

# Phosphorus Uptake by Young Citrus Trees in Low-P Soil Depends on Rootstock Varieties and Nutrient Management

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*Because low-phosphorus (P) availability limits citrus growth, rootstocks with a relatively high capacity for P uptake are desirable. An experiment was conducted with trees on Cleopatra mandarin (CM) and Rangpur lime (RL). Treatments consisted of P rates (20, 40, and 80 mg kg<sup>-1</sup> of soil) applied in soil layers of 0–0.30 m and/or 0.31–0.60 m, besides an unfertilized control. The P fertilization increased root and shoot growth, and P nutrition was improved as indicated by greater leaf P concentration, P uptake, and P root uptake efficiency (PUE). The P applied in both soil layers improved shoot growth, P uptake, and PUE. Trees on RL took up 23–126% more P and had root systems with greater growth and PUE compared to those on CM. Thus, P uptake by citrus trees in low-P soils can be improved by augmenting the depth of fertilizer application and the use of more adapted rootstocks.*

**Keywords** Fertilizer distribution, nutrient availability, phosphorus nutrition, root growth

## Introduction

Low-phosphorus (P) availability is one of the major factors in tropical soils that limit the productivity of many crops (Sanchez and Salinas 1981). As a result, increases in fruit yield with P fertilization have been reported in citrus cultivated in these soils (Sobral et al. 2000; Quaggio, Cantarella, and van Raij 1998; Quaggio, Mattos, and Cantarella 2006). The use of genotypes with greater nutrient use efficiency has been suggested as a feasible approach to increase the production efficiency in low-fertility soils (Fageria, Wright, and Baligar 1988; Sinclair and Vadez 2002; Wang, Yan, and Liao 2010). In citrus trees, rootstocks can ameliorate many soil constraints, such as saline stress (Gimeno et al. 2010) and low-nutrient availability (i.e., iron; Pestana et al. 2005). There is also important variation in the ability of citrus rootstocks to acquire P from the soil (Wutscher 1989), which might explain

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differences in responses of citrus trees to P fertilization (Mattos et al. 2006). These authors observed that sweet orange trees on Rangpur lime had greater productivity at the lowest P rate and lower gain in fruit yield per unit of applied P than those on Cleopatra mandarin. Thus, fine tuning of P fertilization has been suggested according to the scion/rootstock combination, because those rootstocks correspond to approximately 75% of the nursery trees produced in Brazil (Pompeu 2005). The distinct nutrient requirement might have resulted from the physiological and the morphological plant roots characteristics that affect P uptake from the soil and its subsequent use by the plants (Hernans et al. 2006; Lambers et al. 2006). However, the specific mechanisms that explain the differential responses of cultivated citrus to P fertilization are not yet fully understood.

Because low-P availability limits root growth (Fujita et al. 2004), the pattern of P distribution in the soil is particularly important because of the high specific P adsorption in well-weathered soils (Fontes and Weed 1996) and depletion zone created at the root surface due to low P mobility in the soil (Barber, Walker, and Vasey 1963; Darrah 1993), which restricts P uptake by the plants. In this context, the effects of P rates and placement might be more pronounced in young trees compared to the mature ones, because the former has relatively greater nutrient demand and lower P absorption capacity due to their limited root system (Quaggio et al. 2004). A practical approach to improve P availability would be to augment the depth of P fertilizer application in the field, which commonly is not deeper than 0.25–0.30 m. However, studies still need to evaluate the efficiency of adopting this strategy for young citrus trees.

Based on the previous discussion, the present experiment was designed to investigate the effects of the rates and placement of P in the soil as well as the influence of two contrasting rootstock varieties on the growth and P nutrition of young citrus trees grown in a typical low-P soil.

