Iron Form and Concentration Affect Nutrition of Container-grown *Pelargonium* and *Calibrachoa*

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Abstract. Two experiments were completed to determine whether the form and concentration of iron (Fe) affected Fe toxicity in the Fe-efficient species *Pelargonium* $\times$ *hortorum* ‘Ringo Deep Scarlet’ L.H. Bail. grown at a horticulturally low substrate pH of 4.1 to 4.9 or Fe deficiency in the Fe-inefficient species *Calibrachoa* $\times$ *hybrida* ‘Trailing White’ Cerv. grown at a horticulturally high substrate pH of 6.3 to 6.9. Ferric ethylenediaminedi(*o*-hydroxyphenylacetic) acid (Fe-EDDHA), ferric ethylenediamine tetraacetic acid (Fe-EDTA), and ferrous sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) were applied at 0.0, 0.5, 1.0, 2.0, or 4.0 $\text{mg} \cdot \text{L}^{-1}$ Fe in the nutrient solution. *Pelargonium* showed micronutrient toxicity symptoms with all treatments, including the zero Fe control. Contaminant sources of Fe and Mn were found in the peat/perlite medium, fungicide, and lime, which probably contributed to widespread toxicity in *Pelargonium*. *Calibrachoa* receiving 0 mg Fe/L exhibited severe Fe deficiency symptoms. *Calibrachoa* grown with Fe-EDDHA resulted in vigorous growth and dark green foliage, with no difference from 1 to 4 $\text{mg} \cdot \text{L}^{-1}$ Fe. Using Fe-EDTA, 4 mg Fe/L was required for acceptable growth of *Calibrachoa*, and all plants grown with FeSO_4 were stunted and chlorotic. Use of Fe-EDDHA in water-soluble fertilizer may increase the upper acceptable limit for media pH in Fe-inefficient species. However, iron and Mn present as contaminants in peat, irrigation water, or other sources can be highly soluble at low pH. Therefore, it is important to maintain a pH above 6 for Fe-efficient species regardless of applied Fe form or concentration, in order to avoid the potential for micronutrient toxicity.

The pH of the substrate in container grown crops has a major influence on micronutrient solubility (Lindsay, 1979; Peterson, 1981) and their subsequent uptake by plants (Marschner, 1995). If the substrate pH is too high, solubility of the micronutrients, boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) can decrease to levels unacceptable for growth, causing a deficiency. If the substrate pH is too low, solubility of these micronutrients can increase to levels that cause toxicity. For most species grown in substrates without soil, a pH range of about 5.8 to 6.2 provides adequate nutrient solubility to meet plant requirements without causing deficiency or toxicity, although recommendations vary ± 0.2 pH units (Bailey and Nelson, 1998; Argo and Fisher, 2002).

When Fe uptake is inadequate (usually induced by high substrate pH), the shoot tip can rapidly become chlorotic followed by necrosis as the deficiency becomes more severe (Argo and Fisher, 2002; Miller et al., 1984). In contrast, excess accumulation of Fe (usually induced by low substrate pH) occurs in the lower leaves of the plant. Toxicity symptoms can include chlorotic and necrotic speckling of leaves, leading to complete leaf death (Albano et al., 1996; Argo and Fisher, 2002) through production of free radicals and resulting oxidative damage of membranes (Bartosz, 1997; Marschner, 1995).

Bedding plants can be classified Fe-efficient or Fe-inefficient based on their ability to accumulate Fe under conditions of limited supply (Römhelt, 1987), usually caused by high substrate pH. At high pH (low Fe solubility), Fe-efficient plants can increase Fe uptake into their tissue using various strategies including acidification of the root zone, extrusion of chelating agents and Fe(III) reductases, and morphological changes to root structure with increased growth of fine root hairs (Guerinot and Yi, 1994; Bienfait, 1988; Römhelt, 1987). Iron deficiency induced by high pH is therefore not generally a problem with Fe-efficient plants. Instead, low pH induced iron and manganese toxicity is the major concern in Fe-inefficient floricultural species, including *Tagetes* species (marigold) and *Pelargonium*

$\times$ *hortorum* (seed or zonal geranium) (Albano and Miller, 1996; Argo and Fisher, 2002; Römhelt, 1987).

Fe-inefficient species, however, are not effective at taking up iron at high substrate pH. Iron deficiency induced at high pH is therefore the major concern with Fe-inefficient plants, rather than iron and manganese toxicity occurring at low pH. Examples of Fe-inefficient species include *Calibrachoa* and *Petunia* hybrids (Argo and Fisher, 2002; Bunt, 1988; Fisher et al., 2003).

