



Article

# New Media Components and Fertilization to Accelerate the Growth of Citrus Rootstocks Grown in a Greenhouse

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Abstract: In Puerto Rico, oranges made up \$6,452,000 of the agricultural gross income for 2014–2015. Today, citrus greening (CG) is the most aggressive disease affecting the citrus industry in the whole world. This disease causes dieback of the plant, among other symptoms, which is resulting in the reduction of citrus trees in the field across the world. Currently, it is recommended to grow citrus rootstocks in nurseries to produce disease-free trees. The objective of this investigation was to evaluate (before and after grafting) the effect of different substrate mixes and quantities of fertilizers on the rootstocks Carrizo citrange and Swingle citrumelo in order to accelerate their development inside of a protected structure. The treatments were: Promix + sand (control) (1:1), Promix + sand + coco peat (1:1:1), Promix + sand + coffee compost (1:1:1) and Promix + sand + rice husk (1:1:1). Two 18-6-2 fertilizer treatments were also evaluated: 5.6 g and 8.5 g. The substrate that contained 33% rice husks negatively influenced every parameter evaluated for both rootstocks. Carrizo presented better development on the coffee compost mix, while Swingle did not exhibit significant differences among any substrates, except on rice husk, for most of the parameters. "Rhode Red Valencia" presented better results for dry weight when grafted on Carrizo with the coffee substrate. The rice husk substrate is not recommended for the citrus tree production at the nursery level.

Keywords: Carrizo citrange; Swingle citrumelo; citrus greening; protected greenhouse; substrates

# 1. Introduction

In 2014–2015, among fruit, oranges were in third place in the gross income of Puerto Rico, contributing \$6,452,000 to the island's economy [1]. However, in the last few years, the production of citrus on the island has decreased remarkably. Citrus greening (CG) disease, caused by the bacteria *Candidatus Liberibacter asiaticus* (*CLas*), has become the principal cause of the worldwide reduction of citrus production [2]. Citrus trees infected with CG present acidic fruits, asymmetric foliar chlorosis, diminished life cycle and, eventually, the tree presents dieback [2,3]. In Puerto Rico, the citrus psyllid *Diaphorina citri* (vector of CG) was first detected in 2001, while the presence of the bacteria, thus the presence of CG, was first detected in 2009 [4,5]. Since then, there has been a reduction in citrus production, from 3500 tons in 2014 to 1080 tons in 2016 [6]. Biological control, harsh pesticide treatments and thermotherapy are some of the controls that are being implemented to control either the vector or disease of CG [7–9]. Another recommendation to maintain a stable citrus production is the elimination of advanced diseased trees, applications of intensive fertilizer practices, production of citrus trees inside protected structures and on-site planting of disease-free citrus trees [10].

Grafted trees produced inside protected structures have the advantage of producing strong, disease-free trees, which can help fight the loss of citrus trees in the field caused by CG disease [11].

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The production of healthy citrus liners in containers inside of a protected nursery facilitates the development of a vigorous root system that results in a stronger/healthier plant [12]. Substrates, fertilizers and hormones can help accelerate the growth of citrus rootstocks, and the combination of rootstock/scion can help shorten the time until transplanting to the field and provide some measure of resistance to diseases [13].

Rootstocks not only provide a supportive root system, they also provide a better absorption of nutrients and water. They can also contribute characteristics to the resulting tree, such as tolerance to low temperature, high salinity, flooding and varying soil pH, and resistance/tolerance to nematodes and diseases [14]. Of the multiple citrus rootstocks used in the industry, we evaluated Carrizo citrange and Swingle citrumelo. Carrizo citrange (*Citrus sinensis x Poncirus trifoliata*) has shown resistance to damage by *Phytophthora* and citrus tristeza virus (CTV), an elevated resistance to the *Tylenchulus semipenetrans* nematode and some resistance to the CG disease [15]. Swingle citrumelo (*Citrus paradise* Macf. x *Poncirus trifoliata*) has presented good adaptation to sandy soils; a high germination percentage; an extensive radicular system; and moderate tolerance to salinity, boron toxicity, and CTV [16,17]. Rootstocks help solve problems with soil, climate, pests and diseases, and can also influence the quality of production of the chosen scion [18]. It is important to choose a good rootstock/scion combination [18]. For the scion, we evaluated the variety Rhode Red Valencia. This orange presents intense color, low acidity, low vitamin C and 400% more antioxidants in comparison to other oranges [19].

