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# Iron Toxicity Stress Causes Bronze Speckle, a Specific Physiological Disorder of Marigold (*Tagetes erecta* L.)

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***Abstract*. A specific physiological disorder, bronze speckle (J.P.A.'s nomenclature), was consistently induced in 'First Lady' and 'Voyager' marigold with Fe-DTPA concentrations greater than 0.018 mM Fe-DTPA (1 ppm) applied to a soilless medium. The disorder was characterized by specific symptomology distinguished visually by speckled patterns of chlorosis and necrosis, and downward curling and cupping of leaves. The percentage of total leaf dry weight affected with symptoms generally increased with increasing Fe-DTPA treatments. Symptomatic leaf tissue had a greater Fe concentration than corresponding asymptomatic leaf tissue. Leaf Mn concentrations in symptomatic and asymptomatic tissue were similar. In 'First Lady', older leaf tissue accumulated more total Fe and was associated with more severe symptoms than younger tissue. Media leachate Fe concentrations increased over 6 weeks and were larger at greater Fe-DTPA treatments. Adjustment of nutrient solution pH to 4.0, 5.25, or 6.5 did not alter media pH, nor did it prevent disorder symptoms. Application of Fe-DTPA containing nutrient solution to a soilless medium resulted in leachate Fe levels 3 times greater than for $FeSO_4$ treatments. Chemical names used: ferric diethylenetriaminepentaacetic acid, monosodium salt (Fe-DTPA).**

A specific physiological disorder affecting African and French marigolds has been reported in the floriculture industries of the United States and Canada throughout the last decade (Albano and Miller, 1993; Biernbaum et al., 1988; Carlson, 1988; Halbrooks and Albano, 1990). Symptoms of this disorder include leaf chlorosis, bronze spotting of the leaf, overall stunting, and delayed flowering (Biernbaum et al., 1988). Symptoms are observed initially on older, mature leaves, and progress to younger growth. Slight chlorotic speckling progresses to necrotic spotting (or pitting), and necrosis of leaf margins (Vetanovetz and Knauss, 1989). Early stages of the disorder may resemble spider mite damage. Affected leaf tissue has been reported to have Fe and Mn concentrations of 400–2500 ppm, which is considered excessive (Biernbaum et al., 1988). Losses in crop quality and production attributed to this disorder have been significant (Biernbaum et al., 1988). Because flowering bedding plants are valued at greater than $750 million annually in the United States, (U.S. Dept. of Agriculture, 1993), economic losses associated with these disorders are significant.

Several trade and extension publications have identified this disorder as an Fe toxicity of some floriculture crops including New Guinea impatiens, Sultana impatiens, cutting and seed geraniums, vinca, and some species of *Brassica* (Vetanovetz and Kanuss, 1989). Similar disorders have been observed in cabbage and tomato transplants, and eliator begonias (Vetanovetz and Knauss, 1989). Cause of the disorder has been suggested to be low media pH, leading to increased availability of Fe and Mn, and subsequently excess accumulation of these metals in leaves. Recommendations for control of this disorder include managing media pH (Biernbaum et al., 1988; Vetanovetz and Knauss, 1989) and water and fertilizer solution pH (Carlson, 1988) above critical values (6.0–6.2), thereby reducing availability of Fe and Mn in soilless media.

Disorders attributed to Fe toxicity include bronzing of rice, freckle leaf of sugarcane (Foy et al., 1978), grey effect in tobacco (Arnold and Binns, 1987), and various unnamed disorders in other plants. The symptomology of Fe toxicity varies with plant species and cultivar, but is generally associated with reduced plant growth and foliar expression of tissue damage. Visual symptoms of Fe toxicity have been reported to include necrotic pitting, spotting or speckling, and bronzing (coalesced tissue necrosis) (Albano and Halbrooks, 1991; Foy et al., 1978; Somers and Shive, 1942; Vetanovetz and Knauss, 1989; Welch and LaRue, 1990). Iron toxicity is affected by age of tissue, with the expression and susceptibility to the disorder more prevalent in older tissue of younger plants (Foy et al., 1978). The most common physiological attribute of Fe toxicity is an increase in both Fe uptake and transport to shoots and leaves (Foy et al., 1978).

Most bedding plants are grown in soilless media. Typical liquid fertilization regimes with marigolds include chelated micronutrients to maintain micronutrient availability over a wide range of rhizospheric conditions, including pH. Iron-DTPA is one of the most commonly used Fe-chelates because DTPA has a very high affinity for Fe and is relatively stable in the presence of other cations (Lindsay, 1974).

Effects of continuous additions of Fe-chelates such as DTPA with fertigation applications, as would be performed in a commercial production regime using a peat-lite fertilizer, have not been studied under controlled conditions for bedding plant crops grown in soilless media. Therefore, the relationship of metal chelates, particularly Fe-DTPA, to the incidence of Fe and Mn-related toxicity disorders in marigolds needs to be established.

