stage.

2. MATERIAL AND METHODS

One trial was carried out in the Federal Rural University of the Semi-Arid (UFERSA) Campus Leste, in the municipality of Mossoró, state of Rio Grande do Norte, Brazil, under greenhouse conditions. Pineapple seedlings of 'Vitória' and 'Imperial' cultivars produced in vitro produced by the BioClone Biotechnology Laboratory and maintained in a regular medium without the addition of growth regulators and vitamins were planted in 200 ml pots.

The seedlings were transferred for the pre-acclimatization greenhouse of the UFERSA Seedling Production Sector on June 15, 2017, where they were kept in Styrofoam trays with 72,120 cm^3 cells and were filled with Plantmax® substrate previously to the day of installation of the trial (July 17, 2017), being watered twice a day by an intermittent nebulizing system.

The trial took place when the seedlings were 32 days old in the pre-acclimatization stage under greenhouse conditions. These seedlings had about 6 cm long, 13 leaves and the leaf rosette were 13 cm of diameter. The 2 L pots were filled with a substrate of proportion (v / v) 70% soil and 30% Ecofertil® industrial organic compound. The materials were sieved previously to the homogenization. One sample of both soil and the organic compound was taken for the chemical analysis (Table 1).

Table 1. Chemical attributes of the substrate used in the trial.

N	MO	P	Na	pH	CE	PST
.....g kg^{-1}.....		.....mg dm^{-3}.....		.....dS m^{-1}.....		.....%.....
0.32	36.03	204.51	1062.40	5.30	1.59	47.22

Comment [H2]: Legend?

Comment [H3]: It's so hight.

The substrate was enhanced with five doses of BioTurbo® Phosphorus 12 organomineral fertilizer (Table 2): 0.0, 2.5, 5.0, 7.5 and 10.0 g.

Table 2. Physical-chemical characteristics of BioTurbo® Phosphorus 12 fertilizer.

P	N	K	C.O	Moisture	pH	CTC
.....%					..dS m^{-1}..	**Mmolc** mmol$_c$ kg^{-1}
12	2	1	8	10	7.0	80

Comment [H4]: Legend?

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The trial was carried out in a randomized complete block design in a 2 x 5 factorial scheme, with two pineapple cultivars ('Imperial' and 'Vitória') and five doses of organomineral fertilizer (0, 2.5, 5.0, 7.5 and 10.0 kg m^{-3}), amounting 10 treatments with 4 replicates and 5 seedlings per plot.

Growth evaluations were performed on the day of transplantation and at 120, 150, 180, 210, 240 and 270 days after planting the seedlings. The variable evaluated were the number of leaves, plant height (cm) and rosette diameter (cm). And at the end of the trial, the leaf number, plant height (cm), leaf rosette diameter, root length (cm) and dry matter mass of root and aerial part (g) were evaluated.

At the end of the trial, the nutritional analysis of the aerial part was carried out. The aerial part was separated from the root, washed with deionized water, wiped with cotton and sent to the UFERSA nutrition laboratory for the chemical analysis of foliar macro- and micronutrients. The contents of N, P, K, Ca and Mg were analyzed.

The statistical analysis was performed with the System for Analysis of Variance – SISVAR software [9]. The averages of the qualitative treatments were compared by the Scott-Knott test, at the 5% probability level; while the quantitative data were submitted to the regression analysis, and the mathematic models adjusted with significance for the t-Test ($p < 0.05$) for the parameters of the equation.

3. RESULTS AND DISCUSSION

Berilli et al. [10] claims a minimum of 17 leaves from acclimated seedlings the appropriate amount for the transfer to the field. The cultivar 'Vitoria' reached the minimum number lately with the dose of 10 g at 180 days (Figure 1A). The cultivar 'Imperial' reached the minimum number of leaves earlier with 5 g of the organomineral fertilizer added to the substrate over 130 days (Figure 1B). Giracca and Nunes [11] state that phosphorus interferes in the processes of storage and transfer of energy, cell division, and cell growth, among others, consequently affecting the number of leaves.

Comment [H5]: Did this happen only because of phosphorus?

Coelho et al. [12] state that the absence of an effect for height, in the first evaluations and the highest growth verified in the seventh month after the transplant indicate a slow growth of the pineapple seedlings in the initial stage of development. In addition, according to Teixeira et al. [13], it is necessary a variable period from the sixth to the eighth months in a greenhouse, so that the plants reach about 20 to 30 cm in height, that is a suitable size for the transfer to the field.