## Material and Methods

The soil used in the experiment was an Oxisol collected from the surface layer (0–0.25 m) of a pasture land. The soil was dried, sieved, and analyzed for physical (clay, 617 g kg<sup>-1</sup>; sand, 333 g kg<sup>-1</sup>) and chemical [pH in calcium chloride (CaCl<sub>2</sub>) 0.01 mol L<sup>-1</sup>, 4.3; P resin, 3.0 mg dm<sup>-3</sup>; potassium (K), 1.0; calcium (Ca), 7.0; and magnesium (Mg), 3.0 mmol<sub>c</sub> dm<sup>-3</sup>] characterizations (Camargo et al. 1986; Raij et al 2001). Dolomite was applied to increase the soil base saturation to 70% and the soil was kept incubated for 60 days. At the end of this period, the soil pH and base saturation reached 5.9 and 65%, respectively.

The P treatments consisted of the combination of P rates and soil layers receiving P fertilization (0–0.30 m and/or 0.31–0.60 m). A standard P rate (mg kg<sup>-1</sup> of soil) was established to increase the soil P resin to 20 mg dm<sup>-3</sup>, which corresponds to the critical level (CL) for maximum fruit yield of citrus trees (Quaggio, Cantarella, and van Raij 1998). This level was attained by applying P at 40 mg kg<sup>-1</sup> of soil, which was based on a relationship previously obtained from soil samples mixed with increasing rates of ammonium dihydrogen phosphate (ADP) after 90 days of incubation. Thus, the P rates consisted of (i) half of the required amount of P to achieve the CL (application of 20 mg P kg<sup>-1</sup> of soil = P<sub>0.5</sub>); (ii) required amount of P to achieve the CL (application of 40 mg P kg<sup>-1</sup> of soil = P<sub>1</sub>); (iii) twofold the required amount of P to achieve the CL (application of 80 mg P kg<sup>-1</sup> of soil = P<sub>2</sub>); and (iv) an unfertilized control (P<sub>0</sub>). Based on these rates, corresponding amounts of ADP were mixed with soil portions that were subsequently transferred into 250-L plastic containers and arranged in layers of 0–0.30 m and/or 0.31–0.60 m deep, each containing 100 kg of soil (total of 200 kg of soil per plastic container). The combination of P rates

and the soil layers receiving P fertilizer resulted in five P treatments, as follows:  $P_0/P_0$ , no P application at either 0–0.30 m or 0.31–0.60 m deep;  $P_1/P_0$ , application of 40 mg P  $\text{kg}^{-1}$  of soil at 0–0.30 m deep and no P at 0.31–0.60 m deep;  $P_{0.5}/P_{0.5}$ , application of 20 mg P  $\text{kg}^{-1}$  of soil at both 0–0.30 m and 0.31–0.60 m deep;  $P_2/P_0$ , application of 80 mg P  $\text{kg}^{-1}$  of soil at 0–0.30 m deep and no P at 0.31–0.60 m deep; and  $P_1/P_1$ , application of 40 mg P  $\text{kg}^{-1}$  of soil at both 0–0.30 m and 0.31–0.60 m deep.

The five P treatments were combined with Pêra sweet orange trees [*Citrus sinensis* (L.) Osbeck] on Cleopatra mandarin (*Citrus reshni* hort. ex Tanaka, CM) or Rangpur lime (*Citrus limonia* Osbeck, RL) rootstocks in a complete factorial design with three replicates. Each experimental plot consisted of one container with a single citrus tree. They were planted in a hole 0.25 m in diameter and 0.25–0.30 m deep in the center of the container immediately after soil deposition. Three representative nursery trees of each scion/rootstock combination were sampled to determine the growth at the beginning of the experiment. Trees on CM and RL had  $35.2 \pm 2.2$  g and  $30.1 \pm 4.2$  g, respectively. The experiment was conducted in a greenhouse under a natural photoperiod, with average day and night temperatures in the ranges of 31–36 °C and 14–21 °C, respectively.

Soil moisture was monitored using tensiometers installed at 0.20 m and 0.40 m deep in three replicate containers. Irrigation was applied at the soil surface when the water potential decreased to –30 kPa (Pires et al. 2005), using a volume of deionized water needed to reach field capacity throughout the profile. The total amounts of N and  $\text{K}_2\text{O}$  supplied to the trees during the experimental period corresponded to 90 and 30 g  $\text{tree}^{-1}$ , respectively. These nutrients were applied proportionally every 10–15 days via fertigation using potassium sulfate, calcium, and magnesium nitrate. Sufficient micronutrients were either foliar sprayed (Quaggio, Mattos, and Cantarella 2005) or fertigated (Furlani et al. 1999).

