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Propagation

Potential of *Pinus elliottii* Engelm. needles as substrate conditioner for the production of "Fepagro C37 Reck" Citrus rootstocks

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Abstract- The production of citrus seedlings in protected environment requires a large amount of substrate, which must have adequate physical and chemical characteristics. The use of acidic conditioners, such as *Pinus* needles mixed with alkaline substrates, allows the cultivation of seedlings with better quality. The objective of this work was to evaluate the development of the "Fepagro C37 Reck" rootstock grown in different alkaline commercial substrate mixtures (Humosolo ES®) with *Pinus elliottii* needles. The experiment was carried out by testing alkaline substrate mixed with 5-month old of decomposition *Pinus* needles in situ decomposition and two granulometry sizes, 3.5 and 8.0 mm. The mixtures were performed in proportions between the needles with the Humosolo, (v / v) 0%; 25%; 50%; 75% and 100%. Expanded cellular polystyrene trays were used to accommodate the mixtures and seeding of the rootstock. Physical and chemical evaluateons of the mixtures were carried out, as well as the development of the seedlings. The use of *Pinus* needles acidified the substrate Humosolo ES®, reduced its electrical conductivity and increased the percentage of porous space of the mixtures and reduced water retention. The treatments containing 25 and 50% of the *Pinus* needles mixture, regardless of the granulometry, provide greater development of rootstock plants "Fepagro C37 Reck".

Index terms: physical-chemical characteristics of substrates, protected environment. seedlings, pH.

Potencial da acícula de *Pinus elliottii* Engelm. como condicionador de substrato para produção do porta-enxerto de citros "Fepagro C37 Reck"

Resumo- A produção de mudas de citros em ambiente protegido demanda grande quantidade de substrato, o qual deve apresentar características físicas e químicas adequadas. O uso de condicionadores ácidos, como acículas de *Pinus*, em mistura com substratos alcalinos, possibilita o cultivo de mudas com maior qualidade. O objetivo foi avaliar o desenvolvimento do porta-enxerto "Fepagro C37 Reck" cultivado em diferentes misturas do substrato comercial alcalino (Humosolo ES®) com acículas de *Pinus elliottii*. O experimento foi realizado testando misturas entre o substrato alcalino e acículas de *Pinus* com cinco meses de decomposição *in situ* e em duas granulometrias: 3,5 e 8,0 mm. As misturas foram realizadas em proporções entre as acículas com o Humosolo, (v/v) de 0%; 25%; 50%; 75% e 100%. Utilizaram-se bandejas alveoladas de poliestireno expandido para acomodação das misturas e semeadura do porta-enxerto. Realizaram-se avaliações físicas e químicas das misturas, bem como do desenvolvimento das mudas. A utilização de acícula de *Pinus* acidificou o substrato Humosolo ES®, reduziu sua condutividade elétrica e tornou as misturas com maior porcentagem de espaço poroso e menor retenção de água. Os tratamentos contendo mistura de 25 e 50% de acícula, indiferentemente da granulometria, proporcionam maior desenvolvimento de plantas do porta-enxerto "Fepagro C37 Reck".

Termos para indexação: ambiente protegido, características físico-químicas de substratos, mudas, pH.

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Introduction

Agribusiness is an extremely important sector for the Brazilian economy. In relation to the fruit industry, citrus is considered one of the most important segments, not only for human nutrition, but also for the economic values obtained with this product, supplying the domestic and international market. However, there are numerous bottlenecks in the production chain, especially in the production of seedlings.

The most used citrus rootstocks in Rio Grande do Sul are "Trifoliata" (*Poncirus trifoliata* (L.) Raf.) and their hybrids, due to their tolerance to lower temperatures. One of these hybrids, "Fepagro C37 Reck", has desirable characteristics such as induction of canopy tolerance to low winter temperatures; reduction of plant size; promotion of the production of quality fruits; in addition to presenting a faster development in the nursery in comparison to *P. trifoliata* (OLIVEIRA et al., 2010; KOLLER, 2013). Hence, the average time for the production of citrus seedlings in Rio Grande do Sul using the open-air system is, on average, three years (KOLLER, 1994), compared to hot climate regions. On the other hand, the production of seedlings in the protected environment allows producing a seedling in up to 22 months, which demands a great amount of substrate since the production is carried out in containers (AZEVEDO et al., 2009).

