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# Photodegradation of FeDTPA in Nutrient Solutions. II. Effects on Root Physiology and Foliar Fe and Mn Levels in Marigold

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***Abstract.*** **Marigold (*Tagetes erecta* L.) grown hydroponically in an irradiated nutrient solution containing FeDTPA had root ferric reductase activity 120% greater, foliar Fe level 33% less, and foliar Mn level 90% greater than did plants grown in an identical, nonirradiated solution, indicating that the plants growing in the irradiated solution were responding to Fe-deficiency stress with physiological reactions associated with Fe efficiency. The youngest leaves of plants grown in the irradiated solution had symptoms of Mn toxicity (interveinal chlorosis, shiny-bronze necrotic spots, and leaf deformation). Plants grown in irradiated solution in which the precipitated Fe was replaced with fresh Fe-chelate were, in general, no different from those grown in the nonirradiated solution. Chemical name used: ferric diethylenetriaminepentaacetic acid (FeDTPA).**

Organic chelating agents like ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), and ethylenediaminedi-o-hydroxyphenylacetic acid (EDDHA) are capable of forming multiple coordinate bonds with Fe, maintaining the metal in a soluble form within a pH range from 4.0 to 6.3, 4.0 to 7.0, and 4.0 to 9.0, respectively (Norvell, 1971), a range generally acceptable for the culture of most plants. The uptake of Fe from Fe-chelates involves reduction of the ferric form of the chelate [Fe(III)-chelate, the form of greatest stability] at the root surface by plasma-membrane-bound Fe(III) reductase to the ferrous form of the chelate [Fe(II)-chelate, generally the form of least stability] prior to the dissociation of the complex and uptake of Fe (Assembly of Life Sciences, 1979; Guerinot and Yi, 1994).

FeDTPA and FeEDTA are chromophores that absorb strongly in the ultraviolet (UV) and blue regions of the spectrum. Absorption of this energy causes the destruction of the chelate complex into ferrous Fe that precipitates as Fe oxides, glyoxylic acid, formaldehyde, $CO_2$, and an amine residue (Frisell et al., 1959; Hamaker, 1956). In a previous study, we found that irradiating FeDTPA-containing nutrient solutions precipitated Fe (Albano and Miller, 2001). The photodegradation of FeEDTA incorporated into tissue-culture medium reduced root growth of *Arabidopsis thaliana* L., due in part to the inhibitory effects of formaldehyde, glyoxylic acid, and precipitated, unavailable Fe (Hangarter and Stasinopoulos, 1991). Similarly, Castillo et al. (1997) observed that growth under microculture conditions of *Carica papaya* L. on FeEDTA- and/or FeEDDHA-containing media, decreased as the level of irradiance increased.

When Fe is not readily available in the rhizosphere, dicots and non-graminaceous monocots undergo specific physiological and morphological modifications that improve Fe uptake. These modifications are collectively referred to as Strategy I Fe-efficiency and include enhanced ferric reductase activity and a greater ability to acidify the rhizosphere; traits that developed in 'First Lady' marigold under Fe-deficiency stress in a previous study (Albano and Miller, 1996). Having established that marigold is an Fe-efficient plant and that FeDTPA photodegrades, the objective of this study was to determine the effects of a photodegraded FeDTPA-containing nutrient solution on plant growth and physiology.

## Materials and Methods

*Treatments*. A base nutrient solution (Albano and Miller, 1996) was prepared as a 5× concentrate (1×: 14.28 mmol·L$^{-1}$ N and 17.9 μmol·L$^{-1}$ FeDTPA). Ten liters of the nutrient-solution concentrate in a 10-L, low-density polyethylene (LDPE) carboy (Nalgene Co., Rochester, N.Y.) was either nonirradiated or irradiated with 1000 μmol·m$^{-2}$·s$^{-1}$ (measured at the outer surface of the container) from a high-intensity discharge (HID), metal halide light source for 28 d at ambient temperature (20 to 30 °C). Treatments, derived from the nonirradiated and irradiated nutrient solutions, consisted of three unaltered solutions: 1) nonirradiated (NI); 2) irradiated with Fe-precipitate remaining in the solution (I+P); and 3) nonirradiated zero-Fe (NI-Fe) and two altered irradiated solutions: 1) Fe-precipitate removed by centrifugation (I-P); and 2) Fe-precipitate removed by centrifugation and 89.5 μmol·L$^{-1}$ FeDTPA added back (I-P+Fe).