Fully expanded mature leaves (six to eight per tree) were sampled 150, 240, and 330 days after transplanting the trees into the containers. The foliar tissue was rinsed with deionized water, oven dried at 60 °C for 72 h, and subsequently ground and digested in nitric–perchloric acid for the colorimetric determination of P (Bataglia et al. 1983).

Tree growth was evaluated approximately 12 months after transplanting by harvesting shoots, which were separated into leaves, twigs, and trunk. In each container, the soil of 0- to 0.30-m and 0.31- to 0.60-m layers was separately collected, sieved to obtain the roots, and sampled to analyze extracted available P (P resin) (Rajj et al. 2001). Tree parts were washed and rinsed with deionized water, oven dried at 60 °C for 72 h, and weighed for dry weight (DW) determinations [leaves DW, LDW; total shoot DW (LDW + trunk and twigs DW), TSDW; roots DW, RDW<sub>0–0.30</sub> and RDW<sub>0.31–0.60</sub> for 0- to 0.30-m and 0.31- to 0.60-m layers, respectively; total root DW (RDW<sub>0–0.30</sub> + RDW<sub>0.31–0.60</sub>), TRDW; and tree DW (TSDW + TRDW), TDW]. These parts were subsequently ground and stored in glass vials until P was analyzed. All leaves that dropped off from the trees during the experimental period were collected, dried, and weighed.

The P uptake by the trees during the experimental period ( $\text{mg tree}^{-1}$ ) was estimated by subtracting total P amounts measured in nursery trees (the mean of three plants for each scion/rootstock combination) just before transplantation from the total amount of P accumulated by the trees at the end of the experiment. The P root uptake efficiency (PUE,  $\text{mg g}^{-1}$ ) was calculated as total P accumulated in the tree ( $\text{mg tree}^{-1}$ ) / (total root DW, g  $\text{tree}^{-1}$ ) (Swiader, Chyan, and Freiji 1994).

Data were analyzed using a two-way variance analysis with five P treatments  $\times$  two scion/rootstock combinations. When interaction terms were significant, the effects of P treatments within rootstocks and the effects of rootstocks within P treatments were

compared using orthogonal contrasts and F test at  $P < 0.1$ , respectively. When the interaction term was not significant and main factors (scion/rootstock combinations and/or P treatments) were significant, orthogonal contrasts and F test at  $P < 0.1$  were run to separate means of the main factors. The following orthogonal contrasts (C) were established to evaluate the effects of P treatments: C<sub>1</sub>, no P application (P<sub>0</sub>/P<sub>0</sub>) vs. P<sub>1</sub>/P<sub>0</sub> and P<sub>0.5</sub>/P<sub>0.5</sub> combined; C<sub>2</sub>, no P application (P<sub>0</sub>/P<sub>0</sub>) vs. P<sub>2</sub>/P<sub>0</sub> and P<sub>1</sub>/P<sub>1</sub> combined; C<sub>3</sub>, P<sub>1</sub>/P<sub>0</sub> and P<sub>0.5</sub>/P<sub>0.5</sub> combined vs. P<sub>2</sub>/P<sub>0</sub> and P<sub>1</sub>/P<sub>1</sub> combined; and C<sub>4</sub> = P<sub>1</sub>/P<sub>0</sub> and P<sub>2</sub>/P<sub>0</sub> combined vs. P<sub>0.5</sub>/P<sub>0.5</sub> and P<sub>1</sub>/P<sub>1</sub> combined. The C<sub>1</sub>, C<sub>2</sub>, and C<sub>3</sub> were used to investigate the influence of P rates, whereas C<sub>4</sub> corresponded to the evaluation of the effect P fertilizer placement. Linear correlation was also used to describe the relationships between the selected variables.