Not only the combination of rootstock/scion can reduce the time of development of the tree—the substrate can also affect the time of development [20]. With the ideal substrate mix for a crop, one can therefore provide the optimal environment for the growth of an extensive root system [21,22]. The citrus plant root system is limited, which results in a poor capacity for nutrient absorption [23]. Therefore, a way of aiding the necessities of the citrus plant is through the components of the substrate mix and the fertilizer [23].

Many substrate components are used at the nursery level and, for this research, a few of them were chosen for evaluation: peat moss, river sand, coco peat, coffee compost and rice husk. Peat moss is the result of decomposition of vegetable matter in an anaerobic environment; it usually exhibits a pH of 5.3–6.5 and more than 80% organic matter [24,25]. River sand, or any sand, is frequently used to provide weight to the pot. Usually, it exhibits a neutral pH and a low capacity for cationic exchange [22,26]. Coco peat is shredded coconut husk. Some characteristics of this substrate are: a pH that ranges from 5.3–6.3, high electric conductivity and water retention of 700–1100% [24,27]. Compost, in general, is the transformation of organic matter that results in a new material that can favor oxygenation of the soil and substrate and promote higher root growth [28]. Rice husk is the product of processing rice, and as a substrate it can provide higher air porosity [29,30]. In Puerto Rico, little to no research has been done with these materials.

## 2. Materials and Methods

The experiment was conducted inside a nursery at the Agricultural Experiment Station (AES) in Corozal, Puerto Rico. The nursery was protected with a mesh ( $0.24~\text{mm}\times0.75~\text{mm}$ , Empresas San Pablo, Lares, PR, USA) to avoid entrance of the psyllid and large insects. The biosecurity protocol to enter the nursery consisted of using a lab coat and hair net and disinfection of footwear with sanitizing solution (sodium hypochlorite (NaClO) at 10%) to avoid the entrance of pathogens and insects. The first door of the nursery had an air curtain (48'', Mars Airs Doors, Gardena, CA, USA), which automatically started when the door was open. When the first door closed, the second door could be opened to enter the nursery.

Two citrus rootstocks were evaluated: Carrizo citrange and Swingle citrumelo. The seeds were obtained from the rootstock collection at AES, Corozal. The seed were germinated in a commercial mix (Pro-Mix BX with mycorrhizae) on 72-hole germination trays. After 3 months, on 14 April 2015, the seedlings were transplanted to  $10.2 \times 30.5$  cm (4  $\times$  12 inch) square pots (CP412CH, Stuewe &

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Sons. Inc., 31933 Rolland Drive, Tanget, OR, USA) containing the different substrate and fertilizer treatments. All the substrates were sterilized with water vapor (100 °C) for 4 h at AES in Adjuntas. The commercial mix used was Pro-Mix Sunshine Mix #4. This product contained Canadian peat moss, dolomitic lime, coarse grade perlite, gypsum and Sun Gro's long-lasting wetting agent. The coco peat used was a commercial block made of coconut husk, compacted and imported by Empresas San Pablo, Lares, PR. The coffee compost was obtained from the AES in Adjuntas and had 4.89% nitrogen, 0.46% phosphorus, 3.33 mS/cm electric conductivity (EC) and a pH ranging from 6.72 to 6.84 [31]. The rice husk was obtained from a rice planting in the Department of Agriculture. The sand used was river sand obtained from Corozal, Puerto Rico. The fertilizer quantity per treatment was calculated from the low (F1—5.6 g) and high (F2—8.5 g) recommendations of the product Caliber Cote 18-6-12 (Homogeneous Trace Pack, Helena Chemical Company, Collierville, TN, USA). There were two applications of fertilizers: at the transplant date or start of the experiment and 3 months later. The experiment was conducted from April 2015 to February 2016. The combinations of control media, substrates and the fertilizer quantities are described in Table 1.

