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Efficacy of Fe(*o,o*-EDDHA) and Fe(*o,p*-EDDHA) Isomers in Supplying Fe to Strategy I Plants Differs in Nutrient Solution and Calcareous Soil

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The FeEDDHA [iron($^{3+}$) ethylenediamine di(*o*-hydroxyphenylacetic) acid] is one of the most efficient iron chelates employed in the correction of iron clorosis in calcareous soils. FeEDDHA presents different positional isomers: the *ortho*−*ortho* (*o*,*o*), the *ortho*−*para* (*o*,*p*), and the *para*−*para* (*p*,*p*). Of these isomers, the *p*,*p* cannot chelate Fe in soil solution in a wide range of pH values, while both *o*,*o* and *o*,*p* can. The objective of this work was to compare the efficiency of both isomers (*o*,*o* and *o*,*p*) to provide Fe to two Strategy I plants (tomato and peach) in nutrient solution (pH $\approx$ 6.0), as well as in calcareous soil (pH $\approx$ 8.4; CALCIXEREPT). For this, chelates of both *o*,*o*-EDDHA and *o*,*p*-EDDHA with ^{57}Fe (a nonradioactive isotope of Fe) were used, where the ^{57}Fe acts as a tracer. The results obtained showed that the *o*,*o* isomer is capable of providing sufficient Fe to plants in both nutrient solution and calcareous soil. However, the *o*,*p* isomer is capable of providing sufficient Fe to plants in nutrient solution but not in calcareous soil.

KEYWORDS: Calcareous soil; *o*,*o*-EDDHA; *o*,*p*-EDDHA; iron chelates; positional isomers

INTRODUCTION

Iron (Fe) is one of the more abundant elements in soils. Nongraminaceous (Strategy I) plants need to reduce Fe^{3+} to Fe^{2+} prior to its absorption (*1*). This reduction is mediated by a plasma membrane ferric reductase [encoded by *AtFRO2*-like ("ferric reductase oxidase") genes (*2*−*5*)]. The Fe^{2+} is then taken up by an iron transporter [encoded by *AtIRT1*-like ("iron-regulated transporter") genes (*5*−*8*)]. In this process, the chelating agent is not taken up by the plants and remains outside (*9*). Iron chlorosis is frequent in dicot (Strategy I) plants, mainly in calcareous soils (*10*). In these soils, Fe bioavailability can be severely limited because of the low solubility of iron oxides and hydroxides at high pH (*10, 11*). Moreover, the elevated bicarbonate concentration of these soils, besides its effect on pH, can inhibit the Fe uptake mechanisms (*12*).

To correct iron chlorosis in Strategy I plants, the most common practice is the application of synthetic Fe-chelates (*13*). One of the most efficient and employed Fe-chelates is FeEDDHA, because of its great stability and solubility at high pH. The synthesis of commercial FeEDDHA usually produces several positional isomers and some unknown byproducts. The positional isomers of FeEDDHA are the *ortho*−*ortho* [*o*,*o*; iron^{3+} ethylenediamine-*N*,*N*′-bis(*o*-hydroxyphenylacetic) acid], the *ortho*−*para* [*o*,*p*; iron^{3+} ethylenediamine-*N*-(*o*-hydroxyphenylacetic)-*N*′-(*p*-hydroxyphenylacetic) acid], and the *para*−*para* [*p*,*p*; iron^{3+} ethylenediamine-*N*-(*p*-hydroxyphenylacetic)-*N*′-(*p*-hydroxyphenylacetic) acid] (*13*−*18*). Of these, the *p*,*p* cannot chelate Fe in soil solution in a wide range of pH values, while both *o*,*o* and *o*,*p* can (*13*). At first, only the *o*,*o* was authorized as a fertilizer in the European Union and the *o*,*p* was considered one impurity (*15*), but since 2003, the *o*,*p* has also been authorized (EC Regulation 2003/2003; *16*). Different experimental assays, all of them performed in nutrient solutions, have shown the capability of the *o*,*p* isomer to provide Fe to plants (*16, 18*). Some authors have shown that Fe-deficient cucumber plants reduce Fe^{3+} from *o*,*p*-EDDHA/Fe^{3+} at higher rates than Fe^{3+} from *o*,*o*-EDDHA/Fe^{3+} (*16, 18*). In the same way, they have shown that soybean plants grown in nutrient solution with *o*,*p*-EDDHA/Fe^{3+} presented higher Fe concentrations in leaves than plants grown with *o*,*o*-EDDHA/Fe^{3+} (*16, 18*).