## Results

At the end of the experiment, extracted available P (P resin) in both soil layers was not affected by scion/rootstock combinations ( $P > 0.1$ ), but varied with P treatments ( $P < 0.1$ ). In the 0- to 0.30-m layer, P resin ranged from  $2 \pm 0.2$  to  $31 \pm 0.8$  mg dm<sup>-3</sup> for P<sub>0</sub>/P<sub>0</sub> and P<sub>2</sub>/P<sub>0</sub> treatments, respectively. For the second layer (0.31–0.60 m), P resin ranged from  $2 \pm 0.2$  to  $19 \pm 0.9$  mg dm<sup>-3</sup> for P<sub>0</sub>/P<sub>0</sub> and P<sub>1</sub>/P<sub>1</sub> treatments, respectively. Tree growth and P resin were significantly and positively correlated ( $0.65 < r < 0.68$ ;  $P < 0.01$ ;  $n = 15$ ), except for trees on CM and the P resin at 0–0.30 m deep.

There was no significant interaction ( $P > 0.1$ ) between P treatments and scion/rootstock combinations for tree growth and leaf P concentration, suggesting that trees on both rootstocks responded in a similar way to the P rates and distribution in the soil (Tables 1 and 2). The P fertilization improved leaf and shoot growth by 15–30% and 11–22%, respectively, compared to the control (P<sub>0</sub>/P<sub>0</sub>) (Table 1). Furthermore, P fertilizer distribution in both of the soil layers instead of the shallow layer only (P<sub>1</sub>/P<sub>0</sub> + P<sub>2</sub>/P<sub>0</sub> vs. P<sub>0.5</sub>/P<sub>0.5</sub> + P<sub>1</sub>/P<sub>1</sub>) favored LDW and TSDW but did not affect root growth (Table 1). RDW in the first layer (RDW<sub>0–0.30</sub>) and second layer (RDW<sub>0.31–0.60</sub>) and TRDW were enhanced by 12%, 20–33% and 16–21%, respectively, with P application in comparison to the control (Table 1). As a result of improved TRDW and TSDW, tree growth at the end of the experiment was increased with P fertilization, but there was no difference between P placement treatments (P<sub>1</sub>/P<sub>0</sub> + P<sub>2</sub>/P<sub>0</sub> vs. P<sub>0.5</sub>/P<sub>0.5</sub> + P<sub>1</sub>/P<sub>1</sub>). Furthermore, across averaged P treatments, trees on RL had 6% and 11% greater TSDW and TRDW, respectively, than those on CM (Table 1).

Leaf P concentration was improved by P fertilization up to 240 days after transplanting, but these differences disappeared by 330 days after transplanting (Table 2). In fact, all treatments had very low leaf P concentrations by the last sampling period, which agreed with the visible symptoms of P deficiency. The symptoms were characterized by a decrease in leaf brightness associated with the development of a brownish-green color and subsequent necrosis of the leaf tissue from the margins to the center, resulting in premature leaf drop from the base to the apex of the twigs. Differences between scion/rootstock combinations for leaf P concentration occurred only at 150 days after transplanting, when trees on RL had a value 13% greater than CM trees (Table 2).

There was significant interaction ( $P < 0.1$ ) between P treatments and scion/rootstock combinations for P uptake but not for PUE (Table 3). The total P uptake by the trees at the end of the experiment was proportional to the increase in P rates; however, trees on RL absorbed more P than those on CM within the same P treatment (Table 3). For instance, P uptake by trees on RL was more than 2.2-fold greater than trees on CM with

**Table 1**

Growth of young citrus trees on two rootstocks in response to phosphorus (P) rates and distribution depths into the soil after 1 year of treatments