Treatments	Substrate Mix	Ratio per Volume	Fertilizer
Control + F1	Commercial mix + river sand	1:1	5.6 g
Control + F2	Commercial mix + river sand	1:1	8.5 g
Coco peat + F1	Commercial mix + river sand + coco peat	1:1:1	5.6 g
Coco peat + F2	Commercial mix + river sand + coco peat	1:1:1	8.5 g
Compost + F1	Commercial mix + river sand + coffee compost	1:1:1	5.6 g
Compost + F2	Commercial mix + river sand + coffee compost	1:1:1	8.5 g
Rice + F1	Commercial mix + river sand + rice husk	1:1:1	5.6 g
Rice + F2	Commercial mix + river sand + rice husk	1:1:1	8.5 g

**Table 1.** Treatment abbreviations with substrate combination and fertilizer amount applied.

# 2.1. Evaluation of Rootstock

# 2.1.1. Height and Diameter

The height (cm) of each plant was taken from the top of the substrate to the petiole of the newest leaf every 2 weeks for 6 months. Tree diameter was taken at 5 cm from the substrate with a caliper (digital caliper, ABSolute AOS Digimatic, Mitutoyo Corp., Takatsu-ku, Japan) every 2 weeks for 6 months.

## 2.1.2. Chlorophyll

Chlorophyll of the rootstock leaves was measured with a chlorophyll meter (Field Scout, Chlorophyll Meter with Data Logger, Spectrum Technologies) every 2 weeks. The leaves sampled were the newest, fully-opened leaves. On each plant, three measurements of the same leaf were taken, and an average was calculated.

## 2.2. Evaluation of Rootstocks Grafted with "Rhode Red Valencia"

## 2.2.1. Height

After 6 months, the rootstocks were grafted with "Rhode Red Valencia" (*Citrus sinensis* L. Osbeck) Vegetative material of "Rhode Red Valencia" was obtained from trees kept inside protected structures at AES at Isabela, Puerto Rico. The height of the developing scion was measured from the grafting site to the petiole of the newest leaf.

# 2.2.2. Chlorophyll

The same instruments and procedure as explained before were used with the scion for chlorophyll.

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## 2.2.3. pH and EC

Samples of all the substrate treatments were sent to the Central Analytical Laboratory of the University of Puerto Rico at the beginning of the experiment for chemical properties testing. The pH and electric conductivity (EC) were measured by the pour-through method [32] (pH/EC/TDS Meter, Milwaukee, MW 802) every 2 weeks, from planting until the end of the experiment.

## 2.2.4. Dry Weight of Shoots and Roots of Trees Grafted with "Rhode Red Valencia"

At 3 months after grafting, the trees were harvested for dry weight. The division of the root and shoot was made at the top of the substrate. The dry weight of shoot and root were taken separately. The samples were dried in an oven (Oven PKN810, Yamato) at  $70 \,^{\circ}$ C for  $144 \,^{\circ}$ h (6 days).

## 2.3. Experimental Design and Statistical Analysis

The design of the experiment was a randomized complete block with four blocks of six replicate trees of each treatment. The data was statistically analyzed using ANOVA and Fisher's least significant difference (LSD) with a P < 0.05.

