IRON SOURCES FOR CITRUS ROOTSTOCK DEVELOPMENT GROWN ON PINE BARK/VERMICULITE MIXED SUBSTRATE

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ABSTRACT: For high technology seedling production systems, nutrition plays an important role, mainly the fertigation with iron chelates to prevent its deficiency. This study had the goal of searching for alternative iron sources with the same nutrient efficiency but lower cost in relation to nutrient solution total cost. An experiment was carried out in 56 cm^3-conic-containers tilled with a pine bark/vermiculite mixed substrate using Fe-DTPA, Fe-EDDHA, Fe-EDDHMA, Fe-EDTA, Fe-HEDTA, $FeCl_3$, $FeSO_4$, $FeSO_4$+citric acid plus a control, and the rootstocks Swingle, Rangpur, *Trifoliata* and Cleopatra, in a randomized complete block design, with four replicates. Seedlings were evaluated for height, relative chlorophyll index, total and soluble iron leaf concentrations. Cleopatra was the only rootstock observed without visual iron chlorosis symptoms. There was a low relative chlorophyll index for Rangpur, Swingle and *Trifoliata* rootstocks in the control plots, in agreement with the observed symptoms. High total iron concentrations were found in the control and Fe-EDTA plots, whereas soluble iron represented only a low percent of the total iron. The economical analysis showed the following cost values of iron sources in relation to the nutrient solution total costs: Fe-HEDTA (37.25%) > $FeCl_3$ (4.61%) > Fe-EDDHMA (4.53%) > Fe-EDDHA (3.35%) > Fe-DTPA (2.91%) > Fe-EDTA (1.08%) > $FeSO_4$+citric acid (0.78%) > $FeSO_4$ (0.25%). However, only plants from Fe-EDDHA and Fe-EDDHMA treatments did not present any deficiency visual symptoms. The relative cost of Fe-EDDHA application is low, its efficiency in maintaining iron available in solution resulted in high plant heights, making it recommendable for citric rootstock production in nurseries.
Key words: seedling production, chelates, fertigation, economical analysis

FONTES DE FERRO PARA O DESENVOLVIMENTO DE PORTA-ENXERTOS CÍTRICOS PRODUZIDOS EM SUBSTRATO DE CASCA DE PINUS E VERMICULITA

RESUMO: No sistema altamente especializado de produção de mudas, a nutrição exerce papel importante, principalmente a fertirrigação com quelatos de ferro para evitar sua deficiência. O objetivo deste estudo foi buscar fontes alternativas de ferro que propiciem a mesma eficiência de aproveitamento e menor custo em relação ao total da solução nutritiva. Avaliaram-se os porta-enxertos Swingle, Cravo, *Trifoliata* e Cleópatra, em tubetes com substrato de casca de pinus/vermiculita, e as fontes de ferro Fe-DTPA, Fe-EDDHA, Fe-EDDHMA, Fe-EDTA, Fe-HEDTA, $FeCl_3$, $FeSO_4$, $FeSO_4$+ácido cítrico e Testemunha. Utilizou-se o delineamento em blocos casualisados, com quatro repetições. Foram analisados altura das plantas, índice relativo de clorofila, teor na folha de Fe total e solúvel. Cleópatra foi o único porta-enxerto que não apresentou sintoma visual de deficiência de ferro. Houve baixo índice relativo de clorofila na Testemunha dos porta-enxertos Cravo, Swingle e *Trifoliata*, comprovando os sintomas visuais observados. Verificou-se alta concentração de Fe total e menor % de ferro solúvel da Testemunha e do Fe-EDTA. Na análise econômica, as fontes de ferro foram classificadas em relação ao custo total da solução nutritiva: Fe-HEDTA (37,25%) > $FeCl_3$ (4,61%) > Fe-EDDHMA (4,53%) > Fe-EDDHA (3,35%) > Fe-DTPA (2,91%) > Fe-EDTA (1,08%) > $FeSO_4$+ácido cítrico (0,78%) > $FeSO_4$ (0,25%). No entanto, somente as plantas dos tratamentos Fe-EDDHA e Fe-EDDHMA não apresentaram sintomas visuais de deficiência. Fe-EDDHA propiciou melhor desempenho sob o aspecto nutricional e na altura das plantas do que Fe-EDDHMA, sendo recomendado para a produção de mudas cítricas nos viveiros.
Palavras-chave: produção de mudas, quelatos, fertirrigação, análise econômica

INTRODUCTION

Brazil holds the position of world's orange producer, with a cultivated area of 820 thousand hectares, yielding 447.81 million boxes of 40.8 Kg, what represents 49% of the total Brazilian fruit production. The citrus industry moves more than US$ 5 billion per year in the productive chain, with investments of US$ 2.2 billion in both new and in-production orchards (Ferrarezi, 2006). To keep high production levels and to assure the position of market leaders, the industries make large investments in equipment, citrus technology, logistics and marketing.

The emergence and aggravation of diseases and pests requires quality scion production under protected nurseries, according to the Rules for Certified Citrus Seedling Production in the State of São Paulo, Brazil. Nowadays, there are 543 nurseries producing more than 12 million citrus seedlings annually in the São Paulo State. More than US$ 50 million were invested in technology in the last 5 years for nursery seedling production and orchard renovation and planting (Ferrarezi, 2006).

Protected citrus seedling production requires adequate nutrition and the substrate requires additional amendment, mainly regular applications of iron to avoid Fe-deficient seedlings. Iron is frequently applied during fertigation through dripping systems, but its availability to the plants would depend on the substrate used and might vary substantially (Gárate & Lucena, 1991).

Iron deficiency alters the photosynthetic apparatus structure and function of superior plants, drastically reducing the quantity of green pigments on deficient leaves and relatively enriching them with yellow pigments, due to the increase in some carotenoids of chloroplasts (Yunta et al., 2003a). As a consequence, Fe-deficiency symptoms occur first in young leaves, being characterized by yellow leaves with green central and side nervures (Römheld, 2001). This affects the mineral nutrient content, the production and quality of fruit trees (Bañuls et al., 2003).