Parameter	LDW (g tree <sup>-1</sup> )	TSDW (g tree <sup>-1</sup> )	RDW <sub>0–0.30</sub> (g tree <sup>-1</sup> )	RDW <sub>0.31–0.60</sub> (g tree <sup>-1</sup> )	TRDW (g tree <sup>-1</sup> )	TDW (g tree <sup>-1</sup> )
P treatments <sup>a</sup>	Scion/rootstock average					
P <sub>0</sub> /P <sub>0</sub>	73.0	165.3	57.3	46.8	104.1	269.4
P <sub>1</sub> /P <sub>0</sub>	79.4	178.8	63.1	55.5	118.6	297.4
P <sub>0.5</sub> /P <sub>0.5</sub>	88.1	188.7	65.2	56.5	121.7	310.4
P <sub>2</sub> /P <sub>0</sub>	92.1	196.1	62.6	63.2	125.8	321.9
P <sub>1</sub> /P <sub>1</sub>	98.0	211.4	65.4	61.0	126.4	337.8
Contrasts						
P <sub>0</sub> /P <sub>0</sub> vs. P <sub>1</sub> /P <sub>0</sub> + P <sub>0.5</sub> /P <sub>0.5</sub>	*	*	*	*	*	*
P <sub>0</sub> /P <sub>0</sub> vs. P <sub>2</sub> /P <sub>0</sub> + P <sub>1</sub> /P <sub>1</sub>	*	*	*	*	*	*
P <sub>1</sub> /P <sub>0</sub> + P <sub>0.5</sub> /P <sub>0.5</sub> vs. P <sub>2</sub> /P <sub>0</sub> + P <sub>1</sub> /P <sub>1</sub>	*	*	ns	ns	ns	*
P <sub>1</sub> /P <sub>0</sub> + P <sub>2</sub> /P <sub>0</sub> vs. P <sub>0.5</sub> /P <sub>0.5</sub> + P <sub>1</sub> /P <sub>1</sub>	*	*	ns	ns	ns	ns
Scion/rootstock	P treatments average					
Pêra on Cleopatra mandarin	82.6 b	182.8 b	60.2 a	53.1 b	113.3 b	296.1 b
Pêra on Rangpur lime	89.6 a	193.4 a	64.2 a	61.7 a	125.9 a	319.3 a

<sup>a</sup>The first “P” in the P treatments indicates the 0- to 0.30-m-deep soil layer and the second one indicates the 0.31- to 0.60-m-deep layer. P<sub>0</sub>, control treatment with no P application; P<sub>0.5</sub>, half of the required amount of P to achieve the critical level (CL) of P in the soil (soil resin extracted P to 20 mg dm<sup>-3</sup>); P<sub>1</sub>, required amount of P to achieve the CL; P<sub>2</sub>, twofold the required amount of P to achieve the CL.

*Notes.* Legend: LDW, leaf dry weight; TSDW, total shoot dry weight; RDW<sub>0–0.30</sub> and RDW<sub>0.31–0.60</sub>, roots dry weight at 0–0.30 m and 0.31–0.60 deep, respectively; TRDW, total root dry weight; and TDW, tree dry weight. Scion/rootstock combination comparison: means followed by different letters within columns are significantly different by the F test ( $P < 0.1$ ). P treatment comparison: orthogonal contrasts were used: ns, nonsignificant ( $P > 0.1$ ) and \* $P < 0.1$ .

**Table 2**

Leaf phosphorus (P) concentration of young citrus trees on two rootstocks in response to P rates and distribution depths into the soil at different sampling times