#### 3. Results and Discussion

## 3.1. Evaluation of Rootstock

## 3.1.1. Height and Diameter

Rootstock height significantly differed (P < 0.05) in the interactions of rootstocks with substrate and substrate with fertilizer. In the interaction of rootstock with substrate (Table 2), the combination of Carrizo with coffee compost was the tallest, with a mean of 66.7 cm. The substrate where rootstocks presented significantly smaller mean height was the mix that contained rice husk. For Swingle, the control, coco peat and compost substrates presented the tallest rootstock, with no significant difference among them (Table 2). Overall, the least favorable treatment was the Swingle with rice husk mix, with a mean of 31.3 cm (Table 2). Therefore, for both Carrizo and Swingle, the use of rice husk is not recommended. This data represents a possible savings for the grower by reducing the amount of commercial mix for coffee compost with Carrizo rootstock, with a significant increase of height. Also, more media substitutes can be used for Swingle, meaning more alternatives for the grower.

**Table 2.** Rootstock height, diameter and leaf chlorophyll as affected by rootstock (R), substrate (S) and fertilizer (F) interactions.

Interactions		Height (cm) <sup>1</sup>	Diameter (mm)	Chlorophyll
R * S				
Swingle	Control	49.0 bc <sup>2</sup>	4.25	61.8 abcd
	Coco peat	41.4 cd	3.68	58.7 cd
	Compost	43.0 cd	4.18	62.2 abcd
	Rice	31.3 e	3.07	60.2 bcd
Carrizo	Control	47.0 cd	3.28	66.6 ab
	Coco peat	56.0 b	3.58	64.3 abc
	Compost	66.7 a	4.00	55.6 d
	Rice	40.3 c	2.66	69.1 a
R * F				
Swingle	F1	39.78	3.77	57.8 a
3	F2	42.53	3.82	63.6 b
Carrizo	F1	47.89	3.14	66.0 b
	F2	57.03	3.61	61.8 ab

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Interactions		Height (cm) <sup>1</sup>	Diameter (mm)	Chlorophyll
F * S				
F1	Control	45.3 b	3.65	71.2
	Coco peat	46.4 b	3.64	68.8
	Compost	56.7 a	4.05	52.4
	Rice	26.9 c	2.49	71.4
F2	Control	50.6 ab	3.89	62.1
	Coco peat	50.9 ab	3.62	59.7
	Compost	52.9 ab	4.13	58.7
	Rice	44.7 b	3.24	66.8

Table 2. Cont.

In the interaction of substrate and quantity of fertilizer, the least effective treatment for the growth of the citrus rootstocks producing the smallest trees was the combination of 5.6 g of fertilizer with rice husk media (Table 2). For any of the other substrate mixtures (control, coco peat and rice husk), a high application of fertilizer did not result in more development of the citrus rootstock. This can translate to economic savings and less labor.

As for diameter, no significant difference (P < 0.05) in any triple or double interaction was found (Table 2). However, main effects showed significant differences (P < 0.05) (Table 3). The rootstock with the largest diameter was Swingle with a 3.80 mm diameter. Between fertilizers, the higher quantity of fertilizer presented a larger diameter mean of 3.72 mm. Also, among the substrates, the larger diameters were found with control and coffee compost substrates—with means of 3.77 mm and 4.09 mm, respectively—though they did not differ.

As in this experiment, the lowest diameter and height of shoots of tamarind (*Dialium guineense* Willd) were found with treatments of distinct proportions of river sand and rice husk (1:1, 1:2, 2:5 and 5:2) [33]. This suggests that rice husk may interfere with the growth of tamarind, as well as citrus as our data shows.

Variables	Diameter (mm)
Rootstock (R)	
Swingle	3.80 a <sup>1</sup>
Carrizo	3.38 b
Substrate (S)	
Rice	2.86 с
Control	3.77 ab
Coco peat	3.63 b
Compost	4.09 a
Fertilizer (F)	
F1	3.46 b
F2	3.72 a

**Table 3.** Main effects on tree diameter.

The most common techniques of grafting used with citrus rootstocks are T-budding and lateral budding, which can be performed effectively when the diameter of the rootstocks reaches 10–20 mm [34]. For this reason, even though Swingle with a mean of 4.09 mm (Table 3) presented a better diameter than Carrizo, we cannot deduce that the time needed for grafting was shortened.