Despite the widespread occurrence of the disorder bronze speckle in marigolds, few data have been published in the literature

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to corroborate these reports and offer recommendations for control. The objectives of current research were 1) to induce, document, and characterize the disorder under controlled conditions; 2) to determine the relationship of the disorder to Fe-DTPA applied to a soilless medium; 3) to determine changes in pH, Fe, and Mn concentrations in the medium over time; 4) to determine the effects of nutrient solution pH adjustment on the occurrence of the disorder; and 5) to determine the distribution of Fe and Mn in symptomatic and asymptomatic leaves and between true-leaf pairs in both peat-based and hydroponic situations. This information will improve understanding of the relationship of applied metal chelates to metal uptake patterns and occurrences of similar physiological disorders in other crops grown in soilless media.

## Materials and Methods

### Growing conditions

*Disorder induction*. African 'First Lady' and 'Voyager' (*Tagetes erecta L.*) marigold seeds were sowed in metro-mix 360 (The Scotts Co., Marysville, Ohio) soilless medium composed of sphagnum peat moss, processed bark ash, and vermiculite in six-celled grow-packs (40 $cm^3$/cell) in a controlled environment growth chamber. A pack of six plants constituted a single replication, and six replications of each treatment and variety combination were made. Iron-DTPA treatments, 0.018 mM (1 ppm), 0.09 mM (5 ppm), 0.27 mM (15 ppm), or 0.36 mM (20 ppm) incorporated into a base nutrient solution were arranged in a completely randomized design within a controlled environment growth chamber programmed to deliver 10-h photoperiods with a peak photosynthetic photon flux density of 760 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and temperatures 18–24C (night/day). The base nutrient solution, prepared in distilled, deionized water, was composed of the following macronutrients (in millimolar concentrations): 2.5, $K_2SO_4$; 2, $MgSO_4$; 0.5, $Ca(H_2PO_4)_2$; 2, $CaSO_4$; 7.14, $NH_4NO_3$, and micronutrients (in micromolar concentrations): 0.046, $H_3BO_3$; 0.76, $ZnSO_4$; 0.32, $CuSO_4$; 0.54, $MoO_3$; and 9.1 Mn-EDTA (manganese ethylenediaminetetraacetic acid). Plants were thinned to one plant per cell at emergence of first true-leaf pairs when treatments began. Treatment solutions (250 ml) were applied per six-cell pack at regular intervals directly to the media (leaching fraction, 15%–25%), avoiding any application to foliage, throughout the experiment to maintain constant moisture. Leachate was collected on a weekly basis beginning one week after first treatment application using a modified pour-through procedure. 12–14 h before leachate collection, the medium received a regular nutrient solution application. At the time of leachate collection, 250 ml distilled, deionized water was applied per six-cell pack to the medium and leachate was collected in a clean, dry container. Leachate was stored at 4C and filtered prior to analysis for pH, electrical conductivity (EC), Fe, and Mn. Plants were harvested when at least one flower was open on all plants, which was 44 days after treatments were initiated. At harvest, visual evaluations of symptom type (e.g., chlorotic speckling, necrotic speckling, or leaf curl) and severity were recorded. For tissue dry weight and elemental analysis, leaves were classified as symptomatic if any symptoms were present (regardless of severity). All remaining leaves were classified as asymptomatic.

*Iron source and pH*. This study was designed to test the hypothesis that adjusting nutrient solution pH is an effective means of avoiding the disorder associated with high levels of Fe in affected tissue of marigold. 'First Lady' marigold germination, environmental conditions, and base nutrient solution formulation were as described in the disorder induction experiment, except that treatments were initiated when true leaf-pair 3 was emerging. The experiment was a completely randomized design with 6 treatments consisting of combinations of nutrient solution pH (4.0, 5.25, or 6.5, adjusted with HCl or NaOH) and Fe source [$FeSO_4$ or Fe-DTPA, at 0.018 mM (1 ppm Fe)]. Plants were harvested 36 days after the emergence of first true-leaf pairs when at least one flower was open on each plant.

*Leaf metal—media*. 'First Lady' marigold germination, environmental conditions, and base nutrient formulation were as described in disorder induction. The major differences in this experiment were that 1) plants were grown in 330 $cm^3$ containers, and 2) tissue harvesting occurred by true-leaf pair, numbering from the stem base upwards. The experiment was a completely randomized design with three Fe-DTPA treatments [0, 0.018 mM (1 ppm), and 0.18 mM (10 ppm)], and six replications per treatment. Plants were irrigated with quarter-strength nutrient solution prior to the initiation of treatments at the emergence of the first true-leaves. Treatment solutions were adjusted to pH 5.8 with NaOH or HCl, and applied directly to the media at intervals appropriate to maintain adequate moisture at the rate of 150 ml per container. At harvest, 28 days after initiating treatments, visual evaluations of symptom type (e.g., chlorotic speckling, necrotic speckling, or leaf curl) and severity were recorded for each true-leaf pair. True-leaf pairs of common stem origin in each treatment were combined to form single pooled samples. Plant height was determined by measuring length of primary shoot from cotyledon node to shoot apex.