*Growing conditions*. 'First Lady' marigold seeds were sown in Redi-Earth germination mix (The Scotts Co., Marysville, Ohio) in 200-cell plug trays in a greenhouse. Roots were washed clean of media under a gentle stream of tap water 14 d after sowing and transferred to 3-L opaque hydroponic containers. Aerated hydroponic nutrient solutions (treatments described above) were formulated as 7.14 mmol·L$^{-1}$ N (8.95 μmol·L$^{-1}$ FeDTPA) and adjusted to pH 5.8 with either 1 N NaOH or 1 N HCl). The I+P and I-P hydroponic nutrient solutions contained <0.895 μmol·L$^{-1}$ (0.05 mg·L$^{-1}$) soluble Fe [Fe determined by atomic absorption spectrophotometry (AA) of the concentrated stock solution]. Four replications of eight plants each treatment were used per treatment, and treatments were arranged in a completely randomized design in a greenhouse with 18 °C night/ 24 °C day temperatures. The study was conducted for 20 d with solution changes on days 8 and 15. On day 17, the degree of leaf chlorosis, necrosis, and deformity, the number of true-leaf pairs, and plant height from the cotyledonary node to the apical meristem of the primary shoot were recorded. The pH of treatment solutions was determined by pH electrode on days 8 and 15 (prior to solution change), and on day 20. Two sets of subsamples, consisting of three plants each, were randomly selected on days 18 and 19 from each replication for determination of root-associated ferric reductase activity. Leaf tissue was saved for foliar analysis. The leaf tissue of the two remaining plants (subreplicates) per replication per treatment was harvested on day 20 and combined with leaf tissue previously harvested; the dry weight and mineral composition were then determined. For mineral analysis, leaf tissue was washed, dried, ashed, and prepared for

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AA as described previously (Albano et al., 1996).

*Quantification of root reduction of Fe(III)*. Root-associated Fe(III) reduction was determined as described by Albano and Miller (1996), with the following modifications: whole root systems were placed in 40 mL of the oxygenated $Na_2$-bathophenanthrolinedisulfonic acid (BPDS) assay solution. Absorbance by the assay solution was read at 535 nm after 20 min and the concentration of Fe(II)-BPDS produced was calculated using an extinction coefficient of 22.14·mM$^{-1}$·cm$^{-1}$ (Welch et al., 1993).

*Statistics*. Data were analyzed by analysis of variance (ANOVA) to determine the effects of treatments. Calculations were performed with the general linear model (GLM) procedure of SAS (SAS Institute, Cary, N.C.). Means were separated and planned comparisons were made using LSD or pairwise *t* tests.

## Results and Discussion

*Effects on growth*. The leaves of 'First Lady' marigold plants grown in solutions of NI and NI-Fe treatments (i.e., the controls), were normal and entirely chlorotic, respectively (Table 1 and Fig. 1A). The leaf symptoms that developed on the NI-Fe plants were typical Fe-deficiency symptoms (i.e., chlorosis of newly developing leaves). The leaves of plants grown in I+P and I-P solutions developed symptoms similar to those of NI-Fe plants except that the newly developing leaves were chlorotic interveinally, not entirely chlorotic (Figs. 1B and 2, and Table 1). Leaves of plants grown in the I-P+Fe solution appeared normal and did not differ from those of plants in NI solutions (Fig. 1A and Table 1), indicating that any soluble toxic byproduct of FeDTPA photodegradation (i.e., glyoxylic acid and possibly formaldehyde) were at nontoxic levels.