In contrast to these results, in another study, rough lemon presented greater height and diameter in a substrate of sand and peat moss [35]. In another study, it was observed that rough lemon grown in

<sup>&</sup>lt;sup>1</sup> Final height and diameter minus initial height and diameter. <sup>2</sup> Means followed by the same letter within interaction set are not significantly different (P < 0.05) by Fisher's least significant difference. Means with absence of letter = F test not significant (P < 0.05).

 $<sup>^{1}</sup>$  Means followed with the same letter are not significantly different (P < 0.05) in accordance with the Fisher's least significant difference.

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a substrate composed of farmyard manure, soil, coco peat and compost (1:2:1) exhibited a higher mean diameter in comparison to other treatments [36]. The addition of compost to the growing media seems to positively impact the height and diameter of citrus varieties.

# 3.1.2. Chlorophyll

A significant difference (P < 0.05) was found for chlorophyll in the interactions of substrate with rootstock and of rootstock with quantity of fertilizer (Table 2). For rootstock and substrate, Carrizo showed the highest mean chlorophyll value, 69.1, in the media with 33% rice husk. This was significantly greater than Carrizo with coffee compost, Swingle with rice husk and Swingle with coco peat. Secondly, for the interaction of rootstock and fertilizer, the quantity of fertilizer did not have a significant effect on the chlorophyll content of Carrizo leaves. On the other hand, for Swingle, an application of 8.5 g of 18-6-12 per pot was the best treatment for this parameter.

It should be mentioned that, during the experiment, the rootstocks had an incidence of mites. These were concentrated on the newest leaves of the tallest plants, which coincided with the rootstocks on coco peat, control and coffee compost substrate treatments. When mites eat, they change the surface of the leaves, which might have a negative effect on the chlorophyll reading and it might be possible that the damage done to the leaves by the mites affected the level of chlorophyll of these two rootstocks on all of the substrate treatments mentioned.

## 3.2. Evaluation of Rootstocks Grafted with "Rhode Red Valencia"

## 3.2.1. Height

Growth of the scion varied between substrates. The substrate with 33% of rice husk resulted in the least favorable media for "Rhode Red Valencia" on both rootstocks (Table 2). Among the other substrate treatments, there was no significant difference (P < 0.05) on the growth of the scion. The only interaction that showed any significant difference or direct effect was the fertilizer by rootstock interaction. The lowest mean was observed with Swingle with 5.6 g of fertilizer (Table 4). On the other hand, for Carrizo, there was no significant difference between fertilizer treatments. Similar to this research, in a study, it was found that in the first year after budding, "Rhode Red Valencia" developed similarly when grafted on Carrizo and Swingle [37]. This data shows that the limited growth on the rootstocks before grafting did not continue limiting the growth of the scion when rice husk was used in the media.

**Table 4.** Interactions of rootstock (R) with fertilizer (F) and with substrate (S) on height, root and shoot dry weights, and chlorophyll of grafted trees.

Interactions		Height (cm)	Root Dry Weight (g)	Shoot Dry Weight (g)	Chlorophyll
R * F					
Carrizo	F1 <sup>1</sup>	24.1 a <sup>2</sup>	6.47	7.45	66.0 a
	F2	24.9 a	7.09	8.98	61.8 ab
Swingle	F1	14.7 b	6.47	7.53	57.8 b
	F2	23.2 a	6.94	8.73	63.6 a
R*S					
Swingle	Control	23	7.79 bc	8.36 bc	61.8 abcd
O	Coco peat	19.81	6.95 cd	9.11 b	58.7 cd
	Compost	20.19	7.31 c	8.46 bc	62.2 abcd
	Rice husk	12.69	5.49 d	6.58 c	60.2 bcd
Carrizo	Control	24.50	5.82 cd	6.29 c	66.6 ab
	Coco peat	25.25	6.56 bcd	8.4 bc	64.3 abc
	Compost	27.13	9.01 a	11.5 a	55.6 d
	Rice ĥusk	21.19	5.74 cd	6.63 c	69.1 a