*Leaf metal—hydroponics*. This experiment was conducted simultaneously with leaf metal—media in the same growth chamber with the following modifications: 1) plants were grown hydroponically in the same nutrient solutions used in the media experiment, and 2) aerated treatment solutions were changed every 3 days.

### Mineral determination

Leaf tissue was washed in 0.2 N HCl and double rinsed with distilled, deionized water, and dried in a forced-air oven at 75C for 24 h. Leaf dry weight was recorded and the tissue was milled or ground to pass through a 20-mesh screen. One g of leaf tissue was dry ashed at 500C for 5 h and prepared for elemental analysis with a modified digestion procedure (Allen et al., 1986) (Perkin-Elmer, 1982). Modifications included 1) 15.8 N $HNO_3$ was added to wet the ash residue after cooling, 2) wet ash residue was placed on a hot plate at ≈80C until dry, 3) ash was rewetted with 8 ml of 6 N HCl and scraped with a plastic spatula, 4) ash solution was quantitatively transferred to a 100 ml volumetric flask and brought to volume with distilled, deionized water, and 5) solution was filtered through filter paper (no. 41; Whatman Paper, Maidstone, Kent., U.K.). Tissue extracts and leachates were analyzed by atomic absorption spectrophotometry for Fe and Mn.

*Statistics*. Data from the disorder induction and Fe source and pH experiments were analyzed to determine the main effect of variety and Fe treatments, and to determine the presence of interaction between these factors by using an ANOVA. Calculations were performed with the general linear model (GLM) procedure of SAS (SAS Inst., Cary, N.C.). Where a significant F test was observed, means were separated and planned comparisons were made using pairwise t tests.

## Results

### Disorder induction

*Disorder development and characteristics*. The earliest visual symptoms of the bronze speckle disorder were patches of interveinal chlorosis (Fig. 1A) that became more speckled and then developed

into, or were associated with, necrotic speckles on leaflets towards the distal end of the leaf blade (Fig. 1B). The necrotic speckles were bronze and occasionally shiny, becoming brown with time. Bronze speckles were concentrated initially on the leaflet margin progressing toward the central vein. Concurrently, the leaflet(s) curled downward (Fig. 1C). As symptoms became more severe, more necrotic speckles formed, downward curling of leaflets became more exaggerated, and a larger portion of the leaf was affected (Fig. 1D). In the most severe cases, necrotic speckles coalesced so that entire leaves became necrotic.

Within 3 days of the first application of 0.36 mM Fe-DTPA, both cultivars exhibited necrotic speckling on cotyledons. Within 7 days, necrotic speckling appeared on first true-leaf pairs of both cultivars treated with 0.27 mM Fe-DTPA. Symptoms predominately affected the older leaves of 'First Lady', with fewer symptoms on younger leaves; whereas in 'Voyager', symptoms occurred more frequently in younger than in older leaves.

*Leaf dry weight.* There were no differences in total leaf dry weight between treatments or cultivars (Table 1); averaging 0.48 g/plant. 'First Lady' and 'Voyager' had little or no symptomatic tissue at 0.018 and 0.09 mM Fe-DTPA; averaging <0.2 g/plant (Table 1). Symptomatic dry weight significantly increased between 0.09 and 0.36 mM Fe-DTPA for 'Voyager' (Table 1). Both 'First Lady' and 'Voyager' treated with 0.27 and 0.36 mM Fe-DTPA had significantly higher symptomatic than asymptomatic leaf tissue (Table 1).

*Leachate pH and Fe concentrations.* Leachate pH decreased over the course of the experiment but was similar for all treatments and both cultivars; averaging 5.9 at week 1 and 4.4 at week 6 (Table 2). Conversely, leachate Fe concentration was highly dependent on the concentration of Fe-DTPA applied (Table 2). Within the first week, leachate Fe concentrations began increasing rapidly, and by week 6, leachate Fe levels were much higher than actual treatment Fe-DTPA concentrations, on average, double the concentration applied (Table 2).

*Leaf Fe and Mn concentrations*. For both cultivars, there was a linear increase in Fe concentration in asymptomatic tissue as Fe-DTPA treatment increased. As a mean of all treatments, 'First Lady' had a significantly greater Fe concentration in asymptomatic tissue than 'Voyager' at 755 and 636 $\mu g \cdot g^{-1}$, respectively (Table 1).