The plant height is an extremely important variable to be taken for picking the seedlings. The cultivar 'Vitoria' reached such height with the 10 g dose of organomineral fertilizer **over** after 270 days (Figure 1C). The cultivar 'Imperial', however, reached a minimum of 20 cm high, with a dose of 5 g of organomineral fertilizer added at 160 days of the trial beginning, which plant height losses were observed with increasing doses of P (Figure 1D).

Comment [H6]: And the others elements? Like N and K?

Berilli et al. [10] states that 30 cm is the best value for the leaf rosette diameter of seedlings suitable for the transfer to the field. The cultivar 'Vitória' reached a diameter of the rosette about 28.9 cm with 10 g of organomineral fertilizer **over** after 270 days of acclimatization. However, the cultivar 'Imperial' expected value was reached early over 210 days with 2.5 g of organomineral fertilizer added to the substrate (Figure 1E and 1F).

This behavior in the 'Vitória' cultivar happened probably due to the short acclimatization time of the seedlings. Albert [14] argues that the short acclimatization time in the greenhouse is not enough for the necessary morphological and physiological changes responsible for a successful transition.

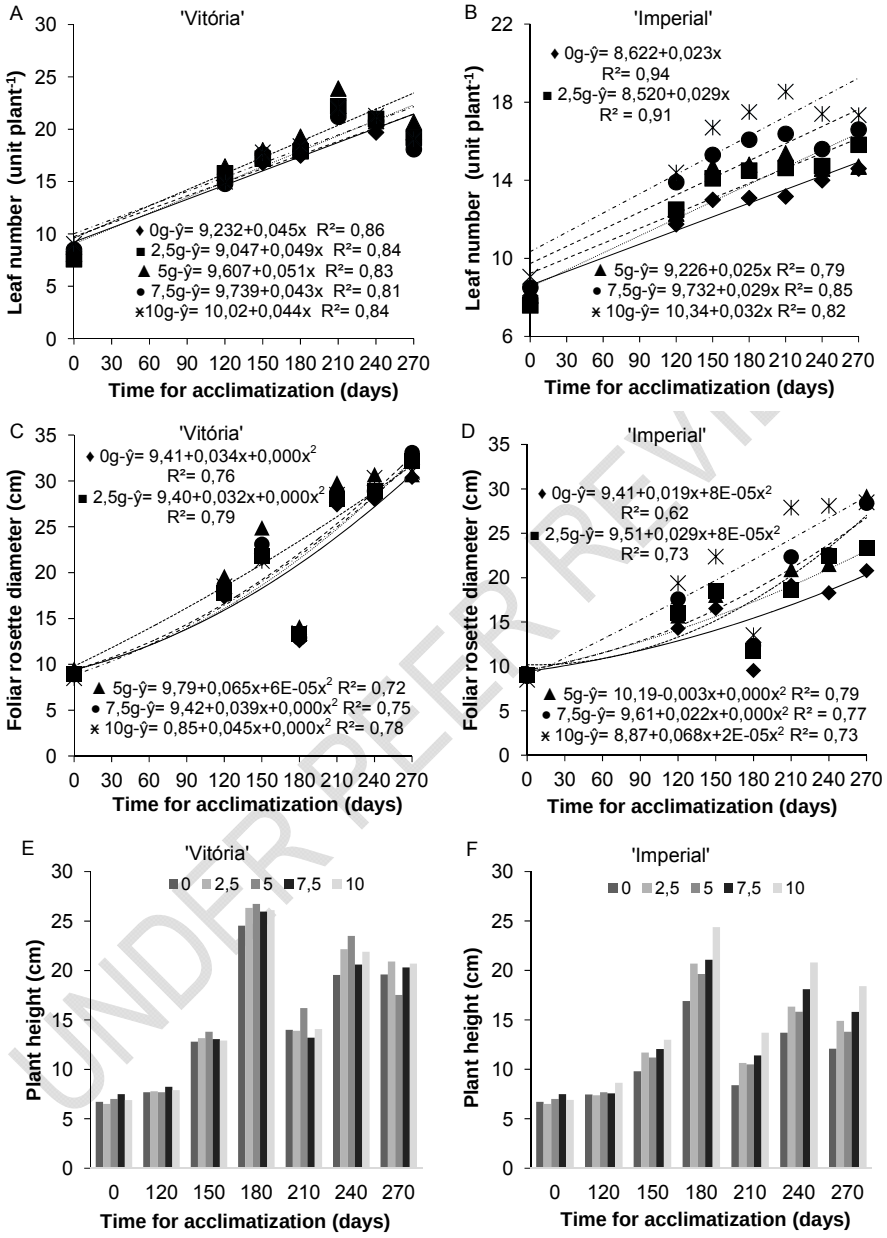

Figure 1. Leaf number of the cultivars 'Vitória' (A) and 'Imperial' (B), foliar rosette diameter (cm) of 'Vitória' (C) and 'Imperial' (D) cultivars, plant height of 'Vitória' (E) and 'Imperial' cultivars (F) (cm) grown under doses of organomineral fertilizer (0, 2.5,

5, 7.5 e 10g) over 0, 120, 150, 180, 210, 240, 270 days of acclimatization. Mossoró-RN, 2017.

Comment [H7]: In the item Material and Methods the unit of measurement is kg m³ and here are grams. Actually, what is it?

At 270 days, when the destructive analyzes of the pineapple seedlings were carried out, the 'Imperial' superiority over 'Vitória' was verified. This superiority was recorded throughout the evaluation process and independent of the treatment applied (Table 2).

Table 2. Average values for leaf number (NF), plant height (AP), leaf rosette diameter (DRF), root system length (CSR), dry shoot mass (MSPA), dry mass of the root system, total dry mass (MST), relation between shoot dry mass and dry mass of the root system (MSPA/MSSR). Mossoró-RN, 2017.

Cultivars	Variables analyzed			
	NF (n°)	AP (cm)	DRF	CSR
'Vitória'	15.8B	15.3B	25.6B	16,3B
'Imperial'	20.7A	20A	31.5A	26,8A
	MSPA (g)	MSSR (g)	MST (g)	MSPA/MSSR (g)
'Vitória'	5.3B	0.5B	5.8B	12,02A