Despite these results, there are other experimental assays that put into question the capability of the *o*,*p*-EDDHA/Fe^{3+} isomer to efficiently provide Fe to plants in calcareous soils. Lucena (*13*) showed that, after 3 days of interaction with different

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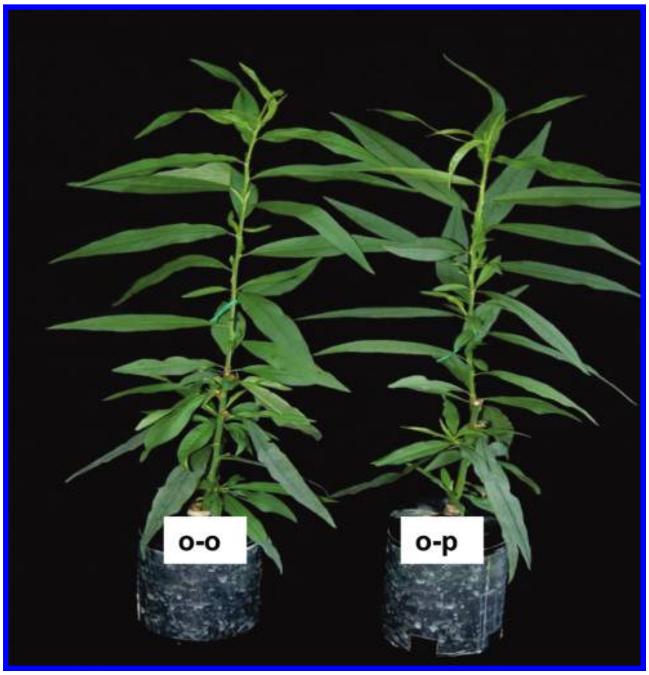

Figure 1. Peach plants grown in nutrient solution with the *o,o* (left) or the *o,p* (right) isomer at the end of the experiment, after 19 days of treatments.

calcareous soils, the percentage of Fe-chelate remaining in the soil solution was much higher when the FeEDDHA was applied as *o,o*-FeEDDHA than when applied as *o,p*-FeEDDHA or FeEDTA. Similarly, Schenkeveld et al. (*17*) found that the *o,p*-FeEDDHA isomer applied to a calcareous soil almost had disappeared from soil solution after 1 week of its application, while the *o,o*-FeEDDHA remained in the soil solution to a much larger extent.

The objective of this work was to compare the efficiency of both isomers (*o,o* and *o,p*) to provide Fe to two Strategy I plants (tomato and peach) in nutrient solution as well as in calcareous soil. For this, chelates of *o,o*-EDDHA and *o,p*-EDDHA with ^{57}Fe (a nonradioactive isotope of Fe) were used, where the ^{57}Fe acts as a tracer. Because the abundance of this isotope in soils is known (approximately 2.1%; *19*), the extra ^{57}Fe present in plant tissues is mainly the one provided by the *o,o*-^{57}FeEDDHA or the *o,p*-^{57}FeEDDHA applied.

MATERIALS AND METHODS

Plant Materials and Treatments. Two plant species were used: peach (*Prunus persica* L. Batsch cv. 'GF305') and tomato (*Lycopersicon esculentum* Mill. cv. 'Tres Cantos'). Peach stones were obtained from the "Instituto Valenciano de Investigaciones Agrarias (IVIA)". For germination, the peach stones were broken and seeds were removed. Seeds were then disinfected by submerging them during 24 h in a solution of TIRAM fungicide at 1%, prepared with sterilized water. After that, seeds were thoroughly washed with sterilized water and disinfected again with bleach (20%) during 5 min. After this, the seed coats were removed and seeds were sown in a tray containing sterilized perlite and situated in a growth chamber. Once germinated and with an adequate size (approximately 45−50 days after sowing), seedlings were transferred (1 seedling per pot) to either 550 mL plastic pots with nutrient solution (**Figure 1**) or to 3 L plastic pots with approximately 2750 g of a calcareous soil (**Figure 2**). The calcareous soil had the following characteristics: carbonates (73.3%), pH (8.4), texture (sand, 69%; lime, 16%; clay, 15%), active lime (13.2%), Fe extracted with oxalate (165 μg/g); according to USDA classification, it could be classified as a CALCIXEREPT. Tomato seeds were germinated on a tray containing sand. When appropriate (approximately after 15−20 days), seedlings were transferred (1 seedling per pot) to either 550 mL plastic pots with nutrient solution or to 3 L plastic pots with calcareous soil.