Fig. 1. Symptom characteristics of the disorder bronze speckle induced with Fe-DTPA are visually observed as interveinal chlorotic patches (**A**), becoming more speckled as symptoms progress (**B**). Severe symptom characteristics of the disorder induced with Fe-DTPA include necrotic speckling (**C**) and leaflet curl (**D**).

Table 1. Leaf dry weight and Fe concentration for asymptomatic and symptomatic marigold tissues at harvest at first open flower, 44 days after treatments began for the disorder induction experiment.

| | | Leaf tissue | | | |
|---|---|---|---|---|---|
| | | Dry wt (g) | | Fe concn ($\mu g \cdot g^{-1}$) | |
| Cultivar | Fe-DTPA (mM) | Asymptomatic | Symptomatic | Asymptomatic | Symptomatic |
| 'First Lady' | 0.018 | 0.46 a[z] | --- | 561 a | --- |
| | 0.09 | 0.50 a | --- | 640 ab | --- |
| | 0.27 | 0.14 b | 0.30 a$^{**}$ | 754 b | 1030 a$^{**}$ |
| | 0.36 | 0.08 b | 0.37 a$^{***}$ | 1144 c | 1647 b$^{***}$ |
| 'Voyager' | 0.018 | 0.52 a | --- | 483 a | --- |
| | 0.09 | 0.39 b | 0.18 a$^{***}$ | 573 ab | 1022 a$^{*}$ |
| | 0.27 | 0.13 c | 0.30 b$^{***}$ | 679 bc | 1195 a$^{**}$ |
| | 0.36 | 0.06 c | 0.42 c$^{***}$ | 808 c | 1171 a$^{*}$ |

[z]Means within a column of a cultivar followed by different letters indicate significant differences between treatments at $P = 0.05$, pairwise *t* test.
$^{*,**,***}$Significant differences between asymptomatic and symptomatic leaf tissue within a treatment at $P = 0.05$, 0.01, and 0.001, respectively, pairwise *t* test.

Table 2. Leachate pH and Fe concentration ($mg \cdot liter^{-1}$) for the disorder induction experiment.

| | Week | |
|---|---|---|
| Fe-DTPA (mM) | 1 | 6 |
| | *pH* | |
| 0.018 | 5.88 | 4.27$^{***}$ |
| 0.09 | 5.87 | 4.32$^{***}$ |
| 0.27 | 5.91 | 4.42$^{***}$ |
| 0.36 | 6.06 | 4.47$^{***}$ |
| | *Fe* | |
| 0.018 | 0.91 | 2.22$^{NS}$ |
| 0.09 | 4.31 | 10.53$^{***}$ |
| 0.27 | 14.54 | 23.52$^{***}$ |
| 0.36 | 19.68 | 31.52$^{***}$ |

$^{NS,***}$Nonsignificant or significant differences between weeks at $P = 0.001$, pairwise *t* test.

Iron concentration in symptomatic tissue was always significantly higher than asymptomatic tissue for both cultivars (Table 1), and Fe concentration in symptomatic tissue, as a mean of all treatments, was again a little higher in 'First Lady' than 'Voyager' at 1339 and 1151 $\mu g \cdot g^{-1}$ Fe, respectively. For both cultivars and for all treatments, the highest Fe concentration in asymptomatic leaf tissue was in 'First Lady' 0.036 mM Fe-DTPA treatment at 1144 $\mu g \cdot g^{-1}$, and the lowest Fe concentration in symptomatic leaf tissue was in 'Voyager' 0.09 mM Fe-DTPA treatment at 1022 $\mu g \cdot g^{-1}$. This suggest a critical toxic Fe concentration in leaf tissue for these cultivars between 1000 and 1200 $\mu g \cdot g^{-1}$.

Leaf Mn was similar for both cultivars at all treatment levels averaging 301 $\mu g \cdot g^{-1}$ Mn (data not shown). Within an Fe-DTPA level, Fe in symptomatic tissue was 2.5, 3.5, and 3.1 times greater than Mn in 'Voyager' treatments delivering 0.09, 0.27, and 0.36 mM Fe-DTPA, respectively; and 3 and 5 times greater for 'First Lady' treatments delivering 0.27 and 0.36 mM Fe-DTPA, respectively (data not shown).

## Iron source and pH

*Disorder development and characteristics*. Generally, symptoms for 'First Lady' were consistent with those expressed on plants in the disorder induction experiment. Interveinal chlorosis was the most prevalent symptom along with a small amount of necrotic speckling and some downward curling of leaves. Symptoms first appeared on third and fourth true-leaf pairs and on axial leaves, which had not been noted in earlier experiments but can probably be explained by the initiation of treatments with development of true-leaf pair 3. Symptoms were similar between all treatments.