A metal-chelate is the result of a special complexing reaction in which the formed complex shows multiple connections in a structure around a metallic ion (Albano & Miller, 1995). Chelates maintain metals soluble in adverse chemical environments where metal ions might precipitate in the form of oxides or hydroxides. Thus, the use of synthetic iron chelates is the most usual and effective way to prevent Fe-deficiency in plants.

When iron-chelates are added to a substrate, large amounts of soluble iron are maintained in solution without being displaced by other cations or retained by the substrate surface negative charges (Lucena, 2003). According to García-Marco et al. (2006), Fe-efficient plants have developed two different strategies to assimilate iron when its availability is low. In Strategy I plants (dicots and nongrass monocots), plant roots are able to increase a plasma membrane-bound Fe^{3+} reductase (FC-R), that directly reduces extracellular Fe^{3+} from the chelates to Fe^{2+} (Bienfait, 1985). The Fe^{2+} can be transported into the roots by a plasma membrane transporter (transporter T) (García-Marco et al., 2006). The greater difference compared to other non-chelated Fe-compounds, is that the chelating agent is transported back to the substrate solid phase to chelatize more irons. When the chelate returns to the solution it can chelatize large amounts of iron in a short period of time (Pérez-Sanz & Lucena, 1995).

Nowadays, chelating agents used to obtain chelated micronutrients are available as different molecules and isomers, all of them as polyamine carboxylic acids used to obtain chelated micronutrients: DTPA (Diethylene triaminepentaacetic acid) (**5** in Figure 1), EDDHA (Ethylene diamine di-[o-hydroxyphenylacetic] acid) (**1**, **2** and **3** in Figure 1), EDDHMA (Ethylene diamine di- [o-hydroxy-methylphenyl] acetic acid) (**4** in Figure 1), EDTA (Ethylene diaminetetraacetic acid) (**6** in Figure 1) and HEDTA (Hydroxyethylene
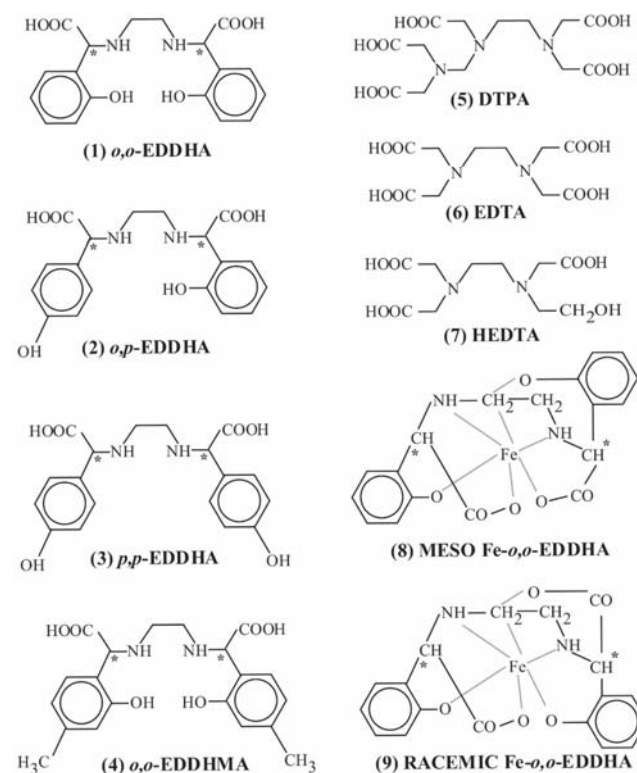

Figure 1 - Chelating agents used to prevent and correct iron deficiency. *Chiral carbons

diaminetriacetic acid) (**7** in Figure 1) (Yunta et al., 2003a). These chelating agents usually have high Fe^{3+} specificity and form highly stable metallic chelates. In absence of competitor ions Fe-EDTA stays unaffected up to pH 6; Fe-EDDHA up to pH 9; Fe-EDDHMA up to pH 11; and Fe-DTPA up to 7.5 (Bermúdez et al., 2002). The Fe-HEDTA is moderately stable under acid and neutral conditions, between pH 4.8 and 6.7. Above pH 7.5 this chelate presents very low stability (Norvell, 1972).

During the industrial synthesis of EDDHA, a mixture of three isomeric products is produced, depending on the position of the hydroxyl and phenyl groups in the aromatic ring (Bermúdez et al., 2002). These isomers are *o,o*- EDDHA, *o,p*-EDDHA and *p,p*-EDDHA (**1**, **2** and **3**, respectively, in Figure 1), which show different abilities to chelate metallic ions, the *o,o*-EDDHA being the most efficient chelating agent, because of its high stability metallic complexes (Yunta et al., 2003b).

In the commercial synthesis, Fe-*o,o*-EDDHA (**1** in Figure 1) and Fe-*o,o*-EDDHMA (**4** in Figure 1), are obtained as a mix of two geometric isomers at a 50% rate each: a meso (**8** in Figure 1) and a racemic (**9** in Figure 1) form (Bermúdez et al., 2002). The Fe-*o,o*-EDDHA racemic form is more stable than a meso isomer, while the opposite happens with the Fe-EDDHMA isomers: the meso form is more stable than the racemic one (Ahrland et al., 1990). However, more stable chelates, like Fe-EDDHA and Fe-EDDHMA, are more expensive, justifying a search for cheaper iron sources in substrate at lower pH. As alternatives, other iron chelates, like Fe-DTPA, Fe-EDTA and Fe-HEDTA might be used. However, their efficiency in correcting iron chlorosis is smaller when compared to other chelates (Lucena et al., 1992; Sánchez-Andreu et al., 1991). In the same way, inorganic fertilizers could be used, like ferrous sulfate ($FeSO_4.H_2O$ or $FeSO_4.7H_2O$) and ferric chloride ($FeCl_3.6H_2O$) (Natt, 1992). On the other hand, iron can easily precipitate in nutrient solution due to Fe displacement from the chelate by other elements, and subsequent precipitation of iron hydroxide, turning Fe unavailable to plants, inducing chlorosis and blocking the drip system. Therefore, complexing substances like citric acid, humic acids and derivates have also been tested to minimize this problem (Álvarez-Fernández et al., 1996).