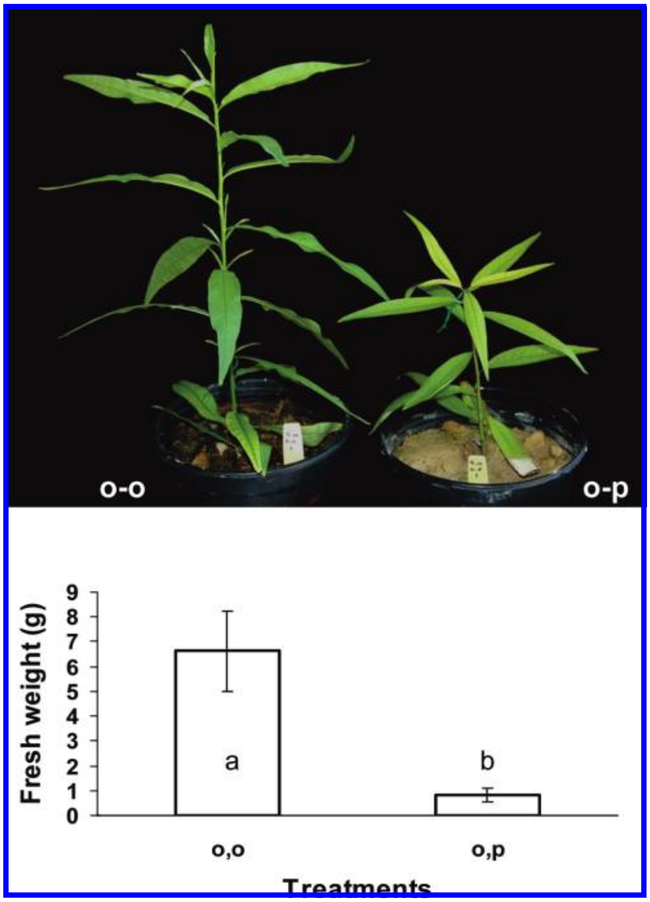

Figure 2. Peach plants grown in calcareous soil with the *o,o* (left) or the *o,p* (right) isomer at the end of the experiment, after 28 days of treatments. Fresh weight corresponds to the new shoot grown since the beginning of treatments. Different letters denote significant differences among treatments according to least significant difference ($\alpha = 0.05$). $x \pm$ standard error (SE) ($n = 4$).

Plants were grown in a growth chamber at 22−24 °C day/20−22 °C night temperatures, with relative humidity between 50 and 70%, and a 16 h photoperiod at a photosynthetic irradiance of 300 μmol m^{-2} s^{-1} provided by fluorescent tubes (Sylvania Cool White VHO, Norampac, Inc., Drummondville, Canada).

For peach plants, the treatments in pots with calcareous soil were applied once plants recovered from the transplant. The treatments consisted of two applications per pot and 1 week of 100 mL of basic nutrient solution containing 10 μM ^{57}FeEDDHA. Half of the plants were treated with *o,o*-^{57}FeEDDHA, and the other half of the plants were treated with *o,p*-^{57}FeEDDHA. For tomato plants grown in pots with calcareous soil, besides the treatments mentioned above for peach, some plants were treated with a similar quantity of Fe, with 50% of *o,o* and the other 50% of *o,p*, and some were not treated with ^{57}Fe. In an additional experiment, some tomato plants were also treated with a similar quantity of ^{57}FeEDTA.

For the treatments of both tomato and peach in nutrient solution, plants were transferred to 550 mL plastic pots continuously aerated (**Figure 1**), with half of them with basic nutrient solution containing 5 μM *o,o*-^{57}FeEDDHA and the other half of them containing 5 μM *o,p*-^{57}FeEDDHA.

In calcareous soil or in nutrient solution, both peach and tomato plants were treated for 18−28 days, depending upon the experiments. Plants grown in nutrient solution were replenished every 4 days with nutrient solution without Fe, and for the rest of the days, plants were replenished with deionized water.