Substrate is any material of mineral and/or organic origin, pure or in a mixture, capable of providing anchoring and supplying the water and oxygen needs of plants (VENCE, 2008). This input of great importance in the cultivation in containers can be altered by using different materials, particle size, humidity, therefore, providing themselves with new material with unique characteristics.

Most of the substrates are produced using composted *Pinus* bark (YAMAGUTI, 2009). In Rio Grande do Sul, because the adequate and available-for-citrus substrates are scarce, there is a need to import products, which result in high costs, mainly for freight. According to Schäfer et al. (2015), most of the substrates evaluated in the southern region show negative aspects such as alkaline pH and high electrical conductivity (salinity). Therefore, materials that are chemically and physically stable and inexpensive are more and more desired.

The cultivation of *Pinus* sp. involves large extensions of land in southern Brazil, representing 26.5% of national reforestation, occupying an area of approximately 1.8 million hectares (GIONGO et al., 2011). This crop stands out as a raw material in several industrial activities, which are an essential base for the economy. However, it generates countless residues in its production such as *Pinus* needles. Piovesan et al. (2012) observed an annual accumulation of 7.1 Mg ha^{-1} of litter in an 8-year-old *Pinus* crop, which was formed by 95.6% of the needles. This material, which has not yet been explored, has already been reported as having low pH (VERDONCK et al., 1981; GOMES et al., 1991; RISTOW et al., 2009), and may be an alternative material to minimize pH alkalinity of certain substrates.

Due to the characteristics of the mentioned material and to the great importance of the substrate in the cultivation of plants in containers, the objective of this work was to evaluate the production of a citrus rootstock ("Fepagro C37 Reck") grown in different proportions of an alkaline commercial substrate, due to the characteristics of the previously mentioned materials and the great importance of the substrate (Hum solo ES®) and crushed *Pinus elliottii* needles on the germination phase.

Material and methods

The experiment was conducted in a protected environment, covered with polyethylene and with anti-aphid screen walls, with no temperature control and relative humidity, at the Faculdade de Agronomia of the Federal University of Rio Grande do Sul, located in Porto Alegre, state of Rio Grande do Sul (30º04'S, 51º08'W; 38 m above sea level). This experiment consisted of different mixtures (v/v) of a commercial substrate using *Pinus elliottii* needles at 5-month old in situ decomposition, at the senescence stage, but still adhered to the plants, without contact with the soil. Under these conditions, samples of adult trees from the same stand were collected. Such trees had been fallen down five months ago at the Agronomic Experiment Station of the Federal University of Rio Grande do Sul (EEA / UFRGS; 30º29'S and 51º06'W; 46 m above sea level).

The needles were dried in a sheltered place over a bench with an average temperature of 25 ºC for 15 days. Afterward, they were crushed in two different particle sizes (3.5 mm and 8.0 mm) in a sieve mill and mixed at five percentages (0; 25; 50; 75 and 100%) to the substrate (Humosolo ES®) from VIDA Company. This input made on the basis of eucalyptus bark has optimum physical characteristics for use in containers, but its pH is slightly alkaline (pH 7.2 ± 0.5).

The mixtures were placed in 121.2 cm^3 expanded polystyrene trays of inverted pyramidal shape (120 x 50 mm - depth x cell width). Sowing of the rootstock "Fepagro C37 Reck" [*Poncirus trifoliata* (L.) Raf. x *Citrus sinensis* (L.) Osbeck.], from matrices located in the EEA/UFRGS, was carried out in October 2014. The seeds were submitted to thermal treatment (immersion in water at 52ºC for 10 minutes), after which a seed per cell was placed at 0.5 cm depth. Irrigation management was automated and consisted of three daily micro-sprinkler cycles, totaling an average of 3 mm day^{-1}. No additional fertilizations were carried out on the substrates over the growing period.