'Imperial'	12.1A	2.7A	14.8A	6,2B

* Means followed by the same letter does not differ by the Scott Knott test at 5% probability ($p < 0.05$).

Different performances of 'Vitória' and 'Imperial' cultivars were observed with the treatments received. Comparative performances of 'Vitória' and 'Imperial' cultivars are not described in the literature, however, in the temperature and humidity conditions of this trial, the 'Imperial' cultivar growth traits stand out despite 'Vitória' cultivar, regardless of the treatments applied.

No significant increase in leaf number was observed for both cultivars with the increasing phosphorus doses in the substrate, that indicates the phosphate fertilization in pineapple seedlings in the acclimatization stage was not efficient (Figure 2A).

Prado et al. [15] studying phosphorus in nutrition and production of passion fruit seedlings, verified that the application of phosphorus in the form of triple superphosphate significantly increased stem diameter, height and number of leaves of passion fruit seedlings and the plants reached a maximum development with the dose about 450 mg of P dm^{-3}. On the other hand, in the highest dose of phosphorus, the plants decreased the development.

The average plant heights of 'Vitória' and 'Imperial' cultivars may be observed for each dose. The 'Imperial' cultivar averages were greater than the 'Vitória' cultivar averages (Figure 2B). In Figure 2C, the mean diameter of the leaf rosette of 'Vitória' and 'Imperial' cultivars may be observed for each dose. Also, the superiority of the 'Imperial' over the 'Vitória' was observed.

Comment [H8]: Why?

No significant interaction was observed from the factors for the cultivar 'Imperial', however, the root length increased up to the dose of 5 g of the organomineral fertilizer, and then decreased (Figure 2D). The cultivar 'Vitória' root length increased up to the major dose applied.

The values observed in the present study differ from those reports that phosphorus is a stimulator of root growth [16].

About the 'Vitória' cultivar, the increase of the P doses increased the root length. Cortez [17] states that phosphorus plays a major role in the development of the root system. When this nutrient is in low levels at the beginning of the vegetative cycle, it can decrease the growth

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and development of the root, harnessing the photosynthesis and the water absorption and nutrients uptake.

Comment [H10]: Was contradictory.

