

## Potential Use of Biodegradable Chelate *N*-(1,2-Dicarboxyethyl)-D,L-aspartic Acid/Fe<sup>3+</sup> as an Fe Fertilizer

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In the past several years, concern about the environmental fate of recalcitrant synthetic ligands (e.g., EDTA) has increased. The use of new biodegradable chelating agents such as imidodisuccinic acid (IDHA) has been proposed as an alternative. However, its application as an iron ligand to correct iron chlorosis in agriculture has not yet been studied. Then the objective of this work is to determine the fertilizer capacity of IDHA/Fe<sup>3+</sup> using interaction assays with soils and soil materials and evaluating Fe nutrition of efficient and susceptible plants. Interaction of IDHA/Fe<sup>3+</sup> with soil materials produces a reduction of the amount of soluble Fe. This is in good agreement with studies on the stability of the IDHA/Fe<sup>3+</sup> chelate. In general, plant response to IDHA/Fe<sup>3+</sup> in hydroponics is acceptable and better than that to EDTA/Fe<sup>3+</sup>. This good behavior seems to be related to the lower coordination of the iron in IDHA/Fe<sup>3+</sup> with respect to EDTA/Fe<sup>3+</sup>.

**KEYWORDS:** Iron; chelates; chlorosis; IDHA; soybean; cucumber

### INTRODUCTION

Iron chlorosis is a nutritional disorder in plants that induces leaf yellowing that affects the development and decreases the yield of many crops (1). It is a widespread agricultural problem, especially in crops grown on calcareous soil, where calcium carbonate buffers soil solution pH in the range of 7.5–8.5 (2) and a high bicarbonate concentration is present (3).

Fertilization with Fe chelates is the most effective agricultural practice to provide iron to deficient crops, and *o,o*-EDDHA (Figure 1) and its analogues are the most efficient chelating agents (4). *o,o*-EDDHA/Fe<sup>3+</sup> and its analogues have two phenolic groups replacing two of the carboxylate groups of EDTA/Fe<sup>3+</sup>, which increases the stability of the iron chelate. Nowadays, in the industrial synthesis of commercial products (5), the isomers *o,o*-EDDHA/Fe<sup>3+</sup> and *o,p*-EDDHA/Fe<sup>3+</sup> are formed. *o,p*-EDDHA has only five functional groups that are able to complex the Fe<sup>3+</sup> ion, so its stability is lower than that of *o,o*-EDDHA/Fe<sup>3+</sup> (6), but higher than the stability of EDTA/Fe<sup>3+</sup>. Recently, García-Marco et al. (7) suggested that *o,p*-EDDHA/Fe<sup>3+</sup> presents several properties that justify its use along with *o,o*-EDDHA/Fe<sup>3+</sup>: it was the best substrate tested for the Fe chelate reductase (FeCR) in cucumber plants, it was faster than *o,o*-EDDHA/Fe<sup>3+</sup> at regreening Fe chlorotic soybean plants, and the chelating agent was able to solubilize Fe native from insoluble materials. However, due to its lower stability constant, *o,p*-EDDHA/Fe<sup>3+</sup> is expected to disappear sooner than *o,o*-EDDHA/Fe<sup>3+</sup> from alkaline soil solutions. This behavior may be related to the presence of only five bonds between the Fe and the ligand (8). Thus, while *o,p*-EDDHA/Fe<sup>3+</sup> can be a

good chelate to correct Fe chlorosis, it is likely to be active for only a short time due to its low stability in soils (7).

EDTA/Fe<sup>3+</sup> (see Figure 1) is also used as a ligand for Fe fertilizer, but due to its low stability, its use is recommended for hydroponics, fertigation, or other fertilization practices where a thorough interaction between the chelate and high-pH soils is avoided (9). EDTA is recalcitrant, so the concern about the environmental fate of EDTA applied in agriculture has risen (10). The use of EDTA in agriculture is low with respect to that in other industrial applications; nonetheless, the study of a more environmentally friendly chelate is convenient.

Recently, the biodegradable chelating agent IDHA, commonly known as imidodisuccinic acid, has been proposed for its use in agriculture (11). IDHA shares structural similarities with EDTA, but similarly to *o,p*-EDDHA, it contains only five functional groups able to bind Fe<sup>3+</sup> (see Figure 1). Then the stability of its Fe chelate is expected to be lower than the EDTA/Fe<sup>3+</sup> stability, but its efficacy to provide iron to plants has not yet been demonstrated. Besides the ability to maintain Fe in the soil solution, the efficacy of an Fe chelate depends on the ability of the plant roots to take up Fe from the Fe chelate and on the capacity of the free chelating agent to solubilize native Fe present in the solid phases (12). In this paper we evaluate the chemical stability and reactivity of IDHA/Fe<sup>3+</sup> with several soils and soil materials and its efficacy to provide Fe to cucumber (an Fe-efficient plant) and to soybean (an Fe-susceptible plant).