Electrical conductivity (µS cm^{-1}) and pH (in H_2O) of the substrates were monitored using the non-destructive *Pour Thru* method, according to Cavins et al. (2000) over the cultivation cycle. After 49 days of sowing, the variables emergence percentage and seedling formation, average emergence time and emergence speed index were evaluated, which was determined based on the sum of the number of emerged seedlings, divided by the respective time, and then dividing the final result by the total number of seedlings emerged in each replicate. In addition, at the end of the experiment, 166 days after sowing, the variables height, diameter, dry mass of the aerial part, dry mass of the roots, leaf count and leaf area were measured. However, height was measured with the aid of a measuring tape, from the surface of the substrate to the apical point of the seedling; the diameter was measured using a digital caliper at one centimeter from the base, and the leaf area was obtained on a Li-Cor Li-3100 leaf area meter.

The different substrate formulations were submitted to chemical and physical analysis at the Substrates Laboratory of the Department of Horticulture and Forestry (DHS) at the Faculty of Agronomy (UFRGS), in Porto Alegre, state of Rio Grande do Sul.

The experimental design used in the study was a randomized block design with split-plots. The main plot consisted of the proportions of *Pinus elliottii* needles in the composition of the substrates (0; 25; 50; 75 and 100%), and the subplots were the particle size (3.5 and 8.0 mm). Each treatment consisted of four replicates of 12 cells each. Data were submitted to analysis of variance (ANOVA) by the Costat 6.4 software, and polynomial regression by SigmaPlot 11.0 at the level of 1% (**) and 5% (*) of a probability of error, in order to verify the effect of the percentage of leaf on the substrate composition. The electrical conductivity data did not meet the ANOVA assumptions and because of that, they were therefore transformed into *log x/10*.

Results and discussion

Statistical analysis of the data showed no interaction between particle size and *Pinus* needles proportion for the variables tested in the study. However, the different ratios of the needles used in the composition of the substrates influenced the growth and development of the "Fepagro C37 Reck" rootstock (Table 1).

The *Pinus* needles showed acidic pH, regardless of granulometry, while the pure commercial substrate (0% leaf treatment), alkaline pH. As the percentage of the needles increased, the pH reduced proportionally, reaching intermediate values to the pure materials. However, this pH change was most evident when the percentage of the needles was higher than 75% at the beginning of the cultivation (Figure 1A and B). In addition, greater acidification of the substrate was found 40 days after sowing in the treatments that presented the highest proportions of needles in the mixture.

Although the amplitude between the substrates was greater in the first evaluation, the pH values of the materials tended to be nearer over time probably due to the low potential acidity of the *Pinus* needles and to the low buffering effect of the substrate. The large pH variation occurred mainly in the 100% needles treatment (Figure 1A and B), excluding the possibility of the commercial substrate buffer effect. Several factors may cause changes in the pH of the medium, such as the decomposition of the source material, the release of exudates by the roots of the plants, the alkalinity of the irrigation water and the fertilization (FOCHESATO et al., 2008; SCHÄFER et al., 2008). The variation in the pH verified in this study is likely to be caused by the alkalinity of irrigation water.

The need to leach 20% of the watering so to facilitate gas exchange within the cultivation container could have favored the mentioned effect. This is because the application of a volume of water over irrigation to reach the pot capacity or a volume exceeding that capacity by 20% may have altered the pH of the medium, as well as through leaching, the amount of salts over cultivation could have decreased (Figure 1C and D), since the average value of pH and EC of the irrigation water during the conduction period of the experiment was 6.1 ± 0.5 and 110.0 ± 1.0 µS cm^{-1}, respectively. Therefore, *Pinus* needles provide relative acidity, capable of acidifying the medium if used at a large proportion. However, the maintenance of an adequate pH value can be adjusted with acidic reaction fertilization or pH adjustment of the irrigation water, thus maintaining the ideal pH for the cultivation of potted plants.