The objectives of the present work were to evaluate citrus rootstock efficiencies in the utilization of several iron sources, to estimate the costs of these products in nutrient solution and to evaluate their effects on plant height, leaf soluble and total iron concentration and relative chlorophyll content.

MATERIAL AND METHODS

The experiment was conducted in greenhouse, at Campinas, SP, Brazil (22º54´S, 47º05´W, and 674 m altitude). The temperature and relative humidity were registered by a thermo-hygrograph (Elcometer 6700 Electronic®) during the experimental period. The average of the maximum temperature was 34.4ºC and the minimum 17.2ºC. The average of the maximum relative humidity was 83.6% and the minimum 24.6%.

The treatments consisted of five iron chelates (Fe-DTPA, Fe-EDDHA, Fe-EDDHMA, Fe-EDTA and Fe-HEDTA), three salts ($FeCl_3$, $FeSO_4$ e $FeSO_4$ + citric acid) and an iron-free control; and four citrus rootstocks (Rangpur lime Limeira [*Citrus limonia* Osbeck], Cleopatra mandarin [*Citrus reshni* hort. ex Tanaka], Swingle citrumelo [*Citrus paradisi* Macfad. cv. Duncan x *Poncirus trifoliata* (L.) Raf.], *Poncirus trifoliata* Limeira [*Poncirus trifoliata* (L.) Raf.]. The iron sources chosen are available in the market, as commercial chelates and laboratory high pure grade salts. The products were previously analyzed by atomic absorption spectrometry to determine their iron concentration (Table 1).

Citrus rootstock seedlings were grown (from August 22nd, 2004 to February 2nd, 2005) in 56 cm^3 conic containers (cone-tainers), open at the bottom to allow drainage. A commercial pp plan tray, with dimensions 695 × 420 × 30 mm, for cone-tainers with 228 cells (19 × 12 cells), was divided into four parts for sowing the rootstocks. Fifty-six plants of each rootstock were sown and, after one month, the best 40 plants of each rootstock were selected, totalling 160 plants. Rootstocks were separated by lines, resulting in 18 useful plants of each rootstock for biometric and chemical analyses. The positions of the trays in the greenhouse were weekly changed to minimize the experimental errors.

The used commercial growth medium was composed by ground pine bark and vermiculite. This substrate was characterized according to the Dutch method (1:1.5 substrate:water), adapted from Sonneveld & Elderen (1994). Results were as follows: pH = 5.9, electric conductivity (EC) = 0.1 dS m^{-1} and (in mg L^{-1}) total -N = 6.6 (NO_3^- -N = 0.8 and NH_4^- -N = 5.8); $H_2PO_4^-$ -P = 0.6; K^+ = 7.1; Ca^{2+} = 0.7; Mg^{2+} = 0.3; SO_4^{2-} -S = 4.8; Cl = 1.4; Na = 2.6; B = 0.04; Cu = 0.04; Fe = 2.4; Mn = 0.02; Zn = 0.03.

The calculation of the amount of water to be added, through fertigation, was determined by a pilot test using a tray with 160 cone-tainers full of substrate and a container collector installed below. Water was added until there was percolation from the cone-tainers. As result, 3 L of nutrient solution were used

Table 1 - Trade name, manufacturer, iron source, nominal iron percentage declared in the commercial product pack and iron percentage experimentally determined by atomic absorption spectrophotometry.

Trade name[1]	Manufacturer	Iron source	Declared	Determined
			------------ % ------------	
GeoFe 11®	Rigran	Fe-DTPA	11	13.2
Ultraferro®	TradeCorp	Fe-EDDHA (4% Fe o,o)	6	6.8
Tenso Ferro®	Yara	Fe-EDDHMA (4.8% Fe o,o)	6	7.2
Dissolvine®	Akzo-Nobel	Fe-EDTA	13	14.1
$Fe(NO_3)_3.9\ H_2O$ + HEDTA	Merck P.A.	Fe-HEDTA[2]	0.92	0.92
$FeCl_3.6H_2O$	ECIBRA P.A.	$FeCl_3$	6	6.0
$FeSO_4.7H_2O$	Synth P.A.	$FeSO_4$	6	6.0
$FeSO_4.7H_2O$ + citric acid	Synth P.A.	$FeSO_4$ + citric acid[3]	6	6.0

[1]Actual analysis of the Fe chelated has not been accomplished. Results only apply to the products used. Different commercial products with the same chelating agent may not present the same behavior; [2]This solution was prepared as follows: a) 8.68 g of HEDTA was dissolved in NaOH 1mol L^{-1} solution; b) 13.31 g of Fe $(NO_3)_3.9\ H_2O$ was added to the solution and shacked; c) final pH was adjusted to 4.0 with NaOH 1mol L^{-1} solution; and d) Final volume was adjusted to 1 L. [3]To each 1 mol of $FeSO_4.7H_2O$, 1.1 mols of citric acid were added (10% excess citric acid was added to guarantee the complete iron reaction);

per tray with 160 plants, in each fertigation to supply the citrus nutritional demands, resulting an average of 18.75 mL of nutrient solution per cone-tainer.

The solution, prepared according to Furlani (1998), presented pH = 6.0, EC = 2.0 dS m^{-1} and the following chemical composition (in mg L^{-1}): total-N = 184 (NO_3^--N = 161 and NH_4^--N = 23); $H_2PO_4^-$-P = 41.6; K^+ = 234; Ca^{2+} = 103; Mg^{2+} = 36; SO_4^{2-}-S = 48; B = 0.51; Cu = 0.13; Mn = 0.64; Mo = 0.02 and Zn = 0.23. The iron concentration of the treatments was 1.8 mg L^{-1}, except for the control treatment, prepared without Fe.

Initially concentrated solutions A and B, with macro and micronutrients (except iron) (Furlani, 1998), were used for the preparation of the nutrient solutions. The fertigation was applied five times a week during all the experimental period. At every fertigation, a volume of 120 L of nutrient solution was prepared, the iron sources being added to the solution individually, at the moment of the fertigation, to avoid precipitation. Through the use of a watering can, the plants were submitted to the different treatments.