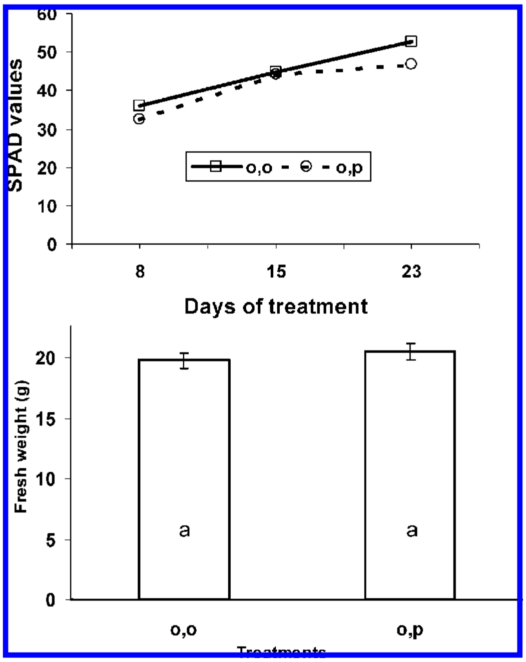

Figure 3. SPAD values along the treatments and shoot fresh weight at the end of the experiment in tomato plants grown in nutrient solution with either the *o,o* or the *o,p* isomer. Different letters denote significant differences among treatments according to least significant difference ($\alpha = 0.05$). $x \pm$ SE ($n = 4$).

The composition of the basic nutrient solution was as follows: 2 mM $Ca(NO_3)_2$, 0.75 mM K_2SO_4, 0.65 mM $MgSO_4$, 0.5 mM KH_2PO_4, 50 μM KCl, 10 μM H_3BO_3, 1 μM $MnSO_4$, 0.5 μM $CuSO_4$, 0.5 μM $ZnSO_4$, 0.05 μM $(NH_4)_6Mo_7O_{24}$, at pH ≈ 6.0. The *o,o*-^{57}FeEDDHA and *o,p*-^{57}FeEDDHA isomers were prepared by Laboratories JAER S.A. using a ^{57}Fe ferric salt and the *o,o*-EDDHA isomer (purity 97%), provided by SIGMA, or the *o,p*-EDDHA isomer (purity 95%), provided by SYNGENTA AG.

Determinations. During the experiments, the degree of chlorosis of the youngest leaves was periodically determined using the SPAD-Minolta chlorophyllmeter. The SPAD values determined by this instrument are positively related to leaf chlorophyll contents. At the end of the experiments, the entire shoots (tomato) or the new shoots grown since the beginning of treatments (peach) were harvested and their fresh weight and total content of natural abundance Fe and ^{57}Fe were determined using isotope pattern deconvolution as described before (*19*). For Fe determinations, samples were dried and then digested with nitric acid in a microwave oven. Total Fe and the contribution of natural abundance Fe and ^{57}Fe were determined using inductively coupled plasma−mass spectrometry (ICP−MS), model Agilent 7500c, at the University of Oviedo (Spain). Natural Fe includes the different isotopes present in nature (^{54}Fe, 5.85%; ^{56}Fe, 91.8%; ^{57}Fe, 2.1%; ^{58}Fe, 0.3%; *19*), while extra ^{57}Fe refers to the extra ^{57}Fe absorbed by plants and is provided by the ^{57}FeEDDHA isomers applied.

Statistical Analysis. Each treatment consisted of four plant replications. Results were statistically treated using the Statgraphics program. Least significant difference ($\alpha = 0.05$) was used to test for differences among means.

RESULTS AND DISCUSSION

When the isomers *o,o*-^{57}FeEDDHA and *o,p*-^{57}FeEDDHA were compared in nutrient solution, plants grew similarly in both treatments and did not show chlorosis symptoms (**Figures 1** and **3**). In both peach (**Figure 4**) and tomato (**Figure 5**), plants took up more ^{57}Fe from the *o,o* isomer than from the *o,p* one, although the differences were not significant.

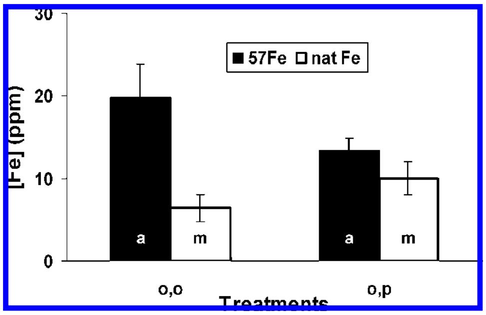

Figure 4. Concentrations of extra ^{57}Fe and natural abundance Fe in shoots of peach plants grown in nutrient solution with either the *o,o* (left) or the *o,p* isomer (right). Within each kind of Fe, different letters denote significant differences among treatments according to least significant difference ($\alpha = 0.05$). $x \pm$ SE ($n = 4$).