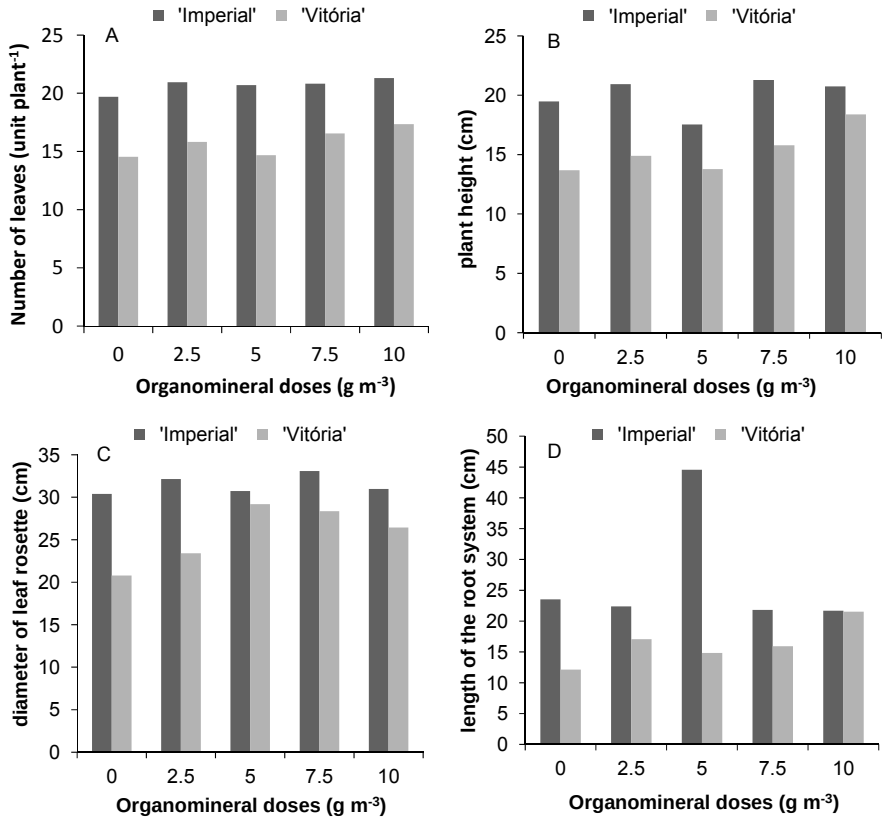


Figure 2: Number of leaves (A), plant height (B), diameter of leaf rosette (C) and length of the root system (D) of seedlings of pineapple cultivars grown under doses of organomineral fertilizer in the acclimatization stage. Mossoró, RN, 2017.

The 'Vitória' cultivar obtained a quadratic shape with a minimum average obtained from 1.6 g plant^{-1} in the dose of 5.5 g of the organomineral fertilizer and a maximum average of 9.2 g plant^{-1} with 10 g of the organomineral fertilizer. The 'Imperial' cultivar data did not fit into any mathematical model (Figure 3A).

The 'Vitória' cultivar obtained a quadratic shape for root dry mass with a minimum average obtained (0.3 g plant^{-1}) in the dose 5.5 g of fertilizer and a maximum average (1.08 g plant^{-1}) with 10 g of the organomineral fertilizer. The 'Imperial' cultivar data did not fit any mathematical models (Figure 3B).

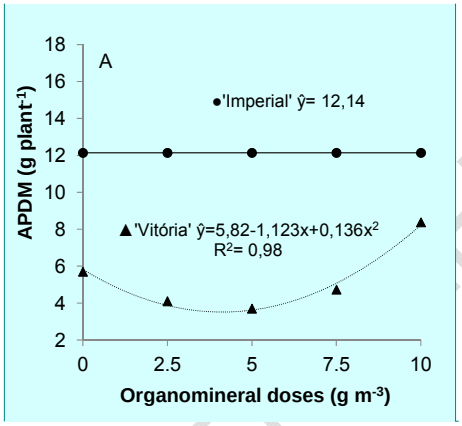
The 'Vitória' cultivar obtained a quadratic shape for total dry mass, the minimum average obtained (4.5 g plant^{-1}) with the dose 4.1 g of the organomineral fertilizer and the maximum