In relation to the electrical conductivity (EC), the results showed that the reduction of the granulometry resulted in higher release of ions to the solution and, consequently, the increase in the electrical conductivity. This fact was verified by the difference between the treatments of 100% needles at the 3.5 mm granulometry showing 600 µS cm^{-1} and in the 8.0 mm granulometry at 231.5 µS cm^{-1}, both on the sowing day (Figure 1C and D).

The value of EC presented by the substrate Humosolo ES® manufacturer is higher than that of the *Pinus* needles, with an average value of 2100 ± 500 µS cm^{-1}, which was verified in the treatment of 0% needles and zero time (Figure 1C and D). It has also been found that as the ratio of the leaf increases, EC decreased in the formulated substrate. In spite of the granulometry and percentage of the needles, the substrates showed low levels - for the treatment without addition of the needles (treatment 0%) - to very low - for the treatments with a large percentage of needles at the beginning of the experiment, according to classification established by

Cavins et al. (2000).

The high EC reduction over the crop is caused by the leaching of the soluble salts through the irrigation system. Thus, none of the treatments exceeded the EC index considered normal for substrates (2600 to 4600 µS cm^{-1}) (CAVINS et al., 2000). This feature is positive since plant nutrition can be managed by precisely meeting the crop requirement. Fagundes et al. (2015), when evaluating the development of yellow passion fruit seedlings report that the loss of nutrients by leaching is a serious issue in the seedling production system, since it represents a loss of capital in the form of nutrients and water, as well as extending the time for the formation of seedlings, due to the lower availability of fertilizers.

Regarding the physical characteristics of the substrates, properties such as density, total porosity and water retention capacity influence the development of the plants (COSTA; DANTAS, 2009) and the management to be used over cultivation. The reduction in the particle size resulted in an increase in the density of *Pinus* needles. However, the increase in the needles percentage to the commercial substrate provided a reduction in density because Humosolo ES® had a density as many as four times of that of the needles. According to Kämpf et al. (2006), substrates with a density of less than 300 kg m^{-3} are recommended for use in multicellular trays; with density between 200 and 400 kg m^{-3}, they are recommended for pots up to 15 cm high; and density of 300 to 500 kg m^{-3} is suggested for pots with 20 to 30 cm in height. According to this classification, all the formulations tested in this study had adequate density to be used in pots. This result confirms the hypothesis of the use of *Pinus* needles in the composition of substrates since the search for new organic materials that meet these requirements is fundamental for the development of a new substrate as a large part of the organic materials such as barks and bagasse showed higher densities than those recommended for cultivation in pots.

The density of a substrate can be altered by the packing density, associated with the moisture content of the material. Changes in density over cultivation of the plants modify the physical properties of the substrate. Therefore, the increase in solids content per volume unit may influence the voids ratio (pore to solids ratio), total porosity, aeration space, water retention capacity and root penetrability (mechanical impedance) (FERMINO; KÄMPF, 2012). Among these changes, those occurring in water retention and mechanical impedance standout as problems with excess water and difficulties in the penetrability of the substrate may occur, damaging the development of the plants. Hence, the higher the substrate compaction, the greater the percentage of malformed seedlings.

It was possible to observe from the water retention curve that the substrates with higher density had a higher percentage of solids, while the use of needles in the formulations provided a larger aeration space. Needles with 3.5 mm provided a higher percentage of solids, a smaller aeration space and a greater amount of readily available water in relation to the use of 8.0 mm granulometry needles (Figure 2). According to Faria et al. (2013) and Souza et al. (2010), the aeration space is considered a determinant factor in the quality of the substrates, as it provides greater root hair development, which promotes an increase in the absorption of water and nutrients (BELLÉ, 2001).

The differences found in the physical characterization for the different formulations do not make unfeasible their use as substrates. However, care should be taken with the frequency of irrigation adopted, since the low percentage of available water compared to the ideal parameters is easily corrected with a higher frequency of irrigation.