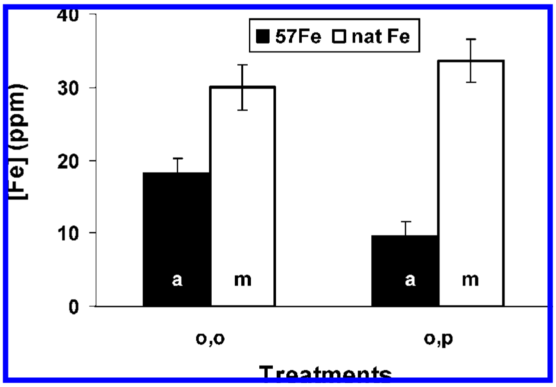

Figure 5. Concentrations of extra^{57}Fe and natural abundance Fe in shoots of tomato plants grown in nutrient solution with either the *o,o* (left) or the *o,p* isomer (right). Within each kind of Fe, different letters denote significant differences among treatments according to least significant difference ($\alpha = 0.05$). $x \pm$ SE ($n = 4$).

When the isomers *o,o*-^{57}FeEDDHA and *o,p*-^{57}FeEDDHA were compared in pots containing calcareous soil, both peach plants (**Figure 2**) and tomato plants (**Figure 6**) grew better and showed less degree of chlorosis (higher SPAD values) in the treatments with the *o,o* isomer than in the ones with the *o,p* isomer. Tomato plants grown with half of the ^{57}Fe applied as *o,o*-^{57}FeEDDHA and the other half applied as *o,p*-^{57}FeEDDHA behaved similarly to the ones treated with the *o,o* isomer alone (**Figure 6**). Both peach (**Figure 7**) and tomato (**Figure 8**) plants took up more ^{57}Fe from the *o,o* isomer than from the *o,p* one, with the differences being greatly significant. In the case of tomato, plants did not take any ^{57}Fe from the *o,p*-^{57}FeEDDHA isomer (**Figure 8**).

The tomato plants grown with half of the ^{57}Fe applied as *o,o*-^{57}FeEDDHA and the other half applied as *o,p*-^{57}FeEDDHA accumulated a quantity of ^{57}Fe in their leaves, intermediate between the ones treated with *o,o*-^{57}FeEDDHA alone and the ones treated with *o,p*-^{57}FeEDDHA alone (**Figure 8**), which suggests that there is not a synergistic effect of both isomers.

The results of ^{57}Fe accumulation indicate that the *o,p* isomer has less capability to provide Fe to plants than the *o,o* isomer, with this difference being more acute when plants are cultivated in calcareous soil. The results of this work agree with previous results, showing that the *o,o* isomer is able to keep Fe in the soil solution in a way much more efficient than the *o,p* isomer (*13, 17*). Consequently, although the *o,p*-FeEDDHA can be easily reduced by Strategy I plants, as occurred with FeEDTA, and to provide Fe to plants in nutrient solution (*16, 18, 20*), this behavior cannot be used as a criterion to define its efficacy in calcareous soils. In soils, Fe should be in the soil solution to be easily reduced by roots. As shown in **Figure 9**,