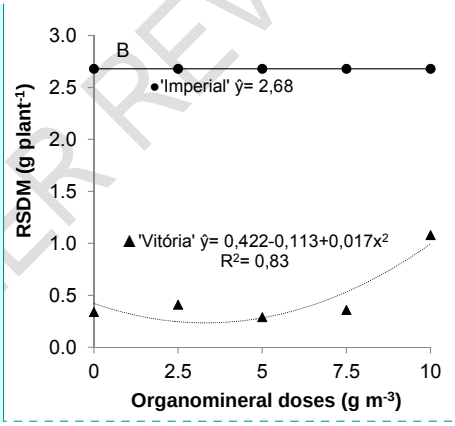
average (8.8 g plant^{-1}) with 10 g of the organomineral fertilizer. The 'Imperial' cultivar data did not fit any mathematical models (Figure 3C).

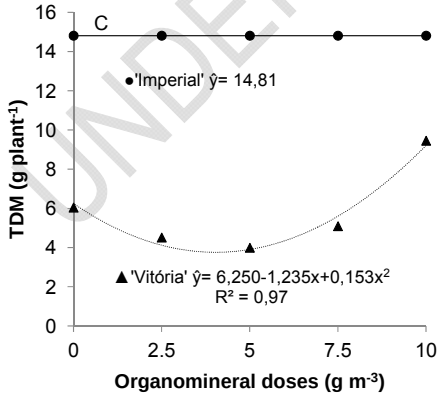
In the case of dry aerial and root matter, Almeida Júnior et al. [6] studying the effect of doses of phosphorus on the initial development of castor bean plants, found that castor bean plants responded positively up to 8.8 g plant^{-1}, and declined from this value.

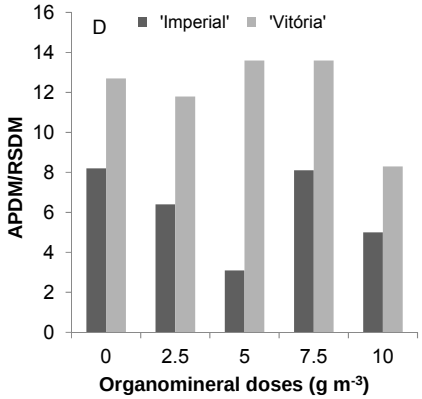
The aerial part dry mass of 'Vitória' cultivar was greater than that of the 'Imperial' cultivar for all doses of fertilizers studied about the dry mass of the aerial part and dry mass of the root system ratio (Figure 3D). This fact was already expected since the aerial part dry mass and the root dry mass of the 'Imperial' cultivar were very greater than that of the 'Vitória' cultivar.

In the case of the present work, this fact indicates the application of P in cultivar 'Vitória', during the acclimatization stage, favors the establishment of ex vitro plants and, possibly, the establishment in the field. As for the 'Imperial' cultivar, this behavior was not observed, denoting the need of the phosphate fertilizer in the acclimation stage. This may be a genetic issue of not responding to phosphate fertilization.









Comment [H11]: This is more like the effects of N, not just of P.

Comment [H12]: Is not kg m^{3}?

Figure 3. Aerial dry mass (A), root dry mass (B), total dry mass (C) and aerial dry mass and root dry mass ratio (D) of pineapple seedlings grown under doses of organomineral fertilizer in the acclimatization phase. Mossoró-RN, 2017.

The nutritional performance of both 'Vitória' and 'Imperial' cultivars indicate the similar contribution of macronutrients between the two cultivars during the seedling acclimatization stage, but for calcium and manganese contents the cultivar 'Vitória' were higher (Table 3).

Table 3. Mean values of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe) and zinc (Zn) leaf contents in pineapple cultivars' Victory 'and' Imperial '. Mossoró-RN, 2017.

Comment [H13]: Where?

Cultivars	Variables analyzed		
	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})
'Vitória'	8.8A	1.7A	21.3A
'Imperial'	8.3A	1.9A	19.1A
	Ca (g kg^{-1})	Mg (g kg^{-1})	Mn (mg kg^{-1})
'Vitória'	3.2A	0.25A	256.9A
'Imperial'	2.4B	0.25A	241.8A

* Means followed by the same letter does not differ by the Scott Knott test at 5% probability ($p < 0.05$).