### MATERIALS AND METHODS

**1. Stability of IDHA Chelates in Agronomic Conditions. 1.1. Stability of IDHA/Fe<sup>3+</sup> in Solution. Effect of pH.** The amount of soluble and chelated Fe in IDHA/Fe<sup>3+</sup> and EDTA/Fe<sup>3+</sup> remaining in solution

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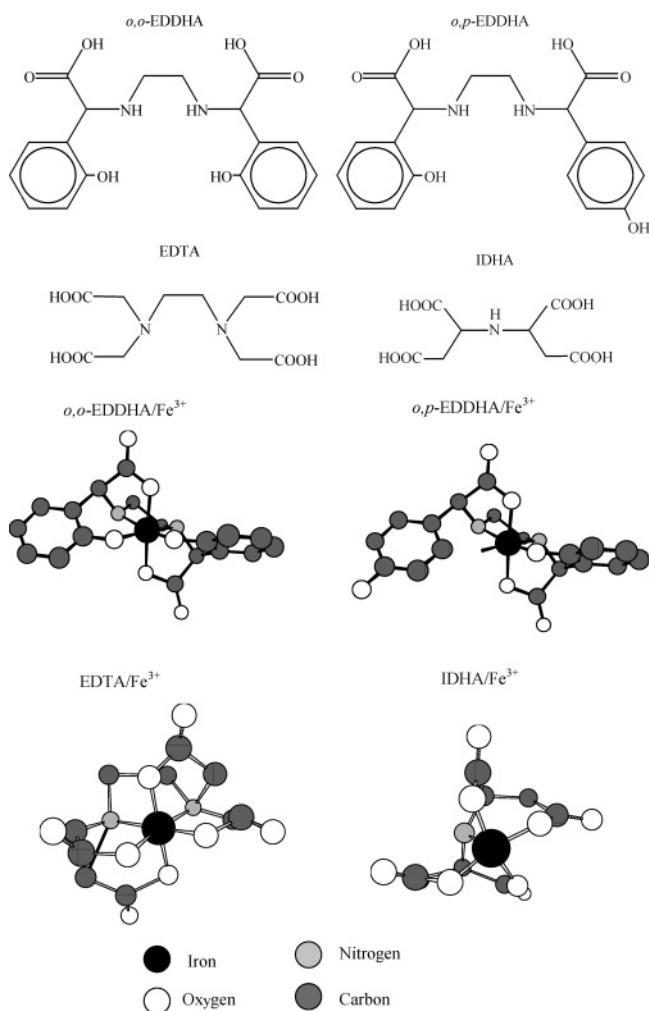


Figure 1. Chelating agents and chelates described in the text.

at different pH values was studied following the method of Álvarez-Fernández et al. (13). Two experimental approaches were used, adjusting the pH initially, or using a pH buffer. For the first one, 5 mL of the  $2.0 \times 10^{-3}$  M IDHA/Fe<sup>3+</sup> or EDTA/Fe<sup>3+</sup> solution was added to 25 mL of type 1 water and 5 mL of 0.10 M CaCl<sub>2</sub> solution. Then the pH was adjusted to 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, or 10.0 with HCl or NaOH and the volume raised to 50 mL. For the second approach IDHA/Fe<sup>3+</sup> solutions were similarly prepared by adding 5 mL of  $2.0 \times 10^{-3}$  M IDHA/Fe<sup>3+</sup>, 5 mL of buffer solution (HEPES, MES, CAPS, AMPSO,  $10^{-2}$  M), and 0.10 M CaCl<sub>2</sub> before the pH was adjusted and the volume raised to 50 mL.

Samples were placed in a shaker bath at 25 °C and 56 min<sup>-1</sup> for 3, 7, or 14 days. Interactions were made in the dark to avoid the possible photodecomposition of the chelates in the light. After agitation samples were filtered through a 0.45 μm Millipore membrane, and the pH was measured using an Orion Research ion analyzer (EA920). One aliquot was prepared by the addition of 6.0 M HCl (3.0 mL of sample, 0.30 mL of 6.0 M HCl), and the total element was quantified by atomic absorption using a Perkin-Elmer Analyst 800 spectrophotometer.