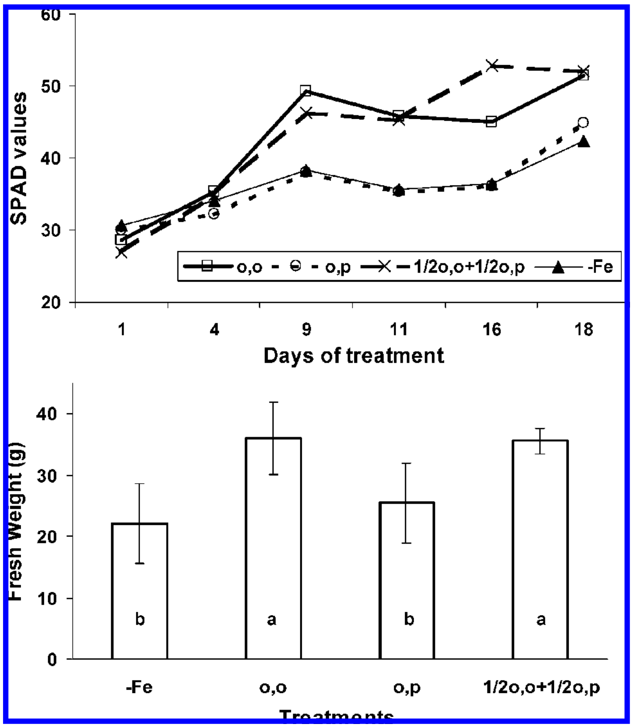

Figure 6. SPAD values along the treatments and shoot fresh weight in tomato plants grown in calcareous soil without Fe (−Fe), with the *o,o* isomer (o,o), with the *o,p* isomer (o,p), or with half of the FeEDDHA applied as *o,o* and the other half applied as *o,p* ($^1/_2$o,o + $^1/_2$o,p). Different letters denote significant differences among treatments according to least significant difference ($\alpha = 0.05$). $x \pm$ SE ($n = 4$).

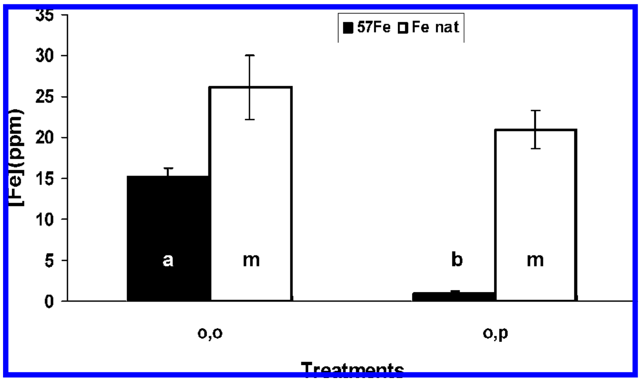

Figure 7. Concentrations of extra ^{57}Fe and natural abundance Fe in shoots of peach plants grown in calcareous soil with either the *o,o* (left) or the *o,p* (right) isomer. Within each kind of Fe, different letters denote significant differences among treatments according to least significant difference (α = 0.05). $x \pm$ SE ($n = 4$).

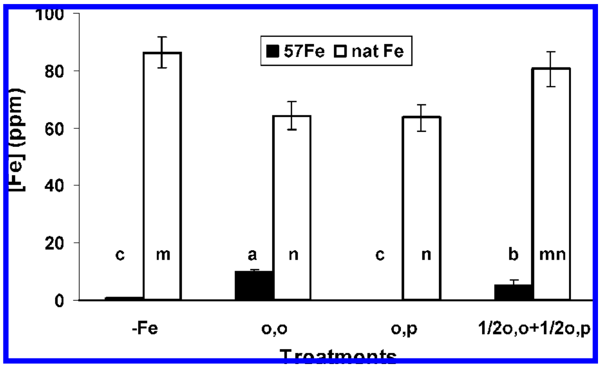

Figure 8. Concentrations of extra^{57}Fe and natural abundance Fe in shoots of tomato plants grown in calcareous soil without Fe (−Fe), with the *o,o* isomer (o,o), with the *o,p* isomer (o,p), or with half of the FeEDDHA applied as *o,o* and the other half applied as *o,p* ($^1/_2$o,o + $^1/_2$o,p). Within each kind of Fe, different letters denote significant differences among treatments according to least significant difference ($\alpha = 0.05$). $x \pm$ SE ($n = 4$).