The comparative nutritional behavior among the cultivars is not described in the literature. The values found in this study agree with several studies for the cultivation of pineapple seedlings, except for calcium content (Prado et al. [15]; Ramos et al. [18]; Leonardo et al. [20]; Cruz et al. [20]; Oliveira et al. [21]) and lower than those found by [4].

The increase in the doses of the phosphate organomineral fertilizer provided a decrease in the average values for N content for 'Vitória', but there was no significant difference. However, no effect for the P and Mg foliar contents was observed with the increasing doses of the phosphate organomineral fertilized (data not shown).

The two cultivars obtained quadratic shape. The K minimum average content (16.2 g kg^{-1}) in ''Vitória' cultivar was observed at the dose of 7.2 g and the K maximum average content (25.1 g kg^{-1}), at the dose of 1.8 g of organomineral fertilizer. The 'Imperial' cultivar obtained a minimum average leaf content (15.4 g kg^{-1}) with the dose 10 g and a maximum average content (22.6 g kg^{-1}) with the dose 4.8 g of organomineral fertilizer (Figure 4A).

The levels of K found in the two cultivars corroborate with those observed by Guarçoni and Ventura [22] when studying NPK fertilization in the development of 'Gold' cultivar (MD-2) and verified that the application of triple superphosphate increased leaf content under a quadratic shape. The increase in the K foliar content, from the application of increasing doses of P_2O_5, may be due to the increase of the active root uptake of K that is generated, according to Fernandes and Souza [23], by the hydrolysis of high energy bonds, as of ATP or PPi accumulated in the plant in greater amounts.

The leaves calcium content data of the 'Vitória' cultivar did not fit any mathematical model. For the 'Imperial' cultivar, a linear shape was observed, with a minimum average content (2.13 mg kg^{-1}) with the dose of 2.2 g and a maximum average content (2.8 mg kg^{-1}) with the dose of 10 g of organomineral fertilizer (Figure 4B).

For Prado et al. [15], the application of phosphorus to the substrate promoted an increasing linear effect on calcium content in the aerial part. Veloso et al. [24] state that when there is greater availability of K, there is less availability of Ca in the soil solution, which competes for

the exchange sites with Ca and Mg. This may have occurred in this work since in the 'Imperial' cultivar presented a quadratic shape for the leaf content of K, when reached a maximum dose of organomineral fertilizer followed by a decrease in leaf content, while Ca increased linearly.

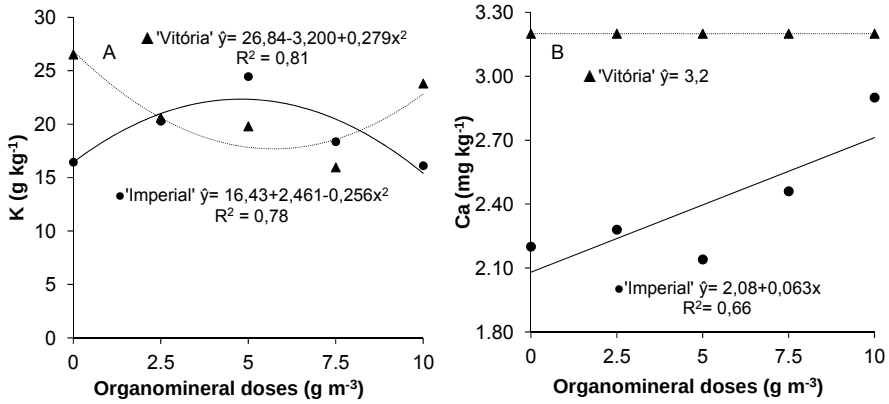


Figure 4: Potassium content (A) and calcium content (B) in leaves of pineapple cultivars grown under doses of organomineral fertilizer in the acclimatization stage. Mossoró-RN, 2017.

4. CONCLUSION

• The cultivars reached growth characteristics suitable for transfer to the field at 210 days; the doses of organomineral fertilizer that provided the greatest gains for the biometric characteristics were 10 g and 5 g for 'Vitória' and 'Imperial' cultivars, respectively.
• Increasing doses of the phosphate organomineral fertilizer applied to 'Vitória' and 'Imperial' cultivars presented similar foliar P and Mg contents; The 'Imperial' cultivar phosphate fertilization increased the K and Ca leaf content. However, for 'Vitória' cultivar, only the leaf content of Ca was affected by the increasing doses.

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