*Leaf dry weight.* Total leaf dry weight did not differ between Fe source or pH treatment, and averaged 0.62 g/plant (data not shown). Across all pH and Fe source treatments, leaf dry weight was much greater in asymptomatic than symptomatic tissue, averaging 0.49 g and 0.13 g/plant, respectively.

*Leachate pH and Fe concentrations*. Iron source affected leachate pH. Nutrient solution pH had no effect. Generally, medium leachate pH decreased with both Fe sources, however, after week 2, pH was lower with Fe-DTPA than $FeSO_4$ (Table 3). Leachate Fe concentrations increased rapidly during the first 4 weeks with Fe-DTPA then decreased; while $FeSO_4$ showed no significant change (data not shown). The final leachate Fe concentration for Fe-DTPA was 3-fold greater than for $FeSO_4$ (2.3 ppm vs 0.73 ppm Fe), and 2.3-fold greater than the concentration of Fe-DTPA being applied (Table 3), consistent with leachate data from disorder induction experiment.

*Leaf Fe and Mn concentrations.* Leaf Fe and Mn concentrations did not differ between Fe source and solution pH treatments (data not shown). Iron and Mn concentrations (as a mean of all pH and Fe treatments) in symptomatic and asymptomatic leaf tissue aver-

Table 3. Leachate pH and Fe concentration ($mg \cdot liter^{-1}$) as a mean of pH treatments 4.0, 5.25, and 6.5 for the Fe source and pH experiment.

| | Week | |
|---|---|---|
| Iron source[z] | 1 | 5 |
| | *pH* | |
| $FeSO_4$ | 5.40 ± 0.06 a[y] | 4.44 a$^{***}$ |
| Fe-DTPA | 5.45 ± 0.03 a | 4.64 b$^{***}$ |
| | *Fe* | |
| $FeSO_4$ | 0.90 ± 0.02 a | 0.75 a$^{NS}$ |
| Fe-DTPA | 1.04 ± 0.07 b | 2.30 b$^{**}$ |

[z]Fe supplied at 0.018 mM.
[y]Means followed by different letters indicates significant differences between Fe source at $P = 0.01$, pairwise t test.
$^{NS,**,***}$Nonsignificant or significant differences between weeks at $P = 0.01$ and 0.001, respectively, pairwise *t* test.

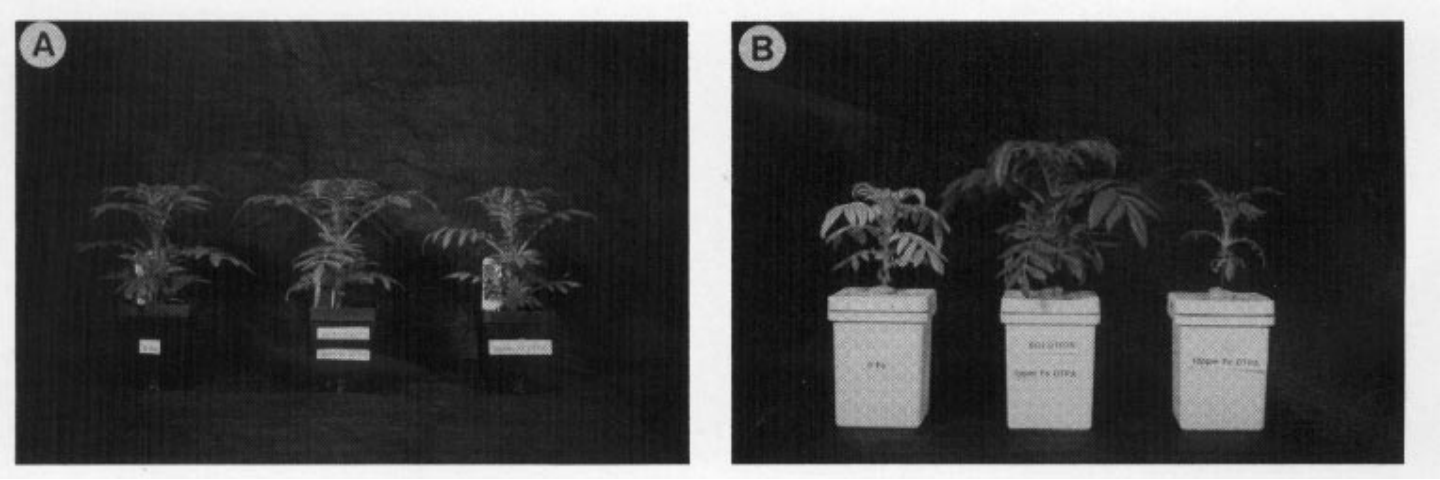


Fig. 2. Representative plants of 0, 0.018 mM (1 ppm), and 0.18 mM (10 ppm) Fe-DTPA treatments (left to right) after 30 days of growth in soilless media (**A**) and hydroponics (**B**).

aged 1586 $\mu g \cdot g^{-1}$ Fe and 1355 $\mu g \cdot g^{-1}$ Mn, and 731 $\mu g \cdot g^{-1}$ Fe and 942 $\mu g \cdot g^{-1}$ Mn, respectively.