 $<sup>^{1}</sup>$  F1 = 5.6 g; F2 = 8.5 g.  $^{2}$  Means followed by the same letter are not significantly different (P < 0.05) in accordance with the Fisher's least significant difference. Means with absence of letter or NS means that the F test was not significant (P < 0.05).

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## 3.2.2. Chlorophyll

Despite the pattern, on the other parameters, rootstocks grown in substrates with 33% rice husk exhibited high values of chlorophyll. In the case of Carrizo specifically, it was the treatment most favorable for leaf chlorophyll content (Table 4). The mite problem noted above may have impacted these results. This infestation occurred on all trees in all substrate treatments, except those with a third of rice husks. There was no incidence of mites from when the rootstock was grafted to the end of the experiment.

In terms of fertilizer, for each substrate, when comparing both quantities of fertilizers, a higher concentration of chlorophyll was present with a higher quantity (8.5 g) of fertilizer (Table 4).

## 3.2.3. pH and EC

In both pour-through results and the analysis of the Central Analytical Laboratory of the University of Puerto Rico (Table 5), the substrates demonstrated a higher (more basic) pH (Tables 5 and 6) than is preferred for optimal citrus growth. An ideal pH for a good citrus development should be between 5.5 and 6.0 [23,38].

An optimal electric conductivity of the substrates should range from 0.20 to 1.0 dS/m. The sensitivity of the plant to the electric conductivity of the substrates will depend on the crop [17]. The results from the Central Analytical Laboratory of the University of Puerto Rico (Table 5) showed that only the compost had an optimal EC. The EC results were 0.0494 dS/m for the control, 0.0711 dS/m for coco peat, 0.433 dS/m for compost, and 0.0666 dS/m for rice husk (Table 5). The EC results were mostly within the ideal range for the first 3 months of the study (Table 7). After 3 months, there was a second fertilizer application that increased the EC values above what is optimal for citrus. The difference in the values across months could have been affected by the water source for irrigation used in the greenhouse, which was river water (Table 5).

**Table 5.** Report of chemical properties of substrates of Central Analytical Laboratory at EAS, Río Piedras.

		Control	Coco Peat	Compost	Rice Husk	Water
pН		7.79	7.34	7.06	7.56	7.5
EC	dS/m	0.0494	0.0711	0.433	0.0666	0.12

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**Table 6.** pH measurements of all substrates and quantities of fertilizer treatments by month.

	April 2015	May 2015	June 2015	July 2015	August 2015	September 2015	October2015	November 2015	December 2015	January 2016	February 2016
CONTROL + F1	7.4	7.4	7.5	7.4	7.4	7.2	7.1	7.0	7.1	7.1	7.0
CONTROL + F2	7.4	7.3	7.2	7.4	7.1	7.0	7.0	6.9	6.9	6.8	7.0
COCO PEAT + F1	7.3	7.3	7.2	7.2	7.3	6.9	7.2	7.1	7.2	7.3	7.1
COCO PEAT + F2	7.3	7.0	7.0	7.2	6.8	6.6	6.7	6.3	6.4	6.5	6.8
COMPOST + F1	7.5	7.5	7.2	7.4	7.3	6.6	7.0	6.9	7.0	7.0	7.0
COMPOST + F2	7.6	7.5	7.2	7.2	7.3	6.7	7.2	7.1	7.1	7.2	7.1
RICE HUSK + F1	7.5	7.6	7.3	7.4	7.4	6.8	7.1	6.9	6.9	6.9	7.2
RICE HUSK + F2	7.5	7.4	7.1	7.1	7.2	7.0	6.7	6.8	6.7	6.5	6.9