Plant height and number of true-leaf pairs per plant varied slightly among treatments, averaging 2.2 cm and 3 true-leaf pairs, respectively, over all treatments (Table 1). Plants in the I-P+Fe and NI-Fe treatments produced the greatest and least dry weight per plant (69 and 27 mg, respectively) (Table 1) and greatest and least root fresh weight per plant (280 and 120 mg, respectively) (Table 2). However, plants grown in the I-P+Fe solution had greater leaf dry weight than did plants grown in the NI solution (control). Although it is generally accepted that the intact, nonphotodegraded, chelating agent is absorbed by roots only in very small quantities (Römheld and Marschner, 1983; Tiffin et al., 1959), we believe that the amine residues produced by photodegradation of the chelating agent may be readily absorbed, serving as an additional N source or stimulating plant growth in some other way. This, however, is only speculation, since N content of leaves and amine composition of the irradiated solutions were not determined.

*Effects on Fe content of leaves*. Foliar Fe was greatest in plants grown in solutions

Table 1. Effects of irradiation of the hydroponic nutrient solution on leaf appearance, plant height, number of true-leaf pairs, leaf dry weight, and leaf Fe and Mn concentrations of 'First Lady' marigold plants.

| | Leaf appearance[y,x] | | | Height[x] | True-leaf pairs[x] | Leaf DW[w] | Leaf mineral concn[w] (µg·g$^{-1}$) | | Fe : Mn |
|---|---|---|---|---|---|---|---|---|---|
| Treatment[z] | Chlorosis | Necrosis | Deformed | (cm) | (no.) | (mg) | Fe | Mn | ratio |
| NI | 1.13 c[v] | 1.00 c | 1.38 c | 1.7 b | 3.0 a | 52 b | 149 b | 435 c | 1:3 |
| I+P | 2.91 b | 4.13 ab | 2.34 b | 3.0 a | 3.0 a | 63 ab | 115 c | 839 b | 1:7 |
| I-P+Fe | 1.00 c | 1.00 c | 1.09 c | 2.4 ab | 3.0 a | 69 a | 276 a | 446 c | 1:2 |
| I-P | 2.87 b | 4.48 a | 2.81 ab | 2.2 ab | 3.0 a | 53 b | 85 d | 986 a | 1:12 |
| NI-Fe | 5.00 a | 4.00 b | 2.84 a | 1.6 b | 2.6 b | 27 c | 55 e | 909 ab | 1:17 |

[z]Treatments consisted of a 0.5× lab-prepared nutrient solution, pH 5.8 (14.28 mmol·L$^{-1}$ N, 17.9 µmol·L$^{-1}$ Fe, 1×) nonirradiated (NI), irradiated-precipitate retained (I+P), irradiated-precipitate removed and FeDTPA added back (I – P + Fe), irradiated-precipitate removed (I-P), and nonirradiated-minus Fe (NI-Fe).
[y]Chlorosis: 1 = normal green, 2 = pale green, 3 = interveinal chlorosis, 4 = leaves mostly chlorotic, and 5 = leaves entirely chlorotic. Necrosis: 1 = none, 2 = light brown, 3 = brown, 4 = bronze, and 5 = shiny bronze. Leaf deformation: 1 = normal, 2 = some leaflet downward cupping and crinkling, 3 = more pronounced leaflet downward cupping and crinkling, 4 = misshapen, and 5 = severely misshapen.
[x]Recorded 17 d after initiating treatments.
[w]Recorded 18–20 d after initiating treatments.
[v]Mean separation within columns by LSD, $P \leq 0.05$.