Parameter	Days after transplanting		
	150	240	330
Leaf P (g kg <sup>-1</sup> ) Scion/rootstock average			
P treatments <sup>a</sup>			
P <sub>0</sub> /P <sub>0</sub>	0.82	0.54	0.55
P <sub>1</sub> /P <sub>0</sub>	1.01	0.59	0.55
P <sub>0.5</sub> /P <sub>0.5</sub>	0.87	0.54	0.57
P <sub>2</sub> /P <sub>0</sub>	0.92	0.58	0.61
P <sub>1</sub> /P <sub>1</sub>	1.02	0.61	0.60
Contrasts			
P <sub>0</sub> /P <sub>0</sub> vs. P <sub>1</sub> /P <sub>0</sub> + P <sub>0.5</sub> /P <sub>0.5</sub>	*	ns	ns
P <sub>0</sub> /P <sub>0</sub> vs. P <sub>2</sub> /P <sub>0</sub> + P <sub>1</sub> /P <sub>1</sub>	*	*	ns
P <sub>1</sub> /P <sub>0</sub> + P <sub>0.5</sub> /P <sub>0.5</sub> vs. P <sub>2</sub> /P <sub>0</sub> + P <sub>1</sub> /P <sub>1</sub>	ns	*	ns
P <sub>1</sub> /P <sub>0</sub> + P <sub>2</sub> /P <sub>0</sub> vs. P <sub>0.5</sub> /P <sub>0.5</sub> + P <sub>1</sub> /P <sub>1</sub>	ns	ns	ns
Scion/rootstock	P treatments average		
Pêra/Cleopatra mandarin	0.88 b	0.59 a	0.58 a
Pêra/Rangpur lime	0.99 a	0.60 a	0.59 a

<sup>a</sup>The first "P" in the P treatments indicates the 0- to 0.30-m-deep soil layer and the second one indicates the 0.31- to 0.60-m-deep layer. P<sub>0</sub>, control treatment with no P application; P<sub>0.5</sub>, half of the required amount of P to achieve the critical level (CL) of P in the soil (soil resin extracted P to 20 mg dm<sup>-3</sup>); P<sub>1</sub>, required amount of P to achieve the CL; P<sub>2</sub>, twofold the required amount of P to achieve the CL.

*Notes.* Scion/rootstock combinations comparison: means followed by different letters within columns are significantly different by the F test ( $P < 0.1$ ). P treatment comparison: orthogonal contrasts were used: ns, nonsignificant ( $P > 0.1$ ) and  $*P < 0.1$ .

no P application (P<sub>0</sub>/P<sub>0</sub>) and 1.2-fold greater in the P<sub>1</sub>/P<sub>1</sub> treatment. Furthermore, the difference between P<sub>0</sub>/P<sub>0</sub> vs. P<sub>2</sub>/P<sub>0</sub> and P<sub>1</sub>/P<sub>1</sub> combined treatments in P uptake was 31% for trees on RL and 120% for those on CM. There was no effect of P fertilizer distribution in the soil (P<sub>1</sub>/P<sub>0</sub> + P<sub>2</sub>/P<sub>0</sub> vs. P<sub>0.5</sub>/P<sub>0.5</sub> + P<sub>1</sub>/P<sub>1</sub>) on P uptake by CM, but P uptake was improved by 16% in the treatments with P fertilizer distributed in both soil layers for trees on RL (Table 3). Moreover, PUE was enhanced with greater P fertilization (P<sub>2</sub>/P<sub>0</sub> and P<sub>1</sub>/P<sub>1</sub> treatments) and maximized when both soil layers received P fertilization (P<sub>1</sub>/P<sub>0</sub> + P<sub>2</sub>/P<sub>0</sub> vs. P<sub>0.5</sub>/P<sub>0.5</sub> + P<sub>1</sub>/P<sub>1</sub>). On the average of P treatments, trees on RL had a 40% greater PUE than those on CM (Table 3).

## Discussion

The P fertilizer application improved P nutrition of young citrus trees as indicated by greater leaf P concentration and P uptake in fertilized trees on both rootstocks compared to the unfertilized ones (Tables 2 and 3). This positive effect of P application on P nutrition resulted in enhanced tree growth as supported by the correlation of TDW with leaf P concentration in the first sampling period ( $r = 0.45$ ;  $P = 0.04$ ;  $n = 30$ ) and with total P