**1.2. Interaction of IDHA/Fe<sup>3+</sup> with Several Soils and Soil Materials.** To test the amount of IDHA/Fe<sup>3+</sup> that can be available to plants after its addition to soils, interaction experiments have been performed following Álvarez-Fernández et al. (13). In brief, the solid phases and amounts used were 0.2 g of peat, 0.1 g of ferrihydrite, 0.2 g of Camontmorillonite, 0.2 g of illite, 2 g of a standard soil, 2 g of Sudanell soil, and 2 g of Carlet soil. The materials have been described elsewhere (13) (see also the Supporting Information, p A). Sudanell and Carlet soil characteristics are presented in Table 1. Each of the materials was allowed to interact with 5 mL of  $0.20 \times 10^{-3}$  M solutions of IDHA/Fe<sup>3+</sup>, EDTA/Fe<sup>3+</sup>, and *o,o*-EDDHA/Fe<sup>3+</sup>, 5 mL of  $2.0 \times 10^{-2}$  M CaCl<sub>2</sub>,

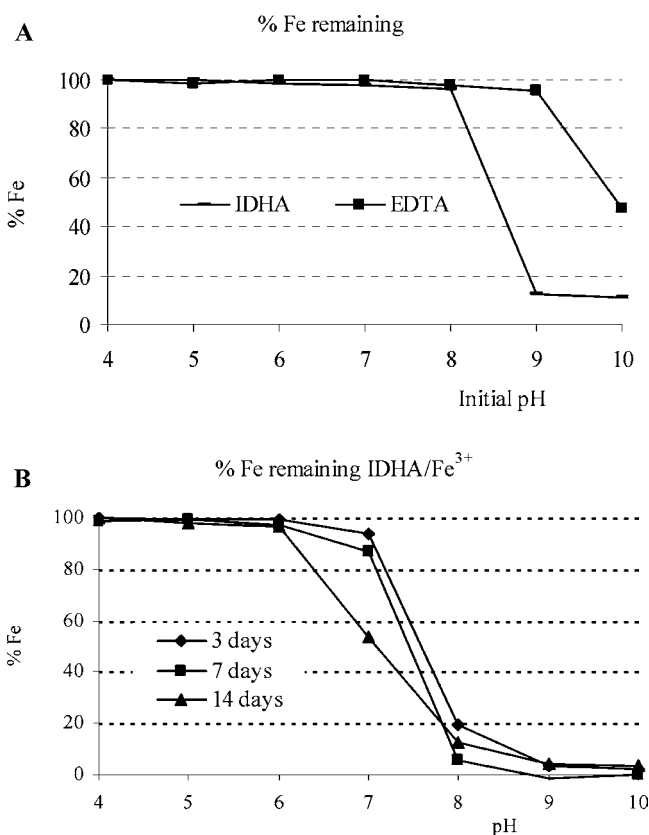


Figure 2. (A, top) Effect of the initial pH on the Fe remaining in 0.01 M Ca<sup>2+</sup> solutions for EDTA/Fe<sup>3+</sup> and IDHA/Fe<sup>3+</sup> after 3 days. (B, bottom) Effect of the pH on IDHA/Fe<sup>3+</sup> remaining in buffered 0.01 M Ca<sup>2+</sup> solutions and for the three periods considered.

Table 1. Chemical and Physical Characteristics of the Soils Used in the Interaction Assays