**Leaf metal—media and leaf metal—hydroponics**

*Disorder development and characteristics.* Plants grown in soilless media were less affected by Fe deficient (0 Fe-DTPA) and excess (0.18 mM Fe-DTPA) treatments than plants grown in hydroponics (Fig. 2). When grown in soilless media, plant height was similar between treatments, averaging 14 cm, whereas in solution culture grown plants, the 0 Fe-DTPA and 0.18 mM Fe-DTPA treatments were half as tall as plants in the 0.018 mM Fe-DTPA treatment which averaged 12 cm. In both the leaf metal—media and leaf metal—hydroponics experiments, plants in the 0.18 mM Fe-DTPA treatment developed characteristic symptomology of the disorder, and symptoms developed 4 and 7 days after initiating treatment, respectively. In hydroponics, plants of the 0 Fe-DTPA treatment showed visual symptoms of Fe deficiency, mild interveinal chlorosis, on newly forming leaves 7 days after initiating treatment. Leaves forming subsequent to day 7 were progressively more Fe-deficient with the most severe leaves entirely chlorotic (yellow to pale yellow-white) with marginal and spotty interveinal necrosis. Plants in the media 0 Fe-DTPA treatment did not develop symptoms of Fe deficiency.

*Leaf Fe and Mn concentrations.* In both soilless media and hydroponic systems, a correlation between leaf Fe accumulation and the occurrence of the disorder bronze speckle was documented with symptom severity and frequency increasing with leaf Fe and leaf age. The disorder developed only in true-leaf pairs 1–5 in the 0.18 mM Fe-DTPA treatment in leaf metal—media , and true-leaf pairs 1–3 in the 0.18 mM Fe-DTPA treatment in leaf metal—hydroponics (Table 4). In both soilless media or hydroponics, symptomatic leaf pairs had at least 4-fold higher Fe concentrations than the corresponding asymptomatic leaf pairs in the 0 Fe-DTPA and 0.018 mM Fe-DTPA treatments (Table 4). Elimination of Fe in hydroponics resulted in high uptake of Mn into leaves, averaging 1172 $\mu g \cdot g^{-1}$, 3.7-times and 1.9-times greater than in leaves of the 0.018 and 0.18 mM treatments averaging 314 $\mu g \cdot g^{-1}$ and 629 $\mu g \cdot g^{-1}$, respectively (Table 4).

In the leaf metal—media experiment, the Mn concentration in leaf tissue was highest in true-leaf pair 1, decreasing with subsequent leaf pairs and remaining nearly constant for leaf pairs 4–7 (Table 4).

## Discussion

*Disorder induction and Fe source and pH.* Symptoms of the disorder induced in marigolds with Fe-DTPA were consistent with those described as an Fe toxicity disorder (Biernbaum et al., 1988; Carlson, 1988). The unique pattern of symptom development that characterizes the disorder begins with patches of interveinal chlorosis of mature leaves, progressing to distinct chlorotic and bronze speckling, and pitting. Increased severity of symptoms with increasing Fe-DTPA concentrations as observed visually was confirmed by leaf dry weight distribution.

At lower Fe-DTPA concentrations [e.g., 0.018 mM (1 ppm Fe) Fe-DTPA], symptoms appeared only on younger leaves and always included chlorotic speckling. Symptom progression to more advanced speckling or necrotic pitting did not always occur. At higher Fe-DTPA concentrations, symptoms appeared earlier, progressed more rapidly, and were more severe at harvest.

In the Iron source and pH experiment, development of symptoms in plants supplied with 0.018 mM Fe-DTPA was not consistent among plants of any Fe source or pH treatment as some plants developed no symptoms at all. Symptoms of the disorder, however, occurred at Fe concentrations similar to those applied in typical production regimes (1 ppm Fe) and in the presence or absence of chelated Fe. However, Mn was supplied as Mn-EDTA,

Table 4. Iron and Mn concentration ($\mu g \cdot g^{-1}$) in true-leaf pairs 1–7 (numbering from stem base upwards) for the Leaf metal—media and Leaf metal—hydroponics experiments, treatments 0, 0.018 mM, and 0.18 mM Fe-DTPA. Plants were harvested 28 days after treatments began.