Sixty, 90 and 120 days from seed emergency, plant height of 18 plants was measured with a ruler, from the base to the upper apical third. At the end of 120 days, the relative chlorophyll index was measured on 18 plants in the first totally expanded leaf, with a non-destructive analysis, using the SPAD-502 equipment (Minolta Camera Co.).

The assessment to the plant tissue analyses was made at the end of the experiment, at 120 days. The plants were removed from the containers and then divided in apical part (corresponding to five totally expanded leaves plus the related stem), base and roots. After identification samples were dried in a forced air oven at 70ºC, until constant mass for dry matter determination. Samples were then ground in a Wiley type mill and analyzed for total iron concentration, by the dry ash method, according to the analytical procedures described in Bataglia et al. (1983).

The soluble iron extraction method with HCl was adapted from Takkar & Kaur (1984). The tissue extract for determination was prepared as follows: 0.1 g of dry sample was transferred to Erlenmeyer flask and 10 mL of HCl 1 mol L^{-1} were added, shaken at each hour for 24 hours, and subsequently filtered in slow filtrage paper, pre-washed with HCl 1 mol L^{-1} solution. The analysis was performed in an atomic absorption spectrometer.

Substrates of all treatments were analyzed according to the Dutch method (1:1.5 substrate:water), adapted from Sonneveld & Elderen (1994), to evaluate the nutrient status at the end of the experiment.

The number of irrigations made during the experiment and the salt quantities (amendments and iron chelates used) were monitored, in order to obtain the cost of iron sources relative to the total cost of nutrient solution. A price quotation of the different products was made at three different places to compose average values and estimate the fertigation costs, simulating a production of 1,000 seedlings, without considering the other costs inherent to the production.

The experiment was carried out in a completely randomized design arranged in a 9 × 4 factorial (eight iron sources + one control and four rootstocks), with four replicates. Each plot was composed of 18 plants. The data of all variables were submitted to analysis of variance, and the means compared at 5% probability against the clustering test of Scott-Knott in SISVAR statistical computer program.

RESULTS AND DISCUSSION

The iron sources provided development differences on the rootstocks. This variability was expected, since the rootstocks presented different genetic characteristics and nutritional requirements, and the iron sources presented differences in chemical composition.

During the experimental period, visual symptoms of iron deficiency were observed in young leaves, on the upper apical third of the plants, allowing the classification of the visual symptoms in a scale, presented in the Table 2, where fertigated plants with salts $FeCl_3$, $FeSO_4$ and $FeSO_4$ + citric acid, with the chelate Fe-DTPA and control showed the iron deficiency symptoms. The results obtained in the present experiment are in agreement with those obtained by Natt (1992), who reported the Fe-DTPA source as the least efficient in iron supply, with plants usually showing visual iron deficiency symptoms; and also, the results reported by Álvarez-Fernández et al. (1996), who classified the chelates Fe-EDDHA and Fe-EDDHMA as the most efficient iron sources for plant growth, in function of the existence of isomeric *orto-orto* in composition, larger solubility and maintenance of high amounts of chelated iron in the solution.

Iron treatments provided significant results for plant height and dry matter depending on the citrus rootstock cultivar (Figures 2 and 3). Rangpur lime seedlings from the control treatment presented the smallest dry matter values (**a** in Figure 2). Similar results were obtained for Cleopatra mandarin seedlings, were the control and Fe-EDTA had the smallest dry matter values, except for the Fe-deficiency visual symptoms, that were not observed in this rootstock cultivar (**b** in Figure 2; Table 2).

Swingle citrumelo seedlings from Fe-EDTA, $FeSO_4$ and $FeSO_4$ + citric acid treatments had lower plant height and dry matter values in relation to those from the control (**a** in Figure 3), at 5% of significance. *Poncirus trifoliata* seedlings from the Fe-DTPA and Fe-EDTA treatments showed lower plant heights than
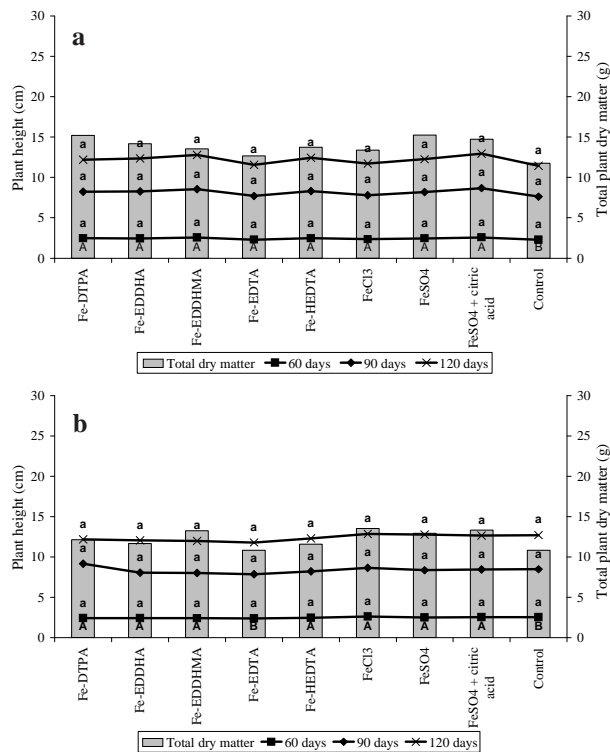

Figure 2 - Rootstock plant height at 60, 90 and 120 days and total dry matter at 120 days grown in 56 cm³ cone-tainers: (**a**) Rangpur lime; and (**b**) Cleopatra mandarin. Average of 18 plants per replicate and 4 replicates. Means followed by the same small letters in plant height and capital letters in total dry matter do not differ (Scott-Knott test, $P > 0.05$).

Table 2 - Visual determination scale of the symptoms of iron deficiency, in the rootstocks Cleopatra mandarin, Rangpur lime, Swingle citrumelo and *Poncirus trifoliata*, in cone-tainers of 56 cm³. Average of four replicates.