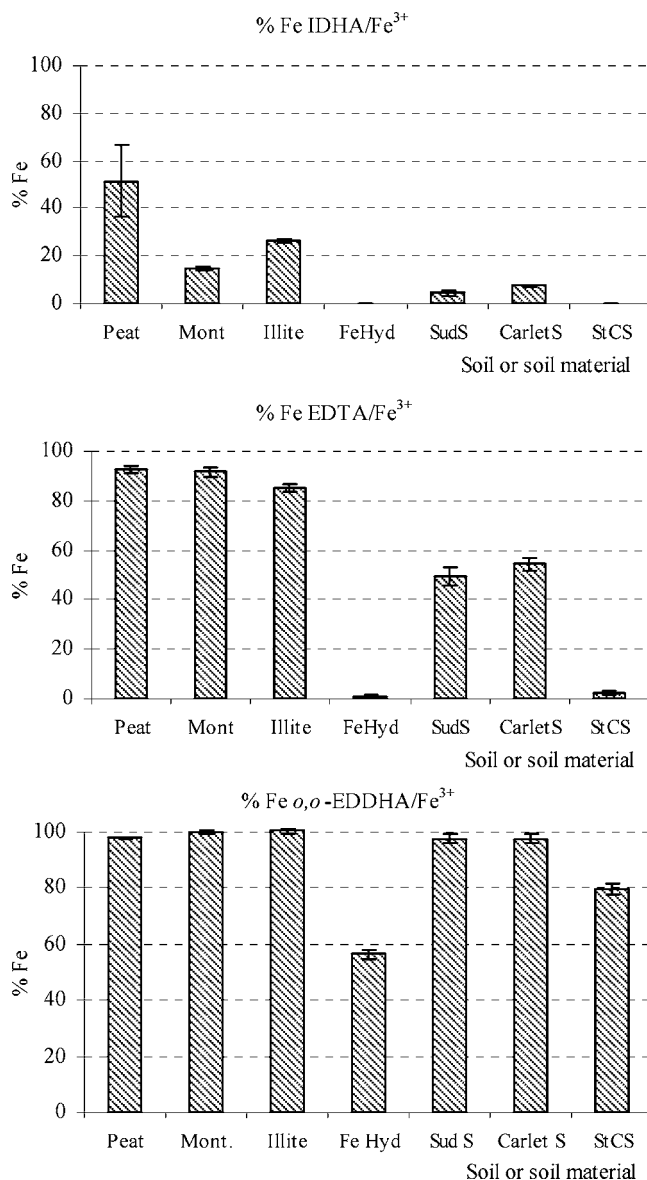
	Sudanell	Carlet
sand fraction (%)	46	65
silt fraction (%)	28	12
clay fraction (%)	26	23
texture	sandy clay loam	sandy loam
pH (H <sub>2</sub> O)	7.82	7.69
pH (KCl)	7.23	7.15
EC, extract, 1:5 (dS·m <sup>-1</sup> )	0.188	0.235
OM oxidizable concn (g·kg <sup>-1</sup> )	24.0	8.0
[N], Kjeldahl (g·kg <sup>-1</sup> )	1.40	0.79
C/N	9.9	10.5
CaCO <sub>3</sub> total concn (g·kg <sup>-1</sup> )	179	150
active lime concn (g·kg <sup>-1</sup> )	52	40
macronutrient concn (Soltampour, cmol <sub>c</sub> ·kg <sup>-1</sup> )		
Ca	1.55	1.76
Mg	0.66	1.72
K	1.02	1.26
Na	0.047	0.150
micronutrient concn (Soltampour, mg·kg <sup>-1</sup> )		
Fe	27.0	14.3
Mn	5.4	13.0
Cu	47.1	1.46
Zn	4.3	1.08

and  $2.0 \times 10^{-3}$  M HEPES (pH 8) in 60 mL sterile polyethylene flasks. Also a blank for the chelate and blanks for the materials were prepared. Interactions were made in the dark to avoid the slow photodecomposition of the chelates in the light. The flasks were shaken for 1 h at 25 °C and 56 min<sup>-1</sup> and then were allowed to stand for 3 days in a thermostated incubator at 25 °C. Finally, the solutions were filtered

**Table 2.** Effect of Different Iron Chelates on the SPAD Index Measured in the Second Leaves in Cucumber Plants<sup>a</sup>

treatment	SPAD at various times following treatment									
	7 days	9 days	11 days	14 days	18 days	22 days	24 days	28 days	30 days	35 days
EDTA/Fe <sup>3+</sup>	38.6 c	35.4 b	41.0 b	34.4 b	33.0 b	30.0 ns	32.4 ns	28.5 ns	27.6 ns	24.8 ns
IDHA/Fe <sup>3+</sup>	43.6 b	42.7 a	46.4 a	40.6 a	39.6 a	32.9	36.6	30.4	28.2	22.4
<i>o,o</i> -EDDHA/Fe <sup>3+</sup>	48.1 a	44.2 a	48.5 a	41.8 a	40.2 a	35.4	28.9	28.7	28.0	21.4

<sup>a</sup> Different letters in the same column denote significant differences among the treatments. ns = nonsignificant.

**Figure 3.** Results of Fe remaining with respect to the total Fe added for the different chelates in the interaction test.

through 0.45  $\mu\text{m}$  Millipore membranes; the pH and soluble Fe, Cu, Mn, and Zn by atomic absorption spectroscopy (AAS) were measured.

**2. Biological Experiments.** Two biological experiments have been carried out to test the ability of IDHA/Fe<sup>3+</sup> to provide Fe to two strategy I model plants: cucumber, which is Fe-efficient, and soybean, which is Fe-susceptible. Both experiments have been done in hydroponics with pH buffer to simulate calcareous soil conditions.