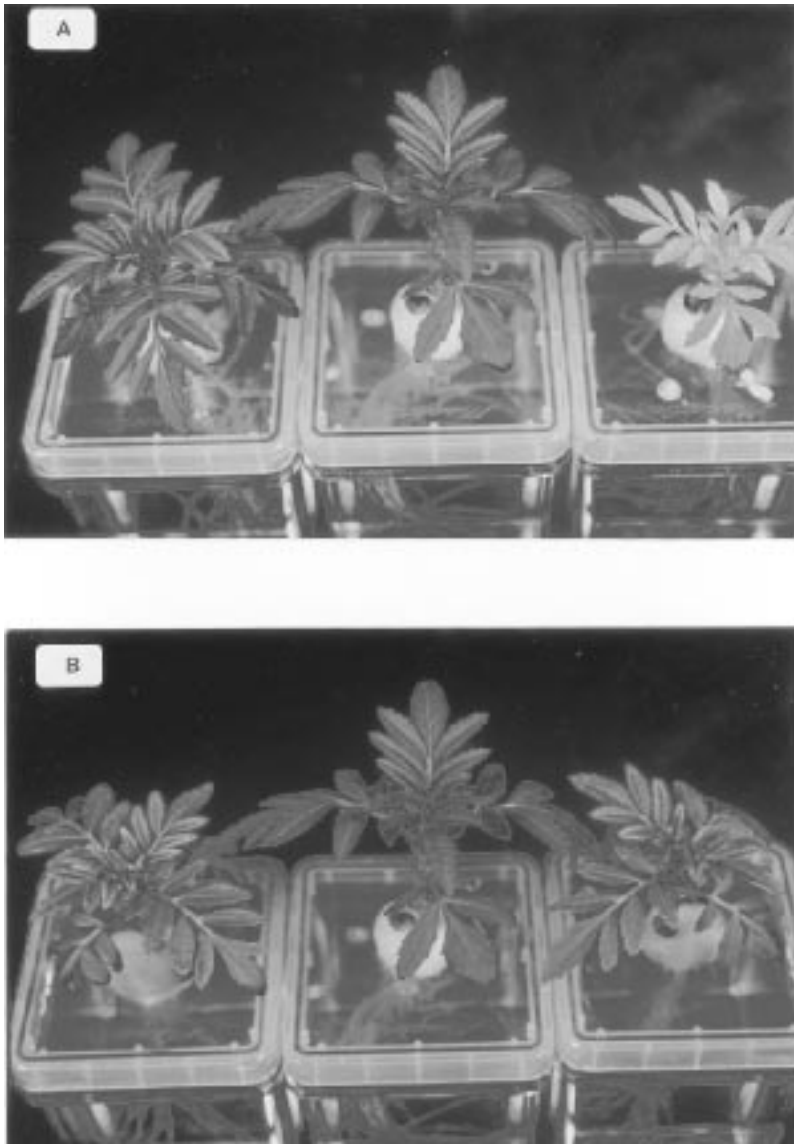


Fig. 1. Representative plants growing hydroponically in 0.5× lab-prepared nutrient solutions, pH 5.8 (14.28 mmol·L$^{-1}$ N, 17.9 µmol·L$^{-1}$ FeDTPA is 1×), 17 d after initiating treatments. Treatments from left to right: (**A**) nonirradiated (NI), irradiated-precipitate removed (by centrifugation) and FeDTPA added back (I-P+Fe), and nonirradiated-minus Fe (NI-Fe); (**B**) irradiated-precipitate retained (I+P), irradiated-precipitate removed (by centrifugation) and FeDTPA added back (I-P+Fe), and irradiated-precipitate removed [(by centrifugation) (I-P)]. Nutrient solutions [10-L of the nutrient-solution concentrate (5×) in 10-L LDPE carboys] were irradiated with 1000 µmol·m$^{-2}$·s$^{-1}$ with a HID light source for 28 d (solution temperature 20 to 30 °C).