| Treatment | | True-leaf pair and metal content ($\mu g \cdot g^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Experiment | Fe-DTPA (mM) | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| | | | | *Fe* | | | | |
| Leaf Metal | 0 | 231 | 154 | 115 | 124 | 133 | 136 | 131 |
| Media | 0.018 | 613 | 359 | 140 | 135 | 140 | 144 | 129 |
| | 0.18 | 3112[z] | 1640[z] | 588[z] | 347[z] | 321[z] | 303 | 280 |
| | | | | *Mn* | | | | |
| | 0 | 465 | 417 | 393 | 313 | 279 | 280 | 286 |
| | 0.018 | 727 | 523 | 305 | 279 | 255 | 237 | 229 |
| | 0.18 | 1286 | 797 | 376 | 273 | 260 | 294 | 281 |
| | | | | *Fe* | | | | |
| Leaf Metal | 0 | 89 | 66 | 50 | 60 | 46 | 92 | 64 |
| Hydroponics | 0.018 | 910 | 515 | 340 | 232 | 224 | 421 | 247 |
| | 0.18 | 13164[z] | 3074[z] | 906[z] | 334 | 289 | 245 | 230 |
| | | | | *Mn* | | | | |
| | 0 | 1519 | 1574 | 1439 | 1200 | 967 | 778 | 729 |
| | 0.018 | 683 | 332 | 252 | 190 | 175 | 371 | 195 |
| | 0.18 | 2499 | 975 | 380 | 145 | 113 | 134 | 158 |

[z]Indicates that at least one true-leaf pair had the disorder bronze speckle at harvest.

which is a relatively unstable chelate complex. Therefore, Fe could have replaced Mn as the ligand-held ion, thereby increasing available Fe levels relative to that which might be available if free Fe was applied in the absence of a chelate (Norvell and Lindsay, 1969). Symptoms were similar among plants treated with pH-adjusted nutrient solution of 4.0, 5.25, or 6.5, indicating that pH adjustment of irrigation water is not a reliable method of preventing this disorder in marigolds.

In the disorder induction experiment, Fe concentrations in symptomatic and asymptomatic leaves increased with increasing Fe-DTPA concentrations. Iron concentrations in symptomatic leaves were consistently higher than in asymptomatic leaves. In contrast, Mn concentrations were similar in symptomatic and asymptomatic tissue in plants grown over a range of Fe-DTPA concentrations. Mn toxicity has been reported for several crops and is typically associated with leaf crinkling, cupping, or curling, interveinal tissue puckering (raised areas of tissue between major veins), interveinal chlorosis, and bronze necrotic spotting and pitting (Hannam and Ohki, 1988). Mn toxicity induced in 'First Lady' in hydroponics with 0.36 mM $MnCl_2$ (20 ppm Mn) was characterized by leaf puckering and crinkling, with shiny bronze or copper necrotic pits of irregular size, shape, and distribution (Fig. 3). Symptoms associated with Mn toxicity were distinguishable from those associated with the disorder bronze speckle induced with Fe-DTPA. These observations and the lack of a consistent accumulation pattern of Mn associated with symptomatic tissue indicates that Mn toxicity is not the direct cause of bronze speckle.

In addition to asymptomatic leaf dry weights decreasing with increasing Fe treatment in experiment disorder induction, the concentration of leaf Fe in asymptomatic leaf tissue increased in both cultivars as well. For 'First Lady', Fe concentration in asymptomatic leaf tissue in the 0.36 mM Fe-DTPA treatment was 1.1-fold greater than in symptomatic leaf tissue in the 0.27 $mM \cdot g^{-1}$ Fe-DTPA treatment. The apparent acquired tolerance to excessive Fe may be due to the induction and production of the Fe storage protein, phytoferritin. Ferritin is an Fe induced protein that can function as a cellular Fe buffer (Bienfait and van der Mark, 1983; Thiel, 1987; van der Mark et al., 1981). It has been proposed that tolerance to Fe toxicity in plants may involve the ferritin system (Verkleji and Schat, 1990). Therefore, future studies should test this hypothesis by quantifying ferritin in symptomatic and asymptomatic leaf tissue at increasing Fe-DTPA treatment levels for 'First Lady'.

The role of the chelating agent, in this case DTPA, to the disorder and increased levels of Fe in affected tissue of marigold is likely as a supplier of Fe to roots rather than as a toxic agent itself. It is accepted that chelates act by keeping the metal available in the soil solution, and therefore enhance Fe absorption and increase Fe concentrations in root and shoot tissues. It is now considered likely that the chelate itself is only absorbed in very small quantities relative to Fe uptake in plant tissue (Chaney, 1988; Romheld and Marschner, 1983). In zinnia (*Zinnia elegans Jacq*.), sunflower (*Helianthus annuus* L.) and soybean (*Glycine max* L.) plants supplied with Fe-EDDHA in nutrient solution, Fe concentrations of exudate from decapitated plants was 8-times higher than the nutrient solution Fe concentration. The average ratio of chelated Fe (Fe-EDDHA) to total Fe in the exudate was 1:12 (Tiffin et al., 1960). Peanut plants (*Arachis hypogaea* L.) subjected to Fe deficiency increased their capacity to take up Fe from a Fe-EDDHA solution with no corresponding increase in the uptake of the chelating agent, EDDHA (Romheld and Marschner, 1983). Thus, it is likely that DTPA itself is not the phytotoxic agent of this disorder in marigold.