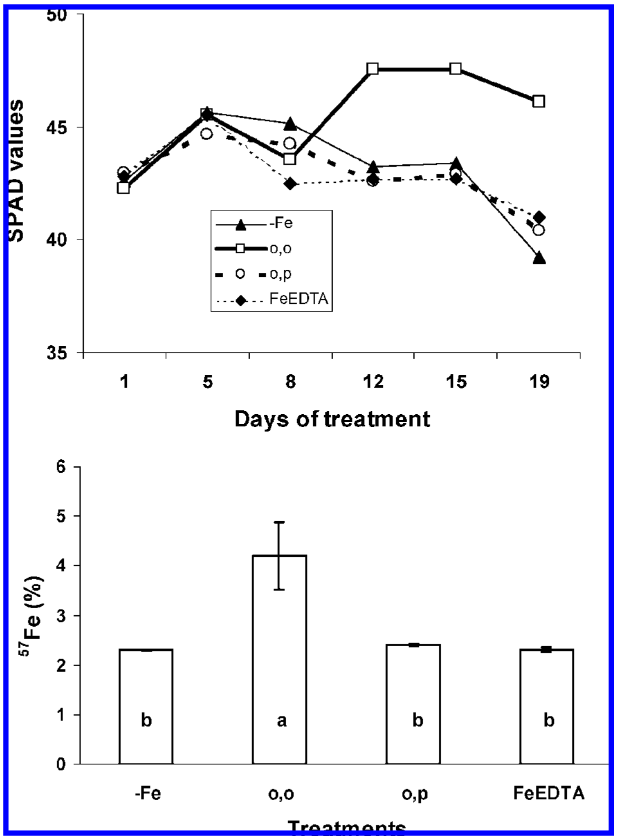

Figure 9. SPAD values along the treatments and percentage of total ^{57}Fe in shoots of tomato plants grown in calcareous soil without Fe (−Fe), with the *o,o* isomer (o,o), with the *o,p* isomer (o,p), or with FeEDTA (FeEDTA). Different letters denote significant differences among treatments according to least significant difference ($\alpha = 0.05$). $x \pm$ SE ($n = 4$).

o,p-^{57}FeEDDHA behaves similarly to ^{57}FeEDTA in its inability to provide ^{57}Fe to tomato plants grown in calcareous soils, while the *o,o*-^{57}FeEDDHA isomer was able to provide ^{57}Fe to plants, which were greener (higher SPAD values) than those in the *o,p*-^{57}FeEDDHA or ^{57}FeEDTA treatments (**Figure 9**). The percentage of total ^{57}Fe in shoots of the −Fe, *o,p*-^{57}FeEDDHA, or ^{57}FeEDTA treatments was around 2.1% (**Figure 9**), which means that none of these treatments supplied any appreciable additional ^{57}Fe to plants (it should be noted that the percentage of ^{57}Fe in soils is around 2.1%; *19*). However, in the *o,o*-^{57}FeEDDHA treatment, the percentage of total ^{57}Fe in shoots was around 4%, which implies that this treatment did provide additional ^{57}Fe to plants. All of these results agree with the fact that FeEDTA and *o,p*-FeEDDHA have less capability to keep Fe in the soil solution as compared to the *o,o*-FeEDDHA (*13, 17, 21*), despite both of them being easily reduced by roots (*16, 18, 20*).

In conclusion, the results of this work and results of other works show that the *o,p*-FeEDDHA isomer is easily reduced by Strategy I plants and has the capability to provide sufficient Fe to plants in nutrient solution but not in calcareous soils, perhaps because it is not stable in these soils, similarly as occurred with FeEDTA.

ABBREVIATIONS USED

o,*o*-FeEDDHA, iron^{3+} ethylenediamine-*N*,*N′*-bis(*o*-hydroxyphenylacetic) acid; *o*,*p*-FeEDDHA, iron^{3+} ethylenediamine-*N*-(*o*-hydroxyphenylacetic)-*N′*-(*p*-hydroxyphenylacetic) acid.

LITERATURE CITED

(1) Chaney, R. L.; Brown, J. C.; Tiffin, L. O. Obligatory reduction of ferric chelates in iron uptake by soybeans. *Plant Physiol.* **1972**, *50*, 208–213.

(2) Robinson, N. J.; Procter, C. M.; Connolly, E. L.; Guerinot, M. L. A ferric-chelate reductase for iron uptake from soils. *Nature* **1999**, *397*, 694–697.

(3) Waters, B. M.; Blevins, D. G.; Eide, D. J. Characterization of FRO1, a pea ferric-chelate reductase involved in root iron acquisition. *Plant Physiol.* **2002**, *129*, 85–94.

(4) Li, L. H.; Cheng, X. D.; Ling, H. Q. Isolation and characterization of Fe(III)-chelate reductase gene LeFRO1 in tomato. *Plant Mol. Biol.* **2004**, *54*, 125–136.

(5) Waters, B. M.; Lucena, C.; Romera, F. J.; Jester, G. G.; Wynn, A. N.; Rojas, C. L.; Alcántara, E.; Pérez-Vicente, R. Ethylene involvement in the regulation of the H^+-ATPase CsHA1 gene and of the new isolated ferric reductase CsFRO1 and iron transporter CsIRT1 genes in cucumber plants. *Plant Physiol. Biochem.* **2007**, *45*, 293–301.