**2.1 Fe Uptake from IDHA/Fe<sup>3+</sup> by Cucumber Grown in Hydroponics.** Cucumber (*Cucumis sativus* L. cv. Ashley) plants were obtained from cucumber seeds germinated in standard seed germination papers. The bottom of the rolled paper was placed in a macronutrient solution

**Table 3.** Effect of the Different Chelate Treatments on the Dry Weight and Leaf Fe, Mn, and Zn Concentrations in Cucumber Plants Grown in Hydroponics 24 and 37 Days after Transplanting<sup>a</sup>

treatment	biomass (g·plant <sup>-1</sup> , dw)	concn in leaves (mg/g of dw)		
		Fe	Mn	Zn
24 Days after Transplanting				
EDTA/Fe <sup>3+</sup>	1.73 ns	29.9 b	38.7 a	20.2 c
IDHA/Fe <sup>3+</sup>	1.83	36.9 a	33.3 ab	23.8 b
<i>o,o</i> -EDDHA/Fe <sup>3+</sup>	1.74	33.1 ab	32.5 b	27.0 a
37 Days after Transplanting				
EDTA/Fe <sup>3+</sup>	2.62 ns	20.5 b	56.0 ns	131.9 ns
IDHA/Fe <sup>3+</sup>	2.84	20.7 b	53.2	128.2
<i>o,o</i> -EDDHA/Fe <sup>3+</sup>	2.98	24.6 a	41.5	100.7

<sup>a</sup> Different letters in the same column denote significant differences among the treatments according to Duncan's multiple range test ( $\alpha = 0.05$ ). ns = nonsignificant.

( $1.0 \times 10^{-3}$  M Ca(NO<sub>3</sub>)<sub>2</sub>,  $9.0 \times 10^{-4}$  M KNO<sub>3</sub>,  $3.0 \times 10^{-4}$  M MgSO<sub>4</sub>,  $1.0 \times 10^{-4}$  M KH<sub>2</sub>PO<sub>4</sub>) in the growth chamber for 7 days (16 h with diffuse light at 30 °C and 8 h at night at 25 °C).

The stems of two plants were wrapped together with foam and placed in 2 L polyethylene vessels (three holes in the lid, six plants per pot). The vessels contained 2 L of continuously aerated solution of the following initial composition: (macronutrients)  $1.0 \times 10^{-3}$  M Ca(NO<sub>3</sub>)<sub>2</sub>,  $9.0 \times 10^{-4}$  M KNO<sub>3</sub>,  $3.0 \times 10^{-4}$  M MgSO<sub>4</sub>,  $1.0 \times 10^{-4}$  M KH<sub>2</sub>PO<sub>4</sub>; [cationic micronutrients (buffered micronutrient solution)]  $2.5 \times 10^{-6}$  M MnSO<sub>4</sub>,  $1.0 \times 10^{-6}$  M CuSO<sub>4</sub>,  $10.0 \times 10^{-6}$  M ZnSO<sub>4</sub>,  $1.0 \times 10^{-6}$  M NiCl<sub>2</sub>,  $1.0 \times 10^{-6}$  M CoSO<sub>4</sub>,  $115.5 \times 10^{-6}$  M EDTANa<sub>2</sub>,  $231 \times 10^{-6}$  M KOH; (anionic micronutrients)  $35.0 \times 10^{-6}$  M NaCl,  $10.0 \times 10^{-6}$  M H<sub>3</sub>BO<sub>3</sub>,  $5.0 \times 10^{-8}$  M Na<sub>2</sub>MoO<sub>4</sub>. The pH was buffered at 7.5 with  $1.0 \times 10^{-6}$  M HEPES and 2 g of CaCO<sub>3</sub> per pot. Water was added every 2 days, and the solution was renewed every week.

Iron ( $5.0 \times 10^{-6}$  M) was added as the following treatments: IDHA/Fe<sup>3+</sup> (considering 2.79% Fe, analytical total Fe 2.79%), *o,o*-EDDHA/Fe<sup>3+</sup> (standard), and EDTA/Fe<sup>3+</sup> (standard). Four replications were prepared for each treatment. During the experiment, SPAD readings with a chlorophyll meter (Minolta SPAD-502) were taken for all the leaf stages (average of three readings per leaf) at several times, although only values measured for the second leaf stage (the youngest fully open leaf at the start of the treatment period) have been presented in the results, since they were the most representative of the whole plant.