Table 2. Effects of irradiation of the hydroponic nutrient solution on root fresh weight (root FW) of 'First Lady' marigold, hydroponic solution pH (rhizosphere pH), and root-associated ferric reduction for 'First Lady' marigold grown hydroponically in 0.5× lab-prepared nutrient solution (14.28 mmol·L$^{-1}$ N, 17.9 µmol·L$^{-1}$ Fe, 1×).

| | Root FW | Rhizosphere pH | | | | Ferric reduction |
|---|---|---|---|---|---|---|
| Treatment[z] | (mg) | Day 8 | Day 15 | Day 20 | Avg. | (µmol·gFM$^{-1}$·h$^{-1}$) |
| NI | 240 ab[y] | 5.35 ab | 4.93 a | 4.86 a | 5.05 a | 0.93 d |
| I+P | 230 b | 5.02 b | 4.45 b | 4.35 c | 4.60 b | 2.02 b |
| I-P+Fe | 280 a | 5.65 a | 4.59 b | 4.57 b | 4.93 a | 0.83 d |
| I-P | 210 b | 5.06 b | 4.22 c | 4.12 d | 4.46 bc | 1.48 c |
| NI-Fe | 120 c | 4.61 c | 4.17 c | 4.48 bc | 4.42 c | 2.73 a |

[z]Treatments consisted of nutrient solutions nonirradiated (NI), irradiated-precipitate retained (I+P), irradiated-precipitate removed and FeDTPA added back (I-P+Fe), irradiated-precipitate removed (I-P), and nonirradiated-minus Fe (NI-Fe).
[y]Mean separation within columns by LSD, $P \leq 0.05$.

Fig. 2. Interveinal chlorosis and some leaf deformation typical of the youngest leaves of plants growing hydroponically in the irradiated [precipitate retained (I+P)] and irradiated [precipitate removed (by centrifugation) (I-P)] nutrient solutions 17 d after initiating treatments. Nutrient solutions (pH 5.8) were 0.5× (14.28 mmol·L$^{-1}$ N, 17.9 µmol·L$^{-1}$ FeDTPA is 1×) and were derived from irradiating 10-L of the nutrient-solution concentrate (5×) in 10-L LDPE carboys with 1000 µmol·m$^{-2}$·s$^{-1}$ with a HID light source for 28 d (solution temperature 20 to 30 °C).

containing soluble Fe, i.e., NI and I-P+Fe treatments, at 149 Fe and 276 µg·g$^{-1}$ Fe, respectively (Table 1). Foliar Fe concentration in plants grown in the I+P solution was intermediate between those of other treatments (115 µg·g$^{-1}$ Fe) (Table 1). This treatment contained the same quantity of Fe as did the NI and I-P+Fe treatments except that Fe was in an insoluble form because of FeDTPA photodegradation; this clearly shows that the solubility of Fe influences Fe uptake. Foliar Fe was least in plants grown in solutions containing little or no soluble Fe, i.e., I-P and NI-Fe at 85 and 55 µg·g$^{-1}$ Fe, respectively (Table 1). In general, 100 µg·g$^{-1}$ Fe is considered sufficient (Taiz and Zeiger, 1991). Our research, however, has shown that normal, nontoxic Fe levels in 'First Lady' leaf tissue can range from 200 to 600 µg·g$^{-1}$ Fe (Albano and Miller, 1996, 1998; and Albano et al., 1996). Also, based on this study and previous work in hydroponics, the critical concentration for inducing Fe deficiency in leaves of 'First Lady' marigold is below 70 µg·g$^{-1}$ Fe (Albano et al., 1996).

*Effects on Mn content of leaves*. Leaves of plants grown in solutions containing soluble Fe (NI or I-P+Fe) had Mn concentrations averaging 441 µg·g$^{-1}$. Although leaf Mn levels of 50 µg·g$^{-1}$ are considered adequate (Taiz and Zeiger, 1991), normal, nontoxic Mn levels in 'First Lady' leaf tissue can range from 100 to 400 µg·g$^{-1}$ Mn (Albano and Miller, 1996, 1998; Albano et al., 1996).