(6) Eide, D.; Broderius, M.; Fett, J.; Guerinot, M. L. A novel iron-regulated metal transporter from plants identified by functional expression in yeast. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 5624–5628.

(7) Eckhardt, U.; Mas Marques, A.; Buckhout, T. J. Two iron-regulated cation transporters from tomato complement metal uptake deficient yeast. *Plant Mol. Biol.* **2001**, *45*, 437–448.

(8) Cohen, C. K.; Garvin, D. F.; Kochian, L. V. Kinetic properties of a micronutrient transporter from *Pisum sativum* indicate a primary function in Fe uptake from the soil. *Planta* **2004**, *218*, 784–792.

(9) Römheld, V.; Marschner, H. Mechanisms of iron uptake by peanut plants. I. Fe^{III} reduction, chelate splitting, and release of phenolics. *Plant Physiol.* **1983**, *71*, 949–954.

(10) Römheld, V.; Marschner, H. Mobilization of iron in the rhizosphere of different plant species. *Adv. Plant Nutr.* **1986**, *2*, 155–204.

(11) Hell, R.; Stephan, U. W. Iron uptake, trafficking and homeostasis in plants. *Planta* **2003**, *216*, 541–551.

(12) Lucena, C.; Romera, F. J.; Rojas, C. L.; García, M. J.; Alcántara, E.; Pérez-Vicente, R. Bicarbonate blocks the expression of several genes involved in the physiological responses to Fe deficiency of Strategy I plants. *Funct. Plant Biol.* **2007**, *34*, 1002–1009.

(13) Lucena, J. J. Synthetic iron chelates to correct iron deficiency in plants. In *Iron Nutrition in Plants and Rhizospheric Microorganisms*; Barton, L. L., Abadía, J., Eds.; Springer: Dordrecht, The Netherlands, 2007; pp 103−128.

(14) Bermúdez, D.; Juárez, M.; Jordá, J. D.; Sánchez-Andreu, J. J.; Lucena, J. J.; Sánchez-Sánchez, A. Effect of pH on the stability of the chelates FeEDDHA, FeEDDHMA and their isomers. *Agrochimica* **2002**, *5*, 202–210.

(15) Gómez-Gallego, M.; Sierra, M. A.; Alcázar, R.; Ramírez, P.; Piñar, C.; Mancheño, M. J.; García-Marco, S.; Yunta, F.; Lucena, J. J. Synthesis of *o*,*p*-EDDHA and its detection as the main impurity in *o*,*o*-EDDHA commercial iron chelates. *J. Agric. Food. Chem.* **2002**, *50*, 6395–6399.

(16) Hernández-Apaolaza, L.; García-Marco, S.; Nadal, P.; Lucena, J. J. Structure and fertilizer properties of byproducts formed in the synthesis of EDDHA. *J. Agric. Food Chem.* **2006**, *54*, 4355–4363.

(17) Schenkeveld, W. D. C.; Reichwein, A. M.; Temminghoff, E. J. M.; Riemsdijk, W. H. The behaviour of EDDHA isomers in soils as influenced by soil properties. *Plant Soil.* **2007**, *290*, 85–102.

(18) García-Marco, S.; Martínez, N.; Yunta, F.; Hernández-Apaolaza, L.; Lucena, J. J. Effectiveness of ethylenediamine-*N*(*o*-hydroxyphenylacetic)-*N′*(*p*-hydroxy-phenylacetic) acid (*o*,*p*-EDDHA) to supply iron to plants. *Plant Soil* **2006**, *279*, 31–40.

(19) Rodriguez-Castrillon, J. A.; Moldovan, M.; García, J. I.; Lucena, J. J.; García-Tomé, M. L.; Hernández-Apaolaza, L. Isotope pattern deconvolution as a tool to study iron metabolism in plants. *Anal. Bioanal. Chem.* **2008**, *390*, 579–590.

(20) Lucena, J. J.; Chaney, R. L. Synthetic iron chelates as substrates of root ferric chelate reductase in green stressed cucumber plants. *J. Plant Nutr.* **2006**, *29*, 423–439.

(21) Cantera, R. G.; Zamarreño, A. M.; García-Mina, J. M. Characterization of comercial iron chelates and their behavior in an alkaline and calcareous soil. *J. Agric. Food Chem.* **2002**, *50*, 7609–7615.

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