Whole plants were sampled 24 days (one pair of plants) and 37 days (two pairs of plants) after transplanting. The sampled roots and leaves were separated and washed first with Tween 80 in 0.1 M HCl for 10 s (14) and then with abundant distilled water, weighed, and dried. Micronutrients were determined in the leaves after the dry digestion procedure by AAS.

**2.2 Fe Uptake from IDHA/Fe<sup>3+</sup> by Soybean Grown in Hydroponics.** Soybean (*Glycine max* L. cv. Oshumi) plants were used in this biological experiment. Plants were obtained from soybean seeds that were germinated in the standard seed growing procedure in closed sterilized trays. The seeds were washed with water and commercial NaClO diluted 10% for 30 min, and then they were rinsed with water and finally with distilled water. The seeds were placed in the trays on a cellulose paper soaked with 50 mL of distilled water, and another

**Table 4.** Effect of Different Iron Chelates on the SPAD Index Measured in the Second Leaves in Soybean Plants<sup>a</sup>

treatment	SPAD at various times following treatment								
	7 days	10 days	12 days	14 days	17 days	19 days	21 days	24 days	26 days
EDTA/Fe <sup>3+</sup>	27.6 b	25.7 b	28.0 b	30.3 c	30.4 b	33.1 b	32.2 b	31.2 b	32.3 c
IDHA/Fe <sup>3+</sup>	29.7 a	28.7 a	32.4 a	33.6 a	34.6 a	37.0 a	36.3 a	36.0 a	37.4 a
<i>o,o</i> -EDDHA/Fe <sup>3+</sup>	29.9 a	28.2 a	31.4 a	32.2 b	33.7 a	36.2 a	35.9 a	35.4 a	34.9 b

<sup>a</sup> Different letters in the same column denote significant differences among the treatments. ns = nonsignificant.

**Table 5.** Effect of the Different Chelate Treatments on the Dry Weight and Leaf Fe, Mn, and Zn Concentrations in Soybean Plants Grown in Hydroponics after 14 and 27 Days<sup>a</sup>

	biomass (g·plant <sup>-1</sup> , dw)	concn in leaves (μg/g of dw)		
		Fe	Mn	Zn
14 Days after Transplanting				
EDTA/Fe <sup>3+</sup>	1.04 b	49.9 b	51.2 a	43.5 b
IDHA/Fe <sup>3+</sup>	1.16 a	59.9 a	48.4 ab	50.7 a
<i>o,o</i> -EDDHA/Fe <sup>3+</sup>	1.11 ab	53.6 ab	44.1 b	53.2 a
27 Days after Transplanting				
EDTA/Fe <sup>3+</sup>	2.55 b	45.0 b	60.3 a	56.2 ns
IDHA/Fe <sup>3+</sup>	3.41 a	50.4 a	44.2 b	54.0
<i>o,o</i> -EDDHA/Fe <sup>3+</sup>	3.23 a	47.4 ab	48.1 b	55.7

<sup>a</sup> Different letters in the same column denote significant differences among the treatments according to Duncan's multiple range test ( $\alpha = 0.05$ ).

paper was placed over them. A 30 mL portion of distilled water and 20 mL of CaSO<sub>4</sub> (1.0 × 10<sup>-3</sup> M) were added. The trays were placed in a thermostated stove, without light, at 28 °C for 3 days. After this time, seedlings of similar development were placed on a holed plate floating over containers containing diluted nutritive solution for 7 days. The device and the treatments are the same as those described in the previous experiment. The SPAD index was also recorded at several times, and whole plants were sampled 14 and 27 days after transplanting. Micronutrients were determined in the leaves after the dry digestion procedure by AAS.

## RESULTS AND DISCUSSION

### 1. Stability of IDHA Chelates in Agronomic Conditions.

**1.1. Stability of IDHA/Fe<sup>3+</sup> in Solution. Effect of pH.** During the experiments without pH buffer, the pH was well maintained for the low pH solutions, but the higher pH solutions presented a considerable pH decrease, in part due to the OH<sup>-</sup> consumption for the Fe(OH)<sub>3</sub> precipitation. In **Figure 2A** the effect of the initial pH on the percentage of soluble Fe remaining after 3 days for each chelate in solution is presented. IDHA/Fe<sup>3+</sup> presents lower stability than EDTA/Fe<sup>3+</sup> in solution. This lower stability could also be predicted from the theoretical models (e.g., MinteqA2; 15) using the stability constants (10), but the theoretical chemical speciation of the Fe chelate of IDHA/Fe<sup>3+</sup> (see the Supporting Information, p B) indicates that it presents low stability even at low pH and in the absence of metal competitors since protons also compete for the ligand. Since the experimental data indicate a better stability at acidic and neutral pH values, the validity of the stability constants presented in the literature is questionable.