**Table 3**  
Phosphorus (P) uptake and P root uptake efficiency (PUE) of young citrus

Parameter	P uptake (mg tree <sup>-1</sup> )		PUE (mg g <sup>-1</sup> )
	Pêra/Cleopatra mandarin	Pêra/Rangpur lime	Scion/rootstock average
<b>P treatments<sup>a</sup></b>			
P <sub>0</sub> /P <sub>0</sub>	41.8 B	94.6 A	0.61
P <sub>1</sub> /P <sub>0</sub>	70.4 A	85.1 A	0.64
P <sub>0.5</sub> /P <sub>0.5</sub>	68.1 B	112.4 A	0.77
P <sub>2</sub> /P <sub>0</sub>	80.5 B	121.4 A	0.82
P <sub>1</sub> /P <sub>1</sub>	103.3 B	126.8 A	0.94
<b>Contrasts</b>			
P <sub>0</sub> /P <sub>0</sub> vs. P <sub>1</sub> /P <sub>0</sub> + P <sub>0.5</sub> /P <sub>0.5</sub>	*	*	ns
P <sub>0</sub> /P <sub>0</sub> vs. P <sub>2</sub> /P <sub>0</sub> + P <sub>1</sub> /P <sub>1</sub>	*	*	*
P <sub>1</sub> /P <sub>0</sub> + P <sub>0.5</sub> /P <sub>0.5</sub> vs. P <sub>2</sub> /P <sub>0</sub> + P <sub>1</sub> /P <sub>1</sub>	*	*	*
P <sub>1</sub> /P <sub>0</sub> + P <sub>2</sub> /P <sub>0</sub> vs. P <sub>0.5</sub> /P <sub>0.5</sub> + P <sub>1</sub> /P <sub>1</sub>	ns	*	*
<b>Scion/rootstock</b>			<b>P treatments average</b>
Pêra/Cleopatra mandarin	—	—	0.65 b
Pêra/Rangpur lime	—	—	0.91 a

*Notes.* Scion/rootstock combinations comparison: means followed by different uppercase letters across paired columns (comparison within each P treatment) or lowercase letter within column (comparison across P treatments average) are significantly different by the F test ( $P < 0.1$ ). P treatments comparison: orthogonal contrasts were used: ns, nonsignificant ( $P > 0.1$ ) and \* $P < 0.1$ .

<sup>a</sup>The first “P” in the P treatments indicates the 0- to 0.30-m-deep soil layer and the second one indicates the 0.31- to 0.60-m-deep layer. P<sub>0</sub>, control treatment with no P application; P<sub>0.5</sub>, half of the required amount of P to achieve the critical level (CL) of P in the soil (soil resin extracted P to 20 mg dm<sup>-3</sup>); P<sub>1</sub>, required amount of P to achieve the CL; P<sub>2</sub>, twofold the required amount of P to achieve the CL.

accumulated by the trees at the end of the experiment ( $r = 0.84$ ;  $P < 0.0001$ ;  $n = 30$ ). It has been demonstrated that reduced leaf area caused by P deficiency results in a lower capacity to supply carbohydrates to the plant and thereby causes growth inhibition (Fredeen, Rao, and Terry 1989; Lynch, Läuchli, and Epstein 1991; Mollier and Pellerin 1999). In the present study, the positive effect of P fertilization was confirmed with increased leaf growth (Table 1), which was positively correlated to TRDW ( $r = 0.65$ ;  $P = 0.0002$ ;  $n = 30$ ) and TDW ( $r = 0.88$ ;  $P < 0.0001$ ;  $n = 30$ ), indicating that under limited P availability, reduced leaf area is an significant factor in defining young citrus tree performance. This pronounced influence of leaf growth on the total DW produced by the trees might support the strategy of more homogenous distribution of P fertilizer in the soil at tree planting, because this approach improved leaf growth (Table 1).

The increased root growth in both soil layers as a result of P fertilization (Table 1) indicated that low-P availability might restrict soil exploration by citrus roots. However,



