Root development of young citrus trees in soil fertilized with phosphorus

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Abstract
Increased plant growth and fruit production of citrus trees occur in soils with high fertility. Phosphorus (P) is a key element for nutrient management, for which adequate supply and soil distribution is required in planting groves. The response of young plants of Pêra sweet orange on two contrasting rootstocks to P fertilization was studied in an experiment conducted in mini-rhizotrons under a screen house. Results demonstrated greater growth of plants on Rangpur lime compared to those on Cleopatra mandarin which corresponded to greater root development as evaluated by root growth rate and architecture. These parameters varied according to P availability in soil.

Key Words
Citrus, rootstock, growth rate, soil fertility, nutrient availability.

Introduction
The present study is part of a major ongoing project to evaluate phosphorus (P) absorption, use efficiency and distribution of P-forms in citrus trees. Earlier research work has demonstrated that limited P availability of low fertility tropical soils, predominant in Brazil, impairs citrus production (Quaggio *et al.* 1998, 2002). Furthermore, growth and fruit yield of citrus trees in response to P fertilization greatly vary according to scion/rootstock combinations (Quaggio *et al.* 2004), what is marked for sweet oranges on Cleopatra mandarin. Trees on Cleopatra appear to be less efficient for P absorption and therefore require additional nutrient supply compared to those either on Rangpur lime or Swingle citrumelo rootstocks (Mattos *et al.* 2006, 2009). This distinct requirement results from physiological and morphological plant characteristics that affect P acquisition from soil and use by plant (Ragothama 1999). However, specific mechanisms that explain such responses for cultivated citrus are not fully understood yet. Additionally, adequate P rates and fertilizer placement in the soil profile are particularly important before tree planting in the field, mostly because of specific adsorption of P-$H_2PO_4^-$ by Fe and Al-oxides what reduces nutrient availability to plants. Based on the above, the objectives of this study were to evaluate the response of Pêra sweet orange grafted on two contrasting rootstocks to P distribution in two layers of the soil profile of mini-rhizotrons by measuring root growth rate, dry mass production and P absorption by plants.

Methods
Young plants of Pêra sweet orange [*Citrus sinensis* (L.) Osb.] grafted on Rangpur lime (*C. limonia* Osb.) or Cleopatra mandarin (*C. reshni* hort. ex Tanaka) were grown in mini-rhizotrons containing 25 kg of soil and maintained under screen house. The rhizotrons in this study were constructed with 1.0 m height and 0.25 m diameter polyvinyl chloride (PVC) half tubes to form a semicircle along its axis. A transparent glass plate (4.0 mm thick) was installed to the flat side of the rhizotron to allow roots to be observed. The mini-rhizotrons were positioned at 80º from the ground level to force roots to grow toward the transparent plate. This plate was covered with a flexible black Plexiglas one, which was only removed for the observation of root development during the course of the study.The experiment was arranged on a 2 x 5 factorial design with treatments defined by the combination of rootstocks and soil P distribution replicated three times. A sandy clay loam Oxisol (333 g/kg clay and 617 g/kg sand) with pH (0.1 mol/L $CaCl_2$) = 5.6 and CEC = 76.5 $mmol_c/dm^3$ collected from the surface layer of a degraded pasture land was used. Portions of the soil were moistened and incubated in plastic bags for 30-d after application of P, K, and micronutrients. Phosphorus was applied as $NH_4H_2PO_4$ (MAP) at three different rates and N supplied was balanced with application of $CaCl_2$ as required. After incubation, soil P extracted by anionic resin levels (Raij *et al.* 1986), in mg/dm^3, were: P_0 = 4 (no P applied), $P_{0.5}$ = 12, P_1 = 19 and P_2 = 47. Two 45 cm soil layers were disposed in the mini-rhizotrons in order to simulate P distribution in the soil profile as follow: P_0/P_0, P_1/P_0, $P_{0.5}/P_{0.5}$, P_2/P_0, and

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P_1/P_1 before plant transplant. The mini-rhizotrons were irrigated with application of water to soil surface and subsurface; in this later case, a perforated PVC tube was installed at 45 cm soil depth was used. Water amounts were applied to replenish evapotranspiration losses.

After 15 days of plant transplant in the mini-rhizotrons, up to 15 root tips visualized on the transparent plate were marked for measurement of root length after 7-day intervals until 84 days. Total measured root length was determined for each treatment and daily root growth rate was calculated. Number of root tips visualized from the 21st to the 84th day after plant transplant was counted and number of fully expanded leaves was evaluated at the 84th day. Plants were destructively harvest 134 days after transplant in the mini-rhizotrons and separated into leaf and stem. Roots were separated from each soil layer soil using a sieve. Plant material was washed in di-water and dried at 60±2 ºC for 48 h for dry weight determinations. A simple analysis of variance (ANOVA) was used to test the hypothesis that means for root growth rate, number of root tips and leaves per plant were equal (*Prob.* = 0.05) using the GLM procedure of the SAS® system (SAS Institute 1996). The study is still in progress for evaluation of P content and uptake efficiency by plants after destructive harvest.

Results

Average root growth rate of Pêra sweet orange on Rangpur lime rootstock was 0.34 cm/day, whereas the same for plants on Cleopatra mandarin rootstock was only 0.24 cm/day (Table 1). Similarly, plants on Rangpur lime showed greater number of root tips and leaves per plant (68 and 22, respectively). These differential responses correlate with observed tree development in the field (Pompeu Jr. 2005).
The significant interaction (*Prob*. < 0.01) between rootstocks and soil P suggested that plants on Cleopatra mandarin lime are responsive to increased P availability in the soil according to increased root growth rate and number of root tips observed in the mini-rhizotrons, even though the same appeared to be less efficient for P acquisition compared to those on Rangpur lime rootstock (Table 1). These results are in line with study conducted in the field for nonbearing Valencia and Natal sweet oranges trees (Mattos Jr. *et al.* 2006).
Plant development was greater with the P_2/P_0 treatment as measured by leaf number per plant (Table 1), what suggested that increased P-fertilizer application to surface soil may overcome difficulties of P incorporation at deeper soil layers in the field similarly to the P_1/P_1 treatment. However, this later need to be validated during long term field experiments.