In **Figure 2B** the amount of Fe remaining in solution is presented for IDHA/Fe<sup>3+</sup> when buffered solutions are used. IDHA/Fe<sup>3+</sup> remains in solution till pH 7 in the first week, but after that a decrease in its presence is observed at this pH. This could be a consequence of the biodegradability of the product. Then it is not expected to have a long-lasting effect.

In equilibrium IDHA/Fe<sup>3+</sup> should not be present in solution in calcareous soils (pH around 8.5) where iron chlorosis is more severe, but it can remain in nutrient solutions of pH 6.5 or lower, such as those used in fertirrigation or hydroponics.

**1.2. Interaction of IDHA/Fe<sup>3+</sup> with Several Soils and Soil Materials.** In **Figure 3** the amount of Fe remaining in solution with respect to the total Fe added in the interaction test is presented. More retention is observed for IDHA/Fe<sup>3+</sup> than for EDTA/Fe<sup>3+</sup> and very little for *o,o*-EDDHA/Fe<sup>3+</sup>. The material that presents a higher retention capacity is the iron hydroxide for all chelates studied.

In this type of study a decrease of the Fe concentration in solution can be due to chelate sorption, Fe displacement from the chelate and precipitation, or chelate degradation. For the different regioisomers of *o,o*-EDDHA/Fe<sup>3+</sup> sorption has been well studied (16), and iron hydroxide has been described as a very reactive phase adsorbing *meso-o,o*-EDDHA/Fe<sup>3+</sup>. Also acidic organic materials present a high retention capacity for *o,o*-EDDHA/Fe<sup>3+</sup>, but that retention is reduced at high pH values. In this case we also observe high retention of EDTA/Fe<sup>3+</sup> in the iron hydroxide and also in the soils. Whether this reduction is a consequence of chelate retention or Fe displacement cannot be elucidated from our data. The IDHA/Fe<sup>3+</sup> concentration is highly reduced after the interaction with most of the soils and soil materials. Since initially the solution was buffered to pH 8, Fe displacement should be the main factor affecting the stability of IDHA/Fe<sup>3+</sup> in these conditions, but surely the other two factors (biodegradation and chelate retention) may play a role in chelate concentration reduction.

Cu and other metal concentrations were measured in the soil extracts to determine the displacement of Fe from the chelate by this cation. Only the Sudanell soil gives a significant increase of the Cu amount in solution with respect to the controls. This is related to the high Cu availability of this soil (see **Table 1**) as a consequence of the heavy Cu fungicide addition on the peach grove during the past few years. Cu solubilization for IDHA/Fe<sup>3+</sup> treatment accounts for around 10% of the Fe displaced, while for EDTA/Fe<sup>3+</sup> it represents 14%. These data support the hypothesis of Fe displacement as a major effect on Fe reduction after IDHA/Fe<sup>3+</sup> reaction with soils.

**2. Biological Experiments. 2.1. Fe Uptake from IDHA/Fe<sup>3+</sup> by Cucumber Grown in Hydroponics.** In **Table 2** the SPAD readings are presented. EDTA/Fe<sup>3+</sup> is the treatment that presents lower values until the 18th day, so a lower chlorophyll content is expected for this treatment. IDHA/Fe<sup>3+</sup> presents lower SPAD indexes than *o,o*-EDDHA/Fe<sup>3+</sup> for the second leaves, but the values are only statistically different in the 7 day reading.

In **Table 3** the biometric data for all the treatments at both sampling times are presented. While there are no significant differences among treatments, EDTA/Fe<sup>3+</sup> presents lower plant growth.

Fe, Mn, and Zn concentrations in the leaves are also presented in **Table 3**. EDTA/Fe<sup>3+</sup> provided less iron to the plants in the first sampling time. There were nonstatistical differences among

*o,o*-EDDHA/Fe<sup>3+</sup> and IDHA/Fe<sup>3+</sup> at that moment, but in the second sampling time significantly higher concentrations of Fe were obtained for *o,o*-EDDHA/Fe<sup>3+</sup>. Mn presents larger concentrations for EDTA/Fe<sup>3+</sup> and Zn for *o,o*-EDDHA/Fe<sup>3+</sup> in the first sampling time, but the differences became nonsignificant at the second sampling time.