Although the substrates showed different physical characteristics, they did not influence the emergence process of the "Fepagro C37 Reck" rootstock (Table 2). The average time of emergency was approximately 35 days; emergence speed index rate was 0.03, and the percentage of seedlings formed was greater than 93% in all treatments. Rieth et al. (2012) when evaluating the development of citrus rootstocks also did not verify the influence of the substrate on the final values of emergence. However, according to Sá et al. (2015), the salinity of the substrate may generate imbalances in the germination, which was not verified in the present study because the low electrical conductivity and the low water retention of the mixtures, associated to the high frequency of irrigation, provided excellent emergency rates.

In relation to height (Figure 3A) and diameter (Figure 3B) of the seedlings, a negative quadratic tendency was found as the proportion of needles was increased in the composition of the substrates, with a maximum point between 25 and 50% leaf treatments. After 40 days of sowing, as the treatments had the same pH range and EC, remaining stable until the end of the experiment, the superior growth of the seedlings grown in the formulations containing 25 and 50% of needles is likely to be explained by the characteristics (Figure 2) where the water retention curves were very similar, which may have benefited from the adopted management.

The treatments that provided the greatest values for the height and diameter of the seedlings also provided the greatest number of leaves (Figure 3C) and leaf area (Figure 3D). The treatment composed of 100% of *Pinus* needles conferred a lower growth rate, due to the low EC and lower retention of water in relation to the other substrates. The pure commercial substrate allowed a superior result only when compared to the 100% needles treatment, evidencing that the addition of this material to the alkaline substrate generated benefit, assuring a better growth of the "Fepagro C37 Reck" rootstock.

In relation to the dry mass of the aerial part and of the root system, a quadratic negative behavior was found in relation to the needles percentage (Figure 3E and F, respectively). The maximum accumulation point of dry biomass of the seedlings was verified between treatments with 25 and 50% needles. This result is justified by the lower retention of water and low fertility of substrates with a high percentage of needles.

The substrate Humosolo ES® showed higher electrical conductivity but did not influence positively the seedlings development, evidencing that the physical characteristics had a greater influence on the growth of these plants. Also, the lack of needles in the substrate composition provided a higher percentage of remaining water, buffer water and solids, so it was evident the greater retention of water in relation to the other treatments, possibly causing damage to the growth of the seedlings. However, the association of the *Pinus elliottii* needles with the substrate Humosolo ES® resulted in a reduction in the amount of solids and, consequently, an increase in total porosity, providing the ideal conditions for the “Fepagro C37” rootstock plants to express their potential growth.

Table 1. Analysis of variance of the development of “Fepagro C37 Reck” rootstock growth in different granulometry and proportions of *Pinus elliottii* Engelm. needles in the substrate composition. Porto Alegre, 2014.

Variables	Proporion of needles	Granulometry	Interaction	CV (%)
AET (days)	0.1004^{ns}	0.5760^{ns}	0.3965^{ns}	5.55
SEI	0.1296^{ns}	0.3996^{ns}	0.4309^{ns}	4.91
Seedling formation (%)	0.8785^{ns}	0.8065^{ns}	0.4666^{ns}	6.23
Height (cm)	<0.001	0.7743^{ns}	0.5491^{ns}	16.25
Diameter (mm)	<0.001	0.9204^{ns}	0.2884^{ns}	13.09
Leaf count	<0.01	0.4485^{ns}	0.7424^{ns}	18.47
Leaf area (cm²)	<0.001	0.7714^{ns}	0.2411^{ns}	24.23
Dry mass of the aerial part (g)	<0.001	0.5148^{ns}	0.4494^{ns}	30.12
Dry mass of the roots (g)	<0.001	0.9813^{ns}	0.1858^{ns}	25.40

ns = not significant; AET = average emergence time; SEI = speed of emergence index; CV = coefficient of variation.

Table 2. Average emergence time (AET), speed of emergence rate (SER) and percentage of “Fepagro C37 Reck” rootstock seedling formation in different granulometry and proportions of *Pinus elliottii* Engelm. needles in the substrate composition. Porto Alegre, 2014.