Changes in leachate pH and Fe concentrations over time were similar in the disorder induction and Fe source and pH experiments. Generally, pH decreased with time due to solubility and leaching of pre-incorporated $CaCO_3$ (lime) from the media. A similar pattern of pH in media is expected in commercial regimes using water low in bicarbonates and fertilizer high in $NH_4$-N. Changing media pH from 4 to 7 by liming is not likely to greatly affect availability of Fe complexed with DTPA because Fe-DTPA is fairly stable within this range (Lindsay, 1979). Boxma (1981) reported that over 95% and 50% of Fe-DTPA applied to a peat-based media at pH 5.65 and 7.25 (pH adjusted with $CaCO_3$), respectively, remained water-soluble after 3 days incubation. Leachate pH changes in the Fe source and pH experiment indicate that adjustment of irrigation water or fertilizer solution pH has no

Fig. 3. Manganese toxicity induced in 'First Lady' marigold with 0.36 mM (20 ppm) $MnCl_2$.

affect on media pH (likely due to buffering capacity of peat, residual $CaCO_3$, and high $NH_4$-N) or the occurrence of symptoms.

Leachate Fe concentrations increased over time in the disorder induction and Fe source and pH experiments, and were higher with correspondingly higher Fe-DTPA treatments and the use of chelated vs. unchelated Fe sources. Availability of metals for plant uptake from solid phase sources of peats has been estimated by extraction methods adapted from those used in mineral soils (Markus et al., 1981) and peats vary widely in metal content, particularly Fe (Handreck, 1989; Mitchell, 1954; Walsh and Barry, 1958). Chelates extract Fe from peat and extractable Fe concentration varies widely with peat source (Broschat and Donselman, 1985; Handreck, 1989). DTPA is a more reliable indicator of medium micronutrient status than EDTA (Berghage et al., 1987). Our data indicate that frequent additions of Fe-chelates, as would be performed in a commercial production regime using a peat-lite fertilizer, will increase the level of leachable Fe from a peat-based medium to a concentration greater than that being applied due to the extraction properties of the chelate.

*Leaf metal—media and leaf metal—hydroponics.* In leaf metal—hydroponics, Mn uptake into leaves of the 0 Fe treatment was greater than for any other treatment of leaf metal—hydroponics or leaf metal—media. This is an example of the competitively antagonistic relationship of Fe and Mn in solution culture where low levels of Fe solution results in high uptake of Mn (Warden and Reisenauer, 1991). The cause of increased Mn uptake when Fe is low may be due to an enhanced rhizodermal reductase activity expressed by Fe-efficient, strategy I plants (dicots and non-graminaceous monocots) (Bienfait, 1988). Marschner et al. (1982) demonstrated that Fe-deficient sunflower plants had a greater ability to reduce $MnO_2$ to Mn(II) as well as Fe(III) to Fe(II) than Fe sufficient sunflower plants.

Visual symptoms of Fe deficiency were not evident in the media 0 Fe treatment over the 28 day course of the experiment. These plants apparently were able to acquire Fe from the soilless medium itself, possibly from the incorporated nutrient starter charge or via Fe-efficiency reactions.

In conclusion, several factors that may influence Fe availability (iron source and pH experiment), uptake, and occurrence of an Fe toxicity physiological disorder in African marigolds (disorder induction, leaf metal—media, and leaf metal—hydroponics ) have been identified in this study. The widespread use of metal chelates in the production of floriculture crops raises the possibility that chelates may be a common factor in the disorder. There does not appear to be a critical concentration of Fe associated with symptom occurrence, suggesting that tolerance to Fe concentrations in tissue varies depending on available Fe levels. Concentrations of Fe-DTPA [e.g., 0.018 mM (1 ppm)], which are typically used in commercial production, were sufficient to cause symptoms. Increased concentrations were associated with increased severity and higher leaf Fe concentrations. Based on these data, we suggest that occurrence of the disorder and its severity in commercial settings may depend on the Fe-chelate concentration in the liquid fertilizer program. Iron-chelate concentrations vary with N concentration of the liquid fertilizer and since N concentrations may vary significantly with each application, either deliberately or due to poor injector calibration, producers may be unaware of the actual Fe-chelate concentrations being applied to the crop. Leachate Fe concentrations increased over time, thus the cumulative effects of repeated Fe-chelate applications are important factors in the occurrence of the disorder. Attempts to control this disorder by managing nutrient solution or medium pH are unlikely to be fully effective as Fe-DTPA remains fairly stable between pH 4–7 and fairly water-soluble (>50%) in a high pH (7.25) peat-based medium several days after application.

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