The results obtained in this experiment suggest that IDHA/Fe<sup>3+</sup> presents a good ability to supply iron for efficient plants grown in hydroponics at pH 7.5. In general it is similar to *o,o*-EDDHA/Fe<sup>3+</sup> in the first sampling time, but slightly worse in the second period, despite the fact that they present similar SPAD indexes.

**2.2. Fe Uptake from IDHA/Fe<sup>3+</sup> by Soybean Grown in Hydroponics.** In **Table 4** SPAD indexes are presented. EDTA/Fe<sup>3+</sup> shows lower values than the other treatments for all the periods and leaf stages (data presented only for the second leaf stage) considered. IDHA/Fe<sup>3+</sup> presented similar, but systematically higher values than *o,o*-EDDHA/Fe<sup>3+</sup>.

The weights of the plants are presented in **Table 5** at both sampling times. The trend is similar to that obtained with the SPAD index. From these data it can be concluded that IDHA/Fe<sup>3+</sup> and *o,o*-EDDHA/Fe<sup>3+</sup> were the best Fe treatments, while EDTA/Fe<sup>3+</sup> gave less yield to the plants.

Also in **Table 5**, Fe, Mn, and Zn concentrations in the leaves are presented. IDHA/Fe<sup>3+</sup> has the higher Fe content. The Mn concentration in the leaves is higher for EDTA/Fe<sup>3+</sup>; however, the Zn concentration increases for IDHA/Fe<sup>3+</sup> and *o,o*-EDDHA/Fe<sup>3+</sup>.

The better Fe nutrition from *o,o*-EDDHA/Fe<sup>3+</sup> than from EDTA/Fe<sup>3+</sup> in hydroponics when they are applied at low concentrations has already been described (9, 17). This is a consequence of the higher stability of *o,o*-EDDHA/Fe<sup>3+</sup> with respect to EDTA/Fe<sup>3+</sup> that reduces Fe displacement from the chelate in the nutrient solution. IDHA/Fe<sup>3+</sup> is less stable than EDTA/Fe<sup>3+</sup>, and a worse Fe nutrition was expected. However, not only the stability is important. In fact, Lucena et al. (18) showed that, for the highly stable chelates (those containing two phenolate, two amino, and two carboxylate donor groups), the less stable ones were better substrates of the Fe chelate reductase of cucumber plants, allowing a better nutrition of the plant (18). Moreover, kinetics is also an important factor. García-Marco et al. (7) showed that *o,p*-EDDHA/Fe<sup>3+</sup> (see **Figure 1**) was faster than highly stable *o,o*-EDDHA/Fe<sup>3+</sup> in providing Fe to plants. In a biological experiment, similar to the one we presented here using soybean plants, both of them were applied at low concentrations, and plants with *o,p*-EDDHA/Fe<sup>3+</sup> presented a better response. In that paper the lower coordination of the Fe on the chelates (five bonds between the chelating agent and the ferric ion; see **Figure 1**) was considered responsible for the faster Fe nutrition. In the experiments presented here, the good results obtained when IDHA/Fe<sup>3+</sup> was applied in hydroponics at pH 7.5 can also be a consequence of the low coordination in the chelate.

Despite the fact that IDHA/Fe<sup>3+</sup> presents low stability and high reactivity in agronomic conditions, it is quite efficient in providing Fe to cucumber (Fe-efficient) and soybean (Fe-susceptible) plants in hydroponics at pH 7.5. This good behavior may be related to the presence of only five bindings between the iron and the chelating agent. Since IDHA is biodegradable, its use as an Fe fertilizer in hydroponics and fertirrigation should be considered.

#### ABBREVIATIONS USED

EDTA, ethylene diamine tetraacetic acid; *o,o*-EDDHA, ethylenediamine-*N,N'*-bis(*o*-hydroxyphenylacetic acid); *o,p*-ED-

DHA, ethylenediamine-*N*-(*o*-hydroxyphenylacetic acid)-*N'*-(*p*-hydroxyphenylacetic acid); *p,p*-EDDHA, ethylenediamine-*N*-(*p*-hydroxyphenylacetic acid)-*N'*-(*p*-hydroxyphenylacetic acid); IDHA, *N*-(1,2-dicarboxyethyl)-D,L-aspartic acid; HEPES, *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid); MES, 2-(*N*-morpholino)ethanesulfonic acid; CAPS, 3-(cyclohexylamino)-1-propanesulfonic acid; AMPSO, *N*-(1,1-dimethyl-2-hydroxyethyl)-3-amino-2-hydroxypropanesulfonic acid; AAS, atomic absorption spectroscopy.

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**Supporting Information Available:** Materials used in the interaction experiments, species distribution, and element species distribution. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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