Iron source	Rootstock[1]			
	Cleopatra mandarin	Rangpur lime	Swingle citrumelo	*Poncirus trifoliata*
	---- Scale of visual deficiency symptoms ----			
Fe-DTPA	–	+++	++++	++++
Fe-EDDHA	–	++	++	++
Fe-EDDHMA	–	+	+	+
Fe-EDTA	–	+++	++++	+++++
Fe-HEDTA	–	++	+++	++++
$FeCl_3$	–	+++	++++	+++++
$FeSO_4$	–	+++	++++	+++++
$FeSO_4$ + citric acid	–	+++	++++	+++++
Control	–	+++	++++	+++++

[1]The sign – indicates absence of visual deficiency. The sign + indicates symptoms of visual deficiency. A larger number of + indicates larger symptoms of visual deficiency.

the control, and the ones from Fe-EDTA treatments had also lower dry matter yields ($P < 0.05$) (**b** in Figure 3). Seedlings treated with FeCl$_3$ presented high plant height and dry matter values, but visual symptoms of Fe-deficiency were observed in these plants (Table 2). *Poncirus Trifoliata* seedlings from Fe-
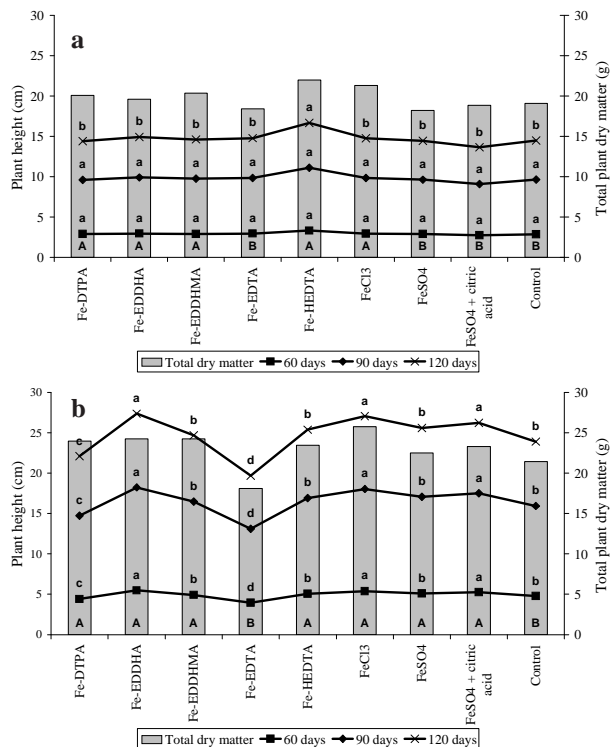

Figure 3 - Rootstock plant height at 60, 90 and 120 days and total dry matter at 120 days grown in 56 cm^3 cone-tainers: (**a**) Swingle citrumelo and (**b**) *Poncirus trifoliata*. Average of 18 plants per replicate and 4 replicates. Means followed by the same small letters in plant height and capital letters in total dry matter do not differ (Scott-Knott test, $P > 0.05$).

EDDHA and Fe-EDDHMA treatments had similar dry matter yields, but those treated with Fe-EDDHA presented higher values for plant height (**b** in Figure 3). This is an important characteristic for rootstock seedlings in nurseries, because plant height is the criterion to define the right moment for seedling transplanting and grafting. Thus, seedlings treated with Fe-EDDHA showed to be ready earlier for transplanting that is, in a shorter period of time in the nursery, which is advantageous from the economical point of view, reducing production costs.

According to Pestana et al. (2001) a positive correlation has been found between the SPAD-502 measurements and the leaf total chlorophyll contents (μmol m^{-2}). Therefore, the relative chlorophyll index allowed evaluating the plant nutritional status at the end of experiment. In the Fe-EDDHMA and Fe-EDTA treatments, the SPAD-502 readings increased about 23% and 17%, respectively, for the rootstock Rangpur lime when compared to the control; while the FeSO$_4$ + citric acid treatment increased the readings in 27% for Swingle citrumelo and 24% for *Poncirus trifoliata* (Table 3). These results are similar to the ones obtained by Bañuls et al. (2003), who showed that the iron application frequency partially affected the SPAD-502 readings during the growth period.

In spite of the high Fe concentrations found in the upper apical third of citrus rootstock plants from the control and Fe-EDTA treatments (Table 4), those plants exhibited severe iron deficiency symptoms (Table 2), probably because iron was present in the form of non-reactive ferric ion (Fe^{3+}), according to Pérez-Sanz & Lucena (1995), not being easily used in photosynthesis reactions and formation of chlorophyll pigments (Cataldo et al., 1988). Furthermore, Mohammad et al.

Table 3 - Relative chlorophyll index in the upper apical third of citrus plants at 120 days (average of 18 plants per replicate and four replicates).

Iron source	Rootstock				
	Cleopatra mandarin	Rangpur lime	Swingle citrumelo	*Poncirus trifoliata*	Mean
	--------------------------------------- SPAD Unit ---------------------------------------				
Fe-DTPA	44.6 aA	37.5 bD	40.4 bC	42.1 cB	41.2
Fe-EDDHA	45.6 aA	38.4 bC	41.1 bB	47.4 bA	43.1
Fe-EDDHMA	45.7 aA	41.7 aB	43.2 bB	47.0 bA	44.4
Fe-EDTA	45.7 aA	39.7 aC	41.9 bB	43.0 cB	42.6
Fe-HEDTA	46.6 a	38.0 bC	41.0 bB	47.3 bA	43.2
FeCl$_3$	46.0 aA	36.5 cC	40.5 bB	40.1 dB	40.8
FeSO$_4$	47.3 aA	37.4 bC	37.1 cC	41.3 dB	40.8
FeSO$_4$ + citric acid	46.6 aB	34.1 dC	46.6 aB	50.3 aA	44.4
Control	45.3 aA	33.8 dD	36.5 cC	40.5 dB	39.0
Mean	45.9	37.4	40.9	44.3	

Mean followed by the same small letters in the columns and capital letters in the lines do not differ (Scott-Knott test, $P > 0.05$).

Table 4 - Total iron concentration in the upper apical third of citrus plants, base and roots, at 120 days (average of 18 plants per replicate and four replicates).