Because Fe is immobile in the plant, continuous supply from a Fe fertilizer source is needed to produce chlorophyll at the growing points (Marschner, 1995). The chemical form of Fe applied to a crop can alter the pH range at which sufficient Fe is supplied. For example, inorganic forms of Fe, such as iron sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) and iron oxide (Fe_2O_3), can be highly soluble in peat-based substrate when the pH is below 5.5, but their solubility decreases rapidly as the substrate pH increases (Lindsay, 1979). In contrast, chelated forms of iron can maintain the solubility of Fe at pH ranges well above 5.5, resulting in elimination of Fe-deficiency symptoms in plants grown at these higher pH ranges (Lindsay, 1979; Hagstrom, 1984). Stability at high pH for Fe chelates is in the order: ferric ethylenediaminedi(*o*-hydroxyphenylacetic) acid (Fe-EDDHA) >> ferric diethylenetriamine pentaacetic acid (Fe-DTPA) > ferric ethylenediamine tetraacetic acid (Fe-EDTA) > N-(2-hydroxyethyl) ethylenedinitrioltriacetic acid (HEDTA) >> Fe-citrate (Lindsay, 1979). Stability of Fe-EDTA and Fe-DTPA decreases rapidly as pH approaches 7.0, whereas Fe-EDDHA remains highly stable at a substrate pH up to 7.85 (Boxma, 1981; Lindsay, 1979). Fe-toxicity can be readily induced at low pH in *Pelargonium* or *Tagetes* using Fe-EDTA or Fe-DTPA (Albano et al., 1996; Smith et al., 2004). We are unaware of reports of how Fe-EDDHA affects Fe toxicity symptoms in bedding plant species grown at low pH compared with other Fe sources.

A common method for optimizing Fe nutrition for either Fe-efficient or Fe-inefficient plants is through manipulation of substrate pH. However, when plants with varying Fe-efficiency are grown in the same greenhouse, optimizing conditions for Fe-inefficient species can cause problems for Fe-efficient species, and vice versa. For example, growing plants at a low substrate pH (pH 4.4 to 5.3) or increasing applied Fe concentration reduced micronutrient deficiency symptoms in *Petunia*, but increased micronutrient toxicity symptoms in *Pelargonium* (Smith et al., 2004, 2005a, 2005b).

The objective of this study was to determine the effect of Fe concentration (0 to 4 $\text{mg} \cdot \text{L}^{-1}$) and Fe form (FeSO_4 , Fe-EDTA, and Fe-EDDHA) on nutritional disorders and plant growth in a peat-based medium under two conditions: 1) an Fe-efficient species grown at a horticulturally low substrate pH; and 2) an Fe-inefficient species grown at a horticulturally high substrate pH.

The Fe-efficient species chosen for this experiment was *Pelargonium* $\times$ *hortorum* L. H.

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Bail. 'Ringo Deep Scarlet', because Hulme and Ferry (1999) found this cultivar was especially susceptible to Fe toxicity at low pH. We have observed that *Calibrachoa* × *hybrida* Cerv. 'Trailing White' is highly prone to Fe deficiency, but responds well to corrective Fe supplementation (Fisher et al., 2003). FeSO₄ and Fe-EDTA are the two most commonly applied sources of Fe in commercial water-soluble fertilizers in the United States, and Fe-EDDHA has been found to be the most effective Fe source for correcting Fe deficiency at high pH in several studies (Fisher et al., 2003; Hagstrom, 1984; Lindsay and Schwab, 1982). A review of commercial water-soluble fertilizer used in the United States found that the median peat-lite formula contained 0.5 mg·L⁻¹ Fe and the median general purpose formula contained 0.25 mg·L⁻¹ Fe for every 100 mg·L⁻¹ N. In our opinion, applying Fe at 0 to 4 mg·L⁻¹ covers the range of Fe concentrations applied in water-soluble fertilizer in most commercial greenhouses.

Materials and Methods

Experimental design. *Calibrachoa* and *Pelargonium* were grown in a peat-perlite substrate with initial pH levels of 6.9 or 4.9, respectively. Treatments were applied in a factorial design with five Fe levels (0, 0.5, 1, 2, or 4 mg·L⁻¹ Fe) supplied from three Fe forms (FeSO₄·5H₂O, Fe-EDTA, and Fe-EDDHA). Plant species were grown separately on two benches in a greenhouse with nutrition treatments completely randomized within a bench. There were 24 replicate pots for *Calibrachoa* and 21 replications for *Pelargonium* for each factorial treatment combination.