RDW<sub>0.31–0.60</sub> 1 year after P fertilization did not depend on fertilizer application in the 0.31- to 0.60-m layer, because there was no difference between P fertilizer placement treatments on root growth ( $P_1/P_0 + P_2/P_0$  vs.  $P_{0.5}/P_{0.5} + P_1/P_1$ , Table 1). The absence of the influence of P treatments on the RDW<sub>0–0.30</sub>/RDW<sub>0.31–0.60</sub> ratio (data not shown) also suggests that P fertilizer placement did not modify root DW partitioning between the soil layers 1 year after transplanting. However, a shorter-term experiment has demonstrated that a P-rich soil patch corresponded to greater proportion of total lateral root length of *Arabidopsis* plants compared to the control with homogenous nutrient distribution (Linkohr et al. 2002). In fact, to increase the adaptation to a low-P soil and to meet increasing shoot demand, citrus trees maximized soil exploration over time as indicated by the positive correlation between TRDW and total P accumulation in the trees ( $r = 0.72$ ;  $P < 0.0001$ ;  $n = 30$ ). It is possible that improved P nutritional status with P fertilization at early stages of tree growth (principally up to 150 days after transplanting, Table 2) contributed to greater leaf photosynthesis and total leaf area (Rao and Terry 1995; Zambrosi, Mattos, and Syvertsen 2011) and further greater amount of carbohydrates to be used for root construction at the 0.31- to 0.60-m layer. In addition, P must be provided in sufficient concentrations to the roots to sustain meristematic and physiological activities (Doerner 2008). In contrast, sink activity of the roots is impaired (Wissuwa, Gamat, and Ismail 2005), leading to lower sucrose export from the leaves (Pieters, Paul, and Lawlor 2001) and reduced total root growth (Zambrosi et al. 2011). Thus, P absorbed by the roots present in the P fertilized 0- to 0.30-m layer was probably transported to growing roots in the deeper soil layer, because P is a mobile nutrient in the phloem of young citrus trees (Zambrosi et al. 2012).

The results of improved leaf and shoot growth with more homogenous distribution of P fertilizer in the soil support the practice of enhancing the depth of P fertilizer application as a strategy to enable young citrus trees to overcome low-P availability. Moreover, total P uptake by trees on RL was positively influenced with the adoption of this strategy of P application (Table 3). This enhanced P uptake resulted because P moves in the soil by diffusion and roots create depletion zones of P uptake (Clarkson 1985; Bates and Lynch 2000), impairing P acquisition by the trees. Thus, a larger volume of roots in contact with the soil having greater content of available P minimized the negative effects of created depletion zones at root surface and favored P uptake by the roots, allowing greater accumulation of P in the trees. This also allowed more efficient root functioning in P acquisition, as indicated by the higher PUE (Table 3), suggesting that when the P fertilizer was more homogeneously distributed in the soil profile, more units of P were acquired for each unit of carbon invested in root system construction of young citrus trees. Thus, the gain in PUE with homogenous P fertilization in the soil might have also contributed to a more vigorous tree growth, as supported by the correlation between PUE and TDW ( $r = 0.68$ ;  $P < 0.0001$ ;  $n = 30$ ).

The effect of the scion/rootstock combination on the leaf P concentration observed at the first sampling date, 150 days after transplanting (Table 2), in association with the greater P uptake by trees on RL (Table 3) confirmed the effects of rootstock varieties on the fruit trees' mineral nutrition status (Kennedy, Rowe, and Samuelson 1980). The greater TRDW and PUE presented by trees on RL contributed to more vigorous growth and P uptake. Root growth and PUE are related and may contribute to define the genotypic differences in P uptake (Nielsen, Eshel, and Lynch 2001). According to Wissuwa (2003) more efficient genotypes for external P uptake are likely to exhibit greater relative root growth because additional P taken up by the plants allows further biomass accumulation, including the root system. The efficiency of P utilization (dry weight produced per unit of P in the tree) was not affected by the scion/rootstock combinations (data not shown), suggesting that the differences in tree growth were mostly influenced by the capacity



of P acquisition by the rootstocks. This confirms the importance of using more suitable rootstocks to improve P-use efficiency in citrus groves.

## Conclusions

The sweet orange trees on Rangpur lime had a root system with greater growth and P uptake efficiency than those on Cleopatra mandarin. The efficiency of the root system of young citrus in acquiring P from the soil was improved with homogenous distribution of P fertilizer in the soil. Thus, this approach associated with rootstocks having root system more vigorous and more efficient in P acquisition might contribute to improve P nutrition of young citrus trees established in low-P soils.

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