Iron source	Rootstock				Mean
	Cleopatra mandarin	Rangpur lime	Swingle citrumelo	*Poncirus trifoliata*	
	------------------------------ Upper apical third (mg kg^{-1})[1] ------------------------------				
Fe-DTPA	61 cB	95 bA	77 bA	64 cB	74.3
Fe-EDDHA	45 cB	70 bA	60 bA	42 cB	53.9
Fe-EDDHMA	56 cA	68 bA	55 bA	51 cA	57.3
Fe-EDTA	115 bA	95 bA	89 bA	89 bA	97.1
Fe-HEDTA	57 cB	80 bA	67 bA	52 cB	63.9
FeCl$_3$	55 cA	70 bA	66 bA	48 cA	59.6
FeSO$_4$	97 bA	76 bB	69 bB	65 cB	76.6
FeSO$_4$ + citric acid	58 cB	79 bA	74 bA	52 cB	65.8
Control	194 aA	196 aA	127 aC	161 aB	169.2
Mean	81.9	91.9	76.0	69.1	
	-------------------------------------- Base (mg kg^{-1}) --------------------------------------				
Fe-DTPA	216 bB	405 aA	477 aA	243 cB	334.9
Fe-EDDHA	88 cA	90 cA	80 eA	69 dA	81.4
Fe-EDDHMA	88 cA	87 cA	84 eA	73 dA	82.9
Fe-EDTA	153 bC	237 bB	231 dB	381 aA	250.4
Fe-HEDTA	149 bB	142 cB	274 cA	303 bA	217.0
FeCl$_3$	214 bA	244 bA	149 eA	185 cA	198.1
FeSO$_4$	129 cA	113 cA	124 eA	90 dA	113.6
FeSO$_4$ + citric acid	393 aA	418 aA	367 bA	215 cB	348.2
Control	168 bA	107 cA	123 eA	107 dA	126.3
Mean	177.4	204.5	212.1	185.1	
	-------------------------------------- Roots (mg kg^{-1})[1] --------------------------------------				
Fe-DTPA	857 aA	540 aB	443 aB	606 aB	611.4
Fe-EDDHA	610 bA	478 aA	511 aA	576 aA	543.8
Fe-EDDHMA	738 bA	525 aB	389 aB	597 aB	562.1
Fe-EDTA	854 aA	596 aB	466 aB	572 aB	622.0
Fe-HEDTA	757 bA	606 aA	414 aB	653 aA	607.2
FeCl$_3$	915 aA	547 aB	432 aC	641 aB	633.7
FeSO$_4$	915 aA	549 aB	509 aB	575 aB	636.9
FeSO$_4$ + citric acid	833 aA	558 aB	430 aB	480 aB	575.4
Control	635 bA	420 aB	368 aB	733 aA	539.0
Mean	790.4	535.5	440.0	603.6	

[1]Analysis of variance of original data transformed to $\sqrt{x}$. Means followed by the same small letters in the columns and capital letters in the lines, do not differ (Scott-Knott test, $P > 0.05$).

(1998) observed that the total iron concentration was always higher in chlorotic leaves as compared to green leaves and was not related to the chlorosis degree.

The application of Fe-EDTA increased young plant leaf iron concentrations in 10% when compared to three-year-old plants treated with and without iron chelates (Natt, 1992). However, opposite results were obtained in this experiment, where plants treated with Fe-EDTA showed severe visual chlorosis symptoms of iron deficiency, despite the high total iron concentration found in them (Table 4). On the other hand, in this experiment, no significant differences were found between Fe-EDDHA and Fe-EDDHA chelate treatments (Table 4) and these results are in agreement with the ones obtained by Lucena et al. (1992). According to these authors, there is large variability among chelates with the same chelating agent, but different formulations.

It is difficult to identify iron deficiency based on plant total Fe concentration, because leaves exhibiting visual symptoms of iron deficiency may present considerably high total iron concentrations (the so-called iron deficiency "paradox", according to El-Baz et al., 1998). The determination of active iron was suggested by Pierson & Clark (1984), that proposed an adapted procedure to determine this iron fraction in plants.

Therefore, in this experiment, once plants treated with Fe-EDTA showed severe visual chlorosis symptoms of Fe-deficiency, despite their high total Fe-concentration (Table 4), an attempt was made to determine the leaf soluble iron (with HCl 1 mol L^{-1}, according to El-Baz et al., 1998) to observe how much of the total iron was available to the photosynthetic reactions and pigment synthesis. Lang et al. (1990) ob-

served that the iron extraction in fresh leaves with HCl 1 mol L^{-1} was a good indicator of the iron status in plants at flowering. Also, Ohwaki & Sugahara (1993) reported that the genotypic differences among Fe-deficient chickpea sensitive and resistant cultivars were attributed to the active iron in leaves grown under iron stress conditions.

The results on leaf soluble iron concentration showed no significant interactions between iron sources and rootstocks (Table 5). However, plants from control and Fe-EDTA treatment presented the highest values, and the soluble iron concentration determined tended to decrease with the increasing chlorosis degree.

The percentage of soluble iron in relation to the total-Fe was found to be closely related to the chlorosis degree (Table 5 and 6). Plants from the control pre-

Table 5 - Soluble iron (in HCl 1 mol L^{-1}) concentration in the upper apical third of citrus plants at 120 days (average of 18 plants per replicate and four replicates).

Iron source	Rootstock				Mean
	Cleopatra mandarin	Rangpur lime	Swingle citrumelo	*Poncirus trifoliata*	
	--- % ---				
Fe-DTPA	38.2	57.2	45.0	37.5	44.5 c
Fe-EDDHA	25.0	44.0	37.5	27.2	33.4 c
Fe-EDDHMA	36.2	49.0	34.7	37.5	39.3 c
Fe-EDTA	85.5	70.0	54.0	60.0	67.3 a
Fe-HEDTA	41.7	64.3	48.0	35.7	47.4 c
$FeCl_3$	35.7	55.5	50.2	31.7	43.3 c
$FeSO_4$	74.5	60.7	43.2	40.2	54.6 b
$FeSO_4$ + citric acid	41.5	55.7	57.0	34.0	47.0 c
Control	88.5	76.0	47.0	64.5	69.0 a
Mean	51.8	59.1	46.3	40.9	

Mean followed by the same small letters in the columns and capital letters in the lines, do not differ (Scott-Knott test, $P > 0.05$).