Treatments	AET (days)	SEI	Seedling formation (%)
Granulometry			
3.5 mm	34.65^{ns}	0.030^{ns}	95.83^{ns}
8.0 mm	34.27	0.030	95.31
Proportion of needles (%)			
0	35.00^{ns}	0.030^{ns}	95.60^{ns}
25	35.52	0.029	96.88
50	34.56	0.030	94.79
75	34.70	0.030	95.83
100	33.05	0.031	94.79

ns = not significant.

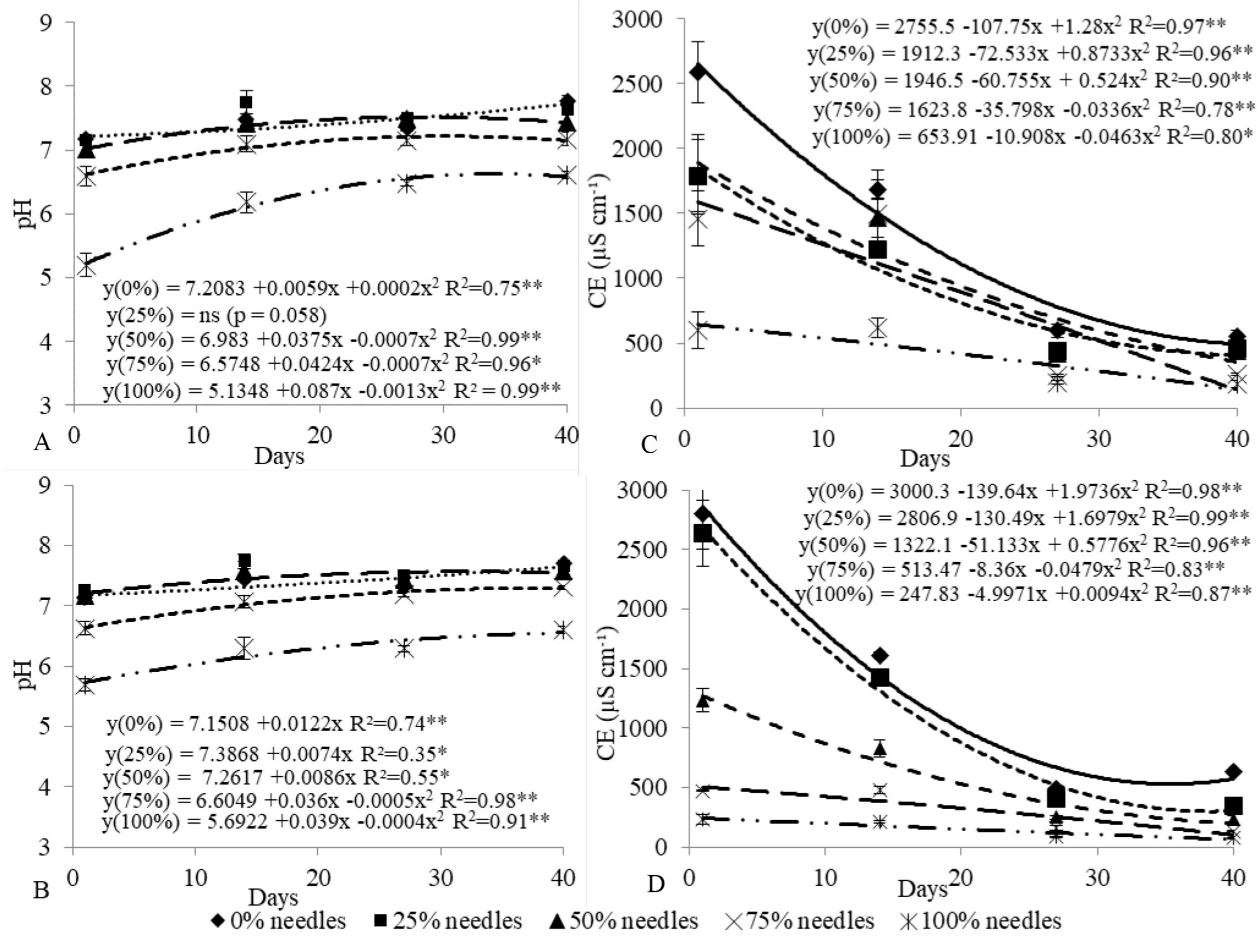

Figure 1. pH variation (A = 3.5 mm and B = 8.0 mm) and electrical conductivity (µS cm^{-1}) (*Pour thru* method) (C = 3.5 mm and D = 8.0 mm) over cultivation of "Fepagro C37 Reck" rootstock seedlings in different proportions of *Pinus