 $\textbf{Table 7.} \ \ \textbf{Electric conductivity measurements (dS/m) of all substrates and quantities of fertilizer treatments by month.}$ 

	April 2015	May 2015	June 2015	July 2015	August 2015	September 2015	October 2015	November 2015	December 2015	January 2016	February 2016
CONTROL + F1	1.02	1.4	0.8	0.72	1.58	2.03	1.58	2.29	1.89	1.49	1.66
CONTROL + F2	1.06	1.52	1.67	1.45	2.93	2.98	2.3	2.38	2.81	3.24	1.33
COCO PEAT + F1	0.81	0.61	0.77	1.2	0.8	1.71	1.6	1.81	1.79	1.75	1.42
COCO PEAT + F2	1.13	1.41	0.99	1.4	2.5	2.81	2.2	4.75	3.61	2.48	1.91
COMPOST + F1	2.04	1.33	0.79	1.91	2.7	3.83	3.2	1.16	2.75	4.35	2.07
COMPOST + F2	1.34	2.35	0.98	2.66	2.92	3.27	1.78	3.13	2.56	2.0	1.55
RICE HUSK + F1	0.57	0.63	1.03	0.58	1.05	1.97	1.65	1.43	1.87	2.3	0.98
RICE HUSK + F2	0.93	0.9	2.87	1.3	1.45	2.45	1.96	2.1	2.33	2.55	1.37

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#### 3.2.4. Dry Weight of Shoots and Roots of Trees Grafted with "Rhode Red Valencia"

For both root and shoot dry weight, the quantity of fertilizer (as an individual variable) had no significant effect (P > 0.05) (Table 4), which indicates that we can obtain similar growth with either fertilizer treatment. This represents an economic savings in terms of fertilizer expenses and application if the lower rate is used. The only significant interaction was that of rootstock and substrate (Table 4). At the first phase of the experiment (Tables 2 and 3), the amount of fertilizer used influenced growth of the rootstock. However, as time passed, the early effects did not persist. In the end, there were similar results in the growth of citrus rootstocks in either of the fertilizer treatments for these substrates mixtures and proportions of its components. Observing the data by rootstock, for Carrizo, the best substrate for shoot and root mass was that with 1/3 coffee compost (Table 4). Carrizo also presented higher root and shoot dry weight than Swingle in the substrate with coffee compost (Table 4).

For Swingle, the lowest value of both shoot and root mass was found with 1/3 rice husk (Table 4). In another study, the effect of different proportions of perlite and rice husk partially boiled with peat moss and grown in different proportions (10%, 15%, 25%, 30% and 35%) on tomato plants resulted in higher root dry weight in comparison with the other treatments [39]. Parboiled fresh rice hull (PFH) is rice that has been partially boiled while still in the husk [39]. For this type of crop (tomato), incorporating rice husk can be positive for the development of the plant. For citrus, as observed in this study, incorporation of rice husk in the substrate had a detrimental effect on rootstock growth.

#### 4. Conclusions

Our results indicated that rice husk without any other treatment should not be incorporated in a substrate mix for the citrus rootstocks used in this experiment. For Carrizo, it is recommended to use coffee compost in the substrate, as it showed increased growth. For Swingle, any of the other substrates (compost, control and coco peat) can be used for production of this rootstock with good development of the plant. The results of different treatments of quantities of fertilizers showed similar results without showing a significant difference between them. This suggests that it is not necessary to apply a higher quantity of fertilizer to achieve a greater development of the rootstock. This experiment demonstrated that other media components can be used for the production of citrus liners without affecting tree growth. For further research, the amounts of compost and coco peat should be increased, as alternatives to the expensive commercial mix.

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