Table 6 - Percentage of soluble iron (in HCl 1 mol L^{-1}) in relation to the total iron concentration in the upper apical third of citrus plants at 120 days (average of 18 plants per replicate and four replicates).

Iron source	Rootstock				Mean
	Cleopatra mandarin	Rangpur lime	Swingle citrumelo	*Poncirus trifoliata*	
	--- % ---				
Fe-DTPA	63.2	60.6	59.2	62.2	61.3 b
Fe-EDDHA	57.6	63.7	63.7	68.5	63.4 b
Fe-EDDHMA	65.1	72.0	63.2	74.2	68.6 a
Fe-EDTA	74.9	73.8	59.3	68.9	69.2 a
Fe-HEDTA	72.8	81.1	71.3	69.1	73.6 a
$FeCl_3$	64.5	78.8	76.6	64.7	71.1 a
$FeSO_4$	76.5	79.8	63.7	62.6	70.6 a
$FeSO_4$ + citric acid	72.1	68.8	76.8	64.9	70.6 a
Control	46.2	42.8	43.8	41.3	43.5 c
Mean	65.9	69.0	64.2	64.1	

Mean followed by the same small letters in the columns and capital letters in the lines, do not differ (Scott-Knott test, $P > 0.05$).

sented the lowest percentages of soluble-Fe in relation to total iron (Table 6), which may explain their typical chlorosis symptoms of iron deficiency (Table 2). The authors could not found a physiological explanation for the high Fe levels found in plants treated with Fe-EDTA, and of the occurrence of visual iron deficiency symptoms in the plants of that treatment. Though, some studies indicate that uptake of hydrophilic solutes such as metal-EDTA complexes is believed to oc-

cur via bypass apoplastic flow, into the plant (Schaider et al., 2006; Epstein et al., 1999). These are open questions that will need further research to be clarified.

When comparing the tissue Fe-concentration among different plant parts, no significant interactions among iron sources and rootstocks were observed (Table 7). In the Fe-EDTA and control treatments, differences among Fe-concentrations of upper apical third of citrus rootstock plants were found (Table 7). In the

Table 7 - Iron accumulation in the upper apical third, base and roots of citrus plants at 120 days (average of 18 plants per replicate and four replicates).

Iron source	Rootstock				Mean
	Cleopatra mandarin	Rangpur lime	Swingle citrumelo	*Poncirus trifoliata*	
	---------------------------- Upper apical third (μg plant^{-1})$^{(1)}$ ----------------------------				
Fe-DTPA	10	20	15	10	13.8 b
Fe-EDDHA	7	12	7	5	7.8 b
Fe-EDDHMA	7	10	10	7	8.5 b
Fe-EDTA	12	17	27	22	19.5 a
Fe-HEDTA	10	22	10	10	13.0 b
FeCl$_3$	10	12	12	7	10.3 b
FeSO$_4$	15	12	10	10	11.8 b
FeSO$_4$ + citric acid	10	15	17	7	12.3 b
Control	22	35	20	32	27.3 a
Mean	11.4 A	17.2 A	14.2 A	12.2 A	
	-------------------------------- Base (μg plant^{-1})$^{(1)}$ --------------------------------				
Fe-DTPA	72	157	260	207	174.0 a
Fe-EDDHA	32	37	45	60	43.5 d
Fe-EDDHMA	35	35	50	55	43.8 d
Fe-EDTA	67	72	155	192	121.5 b
Fe-HEDTA	70	65	170	190	123.8 b
FeCl$_3$	80	77	85	145	96.8 c
FeSO$_4$	50	40	62	62	53.5 d
FeSO$_4$ + citric acid	150	162	197	172	170.3 a
Control	72	20	32	67	47.8 d
Mean	69.8 B	73.9 B	117.3 A	127.8 A	
	-------------------------------- Roots (μg plant^{-1})$^{(1)}$ --------------------------------				
Fe-DTPA	152	155	175	275	189.3 a
Fe-EDDHA	92	102	172	230	149.0 b
Fe-EDDHMA	155	130	150	275	177.5 a
Fe-EDTA	122	110	157	207	149.0 b
Fe-HEDTA	120	135	145	255	163.8 b
FeCl$_3$	207	140	187	315	212.3 a
FeSO$_4$	172	182	165	222	185.3 a
FeSO$_4$ + citric acid	195	145	187	215	185.5 a
Control	95	75	127	270	141.8 b
Mean	145.6 B	130.4 B	162.8 B	251.6 A	

$^{(1)}$Analysis of variance of original data transformed to $\sqrt{x}$. Means followed by the same small letters in the columns and capital letters in the lines do not differ (Scott-Knott test, $P > 0.05$).

basal part of Swingle citrumelo and *Poncirus trifoliata* plants of Fe-DTPA and FeSO$_4$ + citric acid treatments higher iron concentrations were found as compared to the other plants. Root Fe-concentrations in plants of *Poncirus trifoliata* were higher than in the roots of the other rootstocks (Table 7).

Tables 8 and 9 show pH values, EC, macro and micronutrient concentrations in the substrate, at end of experiment. However, there was not significance in the Scott-Knott test ($P < 0.05$).

Among the aspects involved in the complex system of seedling production and commercialization, the economical one is the most important and it will

influence the choice of products and management procedures that assure quality and minimize production costs. Aiming to contribute in this aspect, the price of the products used were quoted at the experiment date, and the economical analysis (Table 10) showed the following decreasing cost values of iron sources in relation to the nutrient solution total costs: Fe-HEDTA (37.25%) > FeCl$_3$ (4.61%) > Fe-EDDHMA (4.53%) > Fe-EDDHA (3.35%) > Fe-DTPA (2.91%) > Fe-EDTA (1.08%) > FeSO$_4$ + citric acid (0.78%) > FeSO$_4$ (0.25%).

The Fe-HEDTA was the most expensive iron chelate, corresponding to 37.25% of the nutrient so-

Table 8 - pH values, CE and macronutrient concentrations in the substrate, at end of experiment, of the rootstocks Cleopatra mandarin, Rangpur lime, Swingle citrumelo and *Poncirus trifoliata*, in cone-tainers of 56 cm^3. Average of four replicates.