elliottii* Engelm. needles in the substrate composition. Porto Alegre, 2014. ns = not significant; ** significant at 1% error probability; * significant at 5% error probability.

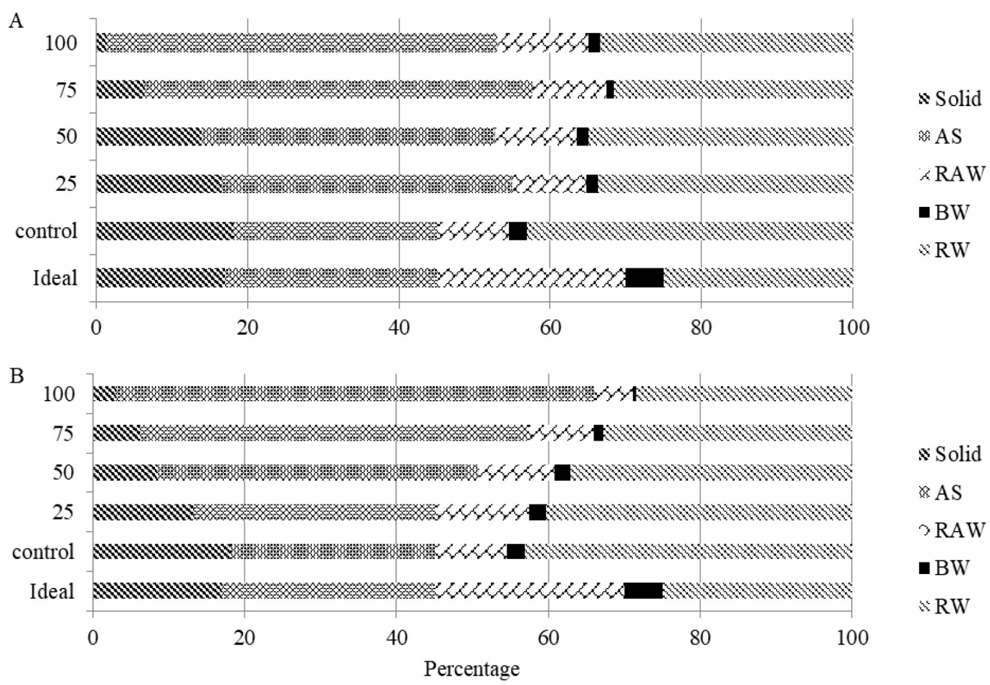

Figure 2. Different proportions of *Pinus elliottii* Engelm. needles (A = 3.5 mm) (B = 8.0 mm) with Humosolo ES® substrate compared to the control (commercial substrate) and ideal range (ABAD et al., 1993; DE BOODT; VERDONCK, 1972; VERDONCK; GABRIELS, 1988). AS: aeration space; RAW: readily available water; BW: buffer water; RW: Remaining water. Porto Alegre, 2014.