Foliar Mn concentration was greatest, averaging 911µg·g$^{-1}$, in plants grown in solutions containing insoluble or no Fe, i.e., I+P, I-P, or NI-Fe treatments (Table 1). This level of foliar Mn is probably toxic, contributing to or causing the symptoms that developed in leaves of those plants. Symptoms of Mn toxicity induced by 0.364 mM $MnCl_2$ in 'First Lady' marigold grown hydroponically included leaf deformity (leaf crinkling/leaf tissue "puckering" between veins) and small, shiny-bronze necrotic spots of irregular shape in young leaves (Albano et al., 1996), symptoms that did not fully match the current symptoms. Other sources indicate that interveinal chlorosis also could be a symptom of Mn toxicity in young leaves (Marschner, 1995). The equilibrium between ferric and ferrous Fe within the plant is Mn-dependent (Somers and Shive, 1942). High leaf tissue Mn : Fe ratios result in the biologically inactive ferric Fe to dominate, leading to Fe deficiency (interveinal chlorosis) and Mn toxicity [small, shiny-bronze necrotic spots ($MnO_2$-induced polyphenol oxidation) and leaf deformity (a result of Mn-induced Ca deficiency)]. Symptoms very similar to these were observed on leaves of plants growing in the I+P and I-P treatments with foliar Fe : Mn ratios of 1:7 and 1:12, respectively (Fig. 1B and 2, and Table 1).

*Effects on ferric reductase activity and rhizosphere acidification*. Plants grown in soluble Fe treatments (NI and I-P+Fe) had lower root-associated ferric reductase activity and less rhizosphere acidification than did plants in the insoluble or no-Fe treatments (I+P, I-P, and NI-Fe) (Table 2). The NI-Fe plants expressed the greatest root reductase activity and ability to acidify the rhizosphere (Table 2). These data indicate that an irradiated Fe-chelate-containing nutrient solution has the potential to influence root physiology associated with Fe acquisition. Root-associated ferric reductase activity increases the plant's capacity to take up Fe, and rhizosphere acidification increases soluble/available Fe, as Fe solubility increases as pH decreases.

In previous work, we found that 'First Lady' marigold grown in nutrient solutions without Fe had foliar Mn concentrations two to four-times greater than in leaves of plants grown in Fe-sufficient nutrient solutions (Albano and Miller, 1996; Albano et al., 1996), as confirmed in this study (Table 1). We also determined that the roots of such plants, as also confirmed in the NI-Fe treatment, had greater ability to acidify the rhizosphere (hydroponic solution) and greater ability to reduce ferric Fe, both of which are traits of Strategy-I Fe-efficiency (Albano and Miller, 1996; Bienfait, 1988). The greater ability of roots to reduce ferric Fe under Fe deficiency conditions results from enhanced expression of root plasma-membrane-bound ferric chelate reductase, which also can reduce Mn (Bienfait, 1988; Guerinot and Yi, 1994; Marschner et al., 1982). Thus, an Fe-efficient plant under Fe stress has the capacity, because of enhanced production of ferric chelate reductase, to take up large amounts of Mn. This study indicates that when the latter occurs, Mn toxicity can result.

*Conclusions*. In conclusion, we have demonstrated that growing plants in an irradiated FeDTPA-containing hydroponic nutrient solution leads to classic Fe-efficiency reactions (i.e., enhanced expression of ferric chelate reductase and proton excretion) that enhance the uptake of Fe. Ferric chelate reductase can also reduce and facilitate Mn uptake (Marschner et al., 1982). Since irradiated nutrient solutions initially containing FeDTPA have insoluble Fe but maintain Mn solubility, marigold plants will exhibit both Fe deficiency and excessive Mn uptake under hydroponic conditions.

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