Table 1. Growth characteristics of young plants of Pêra sweet orange on different rootstocks as affected by soil resin-P availability in mini-rhizotrons.

Rootstock combination	Soil P treatment(1)	Root growth rate(2)	Root tips(3)	Leaves(4)
		cm/day	# per plant	# per plant
Pêra/	P_0/P_0	0.42	94	20
Rangpur lime	P_1/P_0	0.31	46	20
	$P_{0.5}/P_{0.5}$	0.27	52	22
	P_2/P_0	0.42	94	26
	P_1/P_1	0.26	55	21
Mean values		0.34	68	22
Pêra/	P_0/P_0	0.14	27	12
Cleopatra mandarin	P_1/P_0	0.22	29	12
	$P_{0.5}/P_{0.5}$	0.21	28	13
	P_2/P_0	0.30	38	14
	P_1/P_1	0.24	33	14
Mean values		0.22	31	13
Prob. < F				
Rootstock (RS)		17.76**	19.90**	51.02**
Soil P (SP)		0.28^{ns}	0.03^{ns}	2.21^{ns}
RS*SP		6.87**	7.88**	0.01^{ns}

(1) resin-P in each of 45 cm soil layer, in mg/dm^3, $P_0 = 4$, $P_{0.5} = 12$, $P_1 = 19$ and $P_2 = 47$.
(2) mean values for data evaluated at 7-day intervals from 14 to 84 days after transplant in mini-rhizotrons.
(3) mean values for data evaluated at 7-day intervals from 21 to 84 days after transplant in mini-rhizotrons.
(4) number of leaves per plant after 84 days of transplant.

The response of plants on Rangpur lime at the P_0/P_0 treatment suggested that roots of this rootstock variety present greater plasticity at limited P availability in the soil by changing its architecture (Robinson 1994;

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Lynch and Brown 1997). Physiological characteristics may also be involved, however further studies are necessary to better clarify this question. Root growth was greater for plants on the Rangpur lime rootstock (Table 2), which was proportional to the leaf and stem dry matter production. Maximum growth was observed with the P_2/P_0 treatment, whereas the minimum was with the P_0/P_0. Plants on the Cleopatra mandarin differed from those on the Rangpur lime since growth corresponded to soil-P levels with maximum observed with the P_1/P_1 treatment, in which greater amount of P was better distributed in the rhizotrons profile. Furthermore, plants on the Rangpur lime presented lower root to shoot ratio in the P_0/P_0 treatment (0.77) compared to those on Cleopatra (0.96) what together with data presented for root growth rate and number of root tips observed in the vertical plane of the rhizotrons (Table 1) suggested that Rangpur lime presents more plastic roots for P uptake at limited availability in the soil (Ragothama, 1999). Treatment effects on P accumulation will be further discussed.

Table 2. Dry matter production of young plants of Pêra sweet orange on different rootstocks as affected by soil resin-P availability in mini-rhizotrons.

Rootstock combination	Soil P treatment(1)	Dry matter(2)					Root:shoot ratio
		RootU	RootL	Leaf	Stem	Total	
		(--------------------------		g		---------------------------)	
Pêra/	P_0/P_0	6.03	2.59	6.09	5.20	19.91	0.77
Rangpur lime	P_1/P_0	7.58	1.88	5.32	5.58	20.35	0.90
	$P_{0.5}/P_{0.5}$	7.23	2.04	4.03	4.88	18.18	1.04
	P_2/P_0	12.18	4.91	11.17	8.83	37.07	0.89
	P_1/P_1	8.08	2.58	9.25	6.57	26.48	0.67
Mean values		8.22	2.80	7.17	6.21	24.40	0.85
Pêra/	P_0/P_0	3.72	0.75	2.45	2.26	9.18	0.96
Cleopatra mandarin	P_1/P_0	4.16	1.07	3.42	2.60	11.24	0.88
	$P_{0.5}/P_{0.5}$	4.85	0.76	3.52	2.99	12.12	0.87
	P_2/P_0	5.32	1.56	4.61	3.39	14.88	0.85
	P_1/P_1	6.86	1.35	4.30	3.02	15.53	1.15
Mean values		4.98	1.10	3.66	2.85	12.59	0.94
Prob. < F							
Rootstock (RS)		77.06**	21.73**	92.51**	84.64**	138.64**	1.93^{ns}
Soil P (SP)		13.52**	3.37**	19.60**	5.63**	19.15**	0.89^{ns}
RS*SP		7.05**	1.47**	8.61**	2.56*	7.46**	3.03*

(1) resin-P in each of 45 cm soil layer. in mg/dm^3. $P_0 = 4$. $P_{0.5} = 12$. $P_1 = 19$ and $P_2 = 47$.
(2) RootU and RootL = refer to roots in the upper and lower 45 cm soil layers. respectively. harvested 134 days after transplant in mini-rhizotrons.

Conclusion

Young plants of Pêra sweet oranges on Rangpur lime demonstrated more vigorous growth compared to those on Cleopatra mandarin. This was correspondent to observed root system characteristics, which growth rate, architecture and plant dry matter production varied depending on P availability in the soil.

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