Iron source	pH	EC	N-NO$_3$	N-NH$_4$	P	K	Ca	Mg	S
		dS m^{-1}	----------------------------------			mg L^{-1}	----------------------------------		
Fe-DTPA	5.5	1.5	27.4	2.0	3.7	64.6	10.2	8.4	21.2
Fe-EDDHA	5.3	1.5	28.8	2.3	3.3	61.8	11.7	10.0	22.8
Fe-EDDHMA	5.4	1.5	23.1	2.2	3.8	53.3	9.5	7.2	19.0
Fe-EDTA	5.4	1.6	31.2	2.3	3.7	62.7	9.7	7.8	20.2
Fe-HEDTA	5.6	1.5	25.1	2.1	3.6	48.6	10.0	7.7	18.7
FeCl$_3$	5.4	1.5	23.2	2.0	3.8	53.7	7.9	6.4	18.6
FeSO$_4$	5.4	1.5	27.5	2.3	3.6	56.4	10.8	9.3	20.8
FeSO$_4$ + citric acid	5.3	1.5	29.6	1.6	3.3	56.4	11.7	10.6	23.3
Control	5.4	1.5	25.6	1.8	3.1	55.2	10.1	8.8	21.3
Mean	5.4	1.5	26.8	2.1	3.6	56.9	10.2	8.5	20.7
C.V. (%) =	2.81	20.76	29.72	12.75	11.96	13.98	16.35	19.80	6.93
P > F	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

[n.s.]No significant (Scott-Knott test, $P > 0.05$).

Table 9 - Micronutrient concentrations in the substrate, at end of experiment, of the rootstocks Cleopatra mandarin, Rangpur lime, Swingle citrumelo and *Poncirus trifoliata*, in cone-tainers of 56 cm^3. Average of four replicates.

Iron source	B	Cu	Fe	Mn	Zn
	---		mg L^{-1}	---	
Fe-DTPA	0.17	0.06	1.00	0.06	0.04
Fe-EDDHA	0.16	0.05	0.98	0.06	0.04
Fe-EDDHMA	0.15	0.04	1.05	0.05	0.03
Fe-EDTA	0.17	0.05	0.92	0.05	0.04
Fe-HEDTA	0.16	0.06	0.92	0.06	0.04
FeCl$_3$	0.16	0.06	1.06	0.05	0.04
FeSO$_4$	0.17	0.04	0.95	0.06	0.03
FeSO$_4$ + citric acid	0.16	0.05	0.86	0.08	0.03
Control	0.16	0.05	0.84	0.06	0.03
Mean	0.16	0.05	0.95	0.06	0.04
C.V. (%) =	7.93	17.06	18.03	36.68	16.93
P > F	n.s.	n.s.	n.s.	n.s.	n.s.

[n.s.]No significant (Scott-Knott test, $P > 0.05$).

Table 10 - Cost and percentile participation of salts and chelates in the nutrient solution total cost for the production of 1,000 citrus seedlings.

Chelate and salt	Cost		Cost proportion of iron sources in the nutrient solution total cost[2]
	R$	US$[1]	%
Fe-DTPA	0.14	0.06	2.91
Fe-EDDHA	0.16	0.07	3.35
Fe-EDDHMA	0.22	0.10	4.53
Fe-EDTA	0.05	0.02	1.08
Fe-HEDTA	1.80	0.79	37.25
$FeCl_3$	0.22	0.10	4.61
$FeSO_4$	0.01	0.01	0.25
$FeSO_4$ + citric acid	0.04	0.02	0.78

[1]Comercial US dolar in Brazil in 12/12/2005 = R$ 2.26. [2]Total cost of nutrient solution = R$ 4.83 (US$ 2.13).

lution total costs, because of the price of $Fe(NO_3)_3.9H_2O$ used in this chelate composition, a fact that restricts its use for commercial production of citrus seedlings. Besides, plants treated with Fe-HEDTA presented visual symptoms of Fe-deficiency. The salt $FeCl_3$ also presented relatively high percent cost in the solution due to the original material high price. In sequence to the cost classification, the iron chelates Fe-EDDHMA and Fe-EDDHA, although slightly more expensive than the Fe-DTPA, Fe-EDTA, $FeSO_4$ + citric acid and $FeSO_4$ sources, represent an advantage in their use, due to the benefits they provided in the correction of iron deficiencies. The Fe-EDTA and $FeSO_4$ should be only used as alternative sources to reduce even more the costs, but they are not as effective as Fe-EDDHMA and Fe-EDDHA, since plants treated with Fe-EDTA and $FeSO_4$ presented Fe-deficiency symptoms, at the same iron rates.

CONCLUSIONS

The efficiency of iron sources in preventing iron deficiency in citrus rootstock cultivar development, cultivated on pine/bark vermiculite mixed substrate, was initially estimated through visual symptoms, and their efficacy followed the order: Control = $FeCl_3$ = $FeSO_4$ = $FeSO_4$ + citric acid = Fe-EDTA < Fe-DTPA < Fe-HEDTA < Fe-EDDHA < Fe-EDDHMA. Also through visual symptoms, different rootstock cultivar degrees of iron deficiency were found, which followed the order: *Poncirus trifoliata* > Swingle Citrumelo > Rangpur lime > Cleopatra mandarin.

The relative chlorophyll index (SPAD), the total iron concentration and the soluble iron concentration (in HCl 1mol L^{-1}) were not good indicators to diagnose the effect of the different sources of iron in the rootstocks.

Plants treated with Fe-EDDHA did not show any visual symptoms of iron deficiency and this chelate provided better quality seedlings (higher plants), being therefore, recommended for citrus seedling production in nurseries, presenting acceptable cost in relation to the total cost of the nutrient solution. The chelate Fe-EDDHMA presented similar results, but with a slightly higher cost as compared to Fe-EDDHA.

The rootstock Cleopatra mandarin do not show differences in relation to the sources of iron, being able to be grown with the use of salts in hydroponic like cultures, which are of lower costest than the chelates.

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