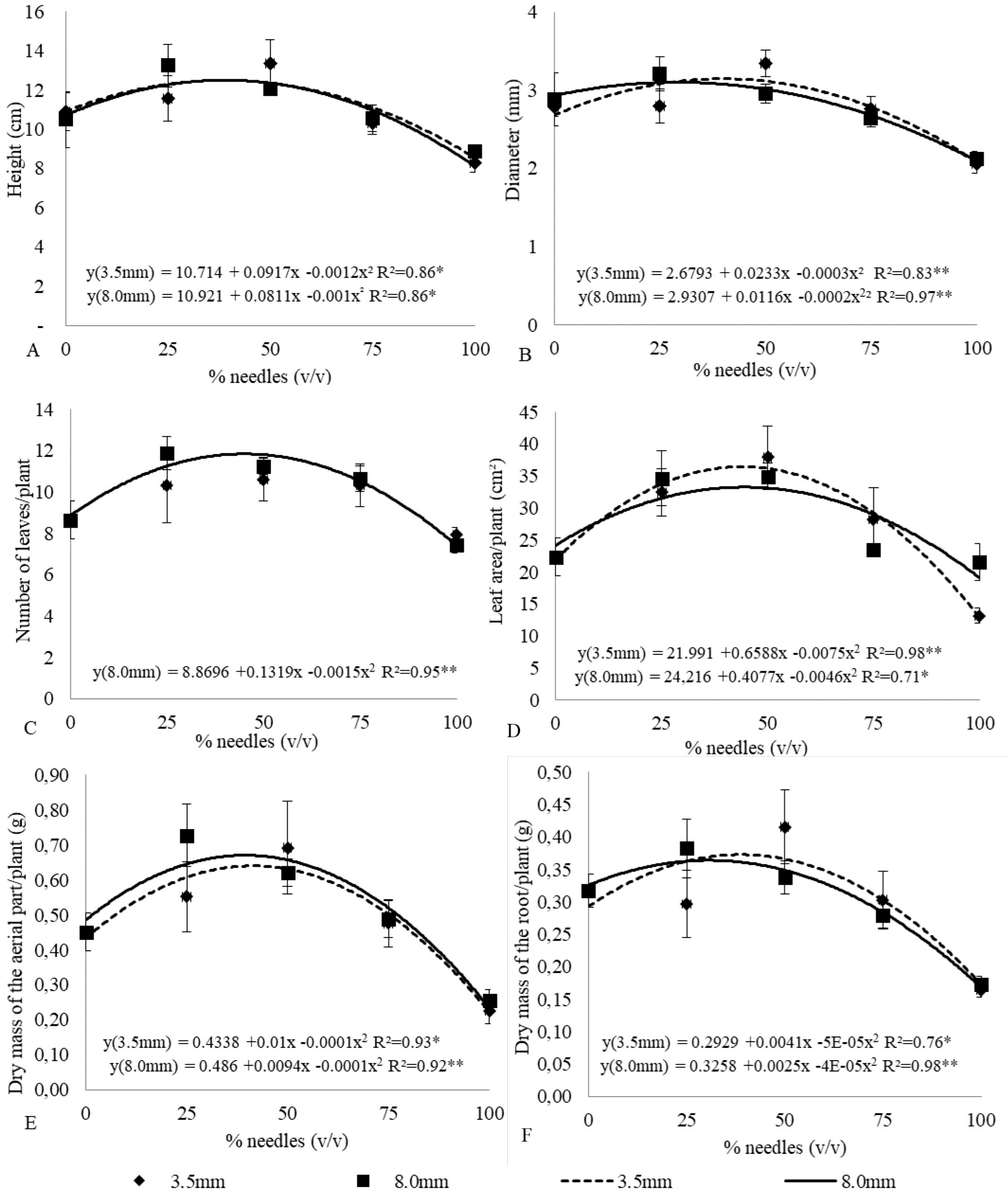

Figure 3. Height (A), diameter (B), number of leaves (C), leaf area (D), dry mass of the aerial part (E) and root (F) of “Fepagro C37 Reck” rootstock at different granulometry and *Pinus elliottii* Engelm. needles proportion in the substrate composition. Porto Alegre, 2014. ** significant at 1% of error probability; * significant at 5% of error probability.

Conclusion

The addition of 25% to 50% of *Pinus elliottii* needles with granulometry between 3.5 mm and 8.0 mm to the substrate Humosolo ES® resulted in a faster growth and vegetative growth of seedlings of the rootstock "Fepagro C37 Reck" over the germination phase.

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