Growing conditions. *Pelargonium* seeds were germinated in 98-cell plug trays (Dillen Products, Middlefield, Ohio), 4 weeks before the beginning of the experiment. *Pelargonium* seedling plugs and size 84 rooted cuttings of *Calibrachoa* (supplied by Pleasant View Gardens, Loudon, N.H.) were planted on 22 Aug. 2001 into 450-cm³ containers (10-cm standard round pots; Dillen Products, Middlefield, Ohio), and placed in 10-cm spacer trays (Dillen Products) spaced 13.5 cm on center. All plants were drenched with a thiophanate-methyl-etrizadiazole fungicide (Banrot, Scotts-Sierra Crop Protection Co., Marysville, Ohio) 3 days after planting. The experiment occurred over four weeks on two 1.2 × 4.6-m benches in a 6.1 × 9.1-m glass house with constant air circulation and cement floors. Greenhouse air temperature was measured by a datalogger (CR10X; Campbell Scientific Inc., Logan, Utah) using an aspirated type-T thermocouple (Omega Engineering, Stamford, Conn.) Mean day and night temperatures were 26.9 ± 4.6°C and 20.3 ± 2.2°C.

Growing medium. Both species were grown in (by volume) 70% Canadian sphagnum peat (Fisons professional black bale peat, Sun Gro Horticulture, Bellevue, Wash.) and 30% coarse perlite (Whittemore Co., Lawrence, Mass.). At mixing, type N microfine dolomitic hydrated lime [97% Ca(OH)₂ · MgO, 92% of which passed through a 45-μm screen, National Lime and Stone, Findlay, Ohio] was used. For the *Pelargonium* medium, the lime was

incorporated into the peat/perlite substrate at 0.9 kg·m⁻³ to achieve an initial pH of 4.9 ± 0.04 (mean ± 95% confidence interval). For the *Calibrachoa* medium, the lime was incorporated into the peat-perlite substrate at 3.1 kg·m⁻³ to achieve an initial pH of 6.9 ± 0.05. A distilled water solution containing 0.04 mL·L⁻¹ of a wetting agent (Psi Matrix; Aquatrols, Cherry Hill, N.J.) was added to all substrates at 0.14 L·m⁻³, and the substrate was allowed to equilibrate in black plastic bags for 5 days before planting.

Substrate pH tended to drop over time for both species. A flowable lime slurry was produced by adding 0.01 L of the LimeStone-F (27.75% CaCO₃, 24.15% MgCO₃) (W.A. Cleary Corp., Dayton, N.J.) per liter of solution, and at day 9, the slurry was applied at a rate of 100 mL/pot to raise media pH.

Fertilization. Plants were fertilized with nutrient solutions containing (in mg·L⁻¹) 200N–29P–223K–191Ca–46.3Mg from 5 mM Ca(NO₃)₂, 5 mM KNO₃, 2 mM MgSO₄, and 1 mM KH₂PO₄ in deionized (DI) water. A micronutrient fertilizer (GreenCare, Chicago, Ill.) containing 4.06% Mn (MnSO₄), 4.10% Zn (ZnSO₄), 2.15% Cu (CuSO₄), 2.40% B (H₃BO₃), and 0.77% Mo ((NH₄)₆Mo₇O₂₄), was also added to provide 0.50Mn–0.50Zn–0.26Cu–0.30B–0.09Mo (mg·L⁻¹).

Tensiometers (Mini LT, Irrrometer, Riverside, Calif.) were randomly placed in two pots per species. When substrate dryness exceeded –5 kPa, all pots in the species were top-watered with treatment solutions. Each pot received approximately 100 mL of solution at each irrigation with <10% leaching. *Pelargonium* were fertigated 7 times (days 0, 6, 9, 13, 17, 20, and 25), and *Calibrachoa* were fertigated 9 times (days 0, 5, 8, 12, 16, 20, 23, 26, and 28).

Data collection. Substrate pH and EC were tested using the saturated media extract method with DI water as the extractant (Warncke, 1995) at 0, 7, 14, and 21 d after planting by sampling 10 randomly selected pots per species. Substrate pH was determined by inserting the pH electrode (model 6165; Orion Technologies, Beverly, Mass.) directly into the saturated medium before extraction, and EC was measured in the extracted solution with a platinum electrode at a standard 25°C (model 130; Orion Technologies). Final pH and EC data were collected 28 or 30 d after planting on 17 or 14 replicate pots for *Calibrachoa* and *Pelargonium*, respectively.

Plant growth data. Plant height from the substrate surface to tallest point on foliage, shoot dry mass, SPAD chlorophyll index, leaf count, proportion of leaves per plant showing necrosis, were collected at the end of experiment. Final plant growth data were collected 28 or 30 d after planting on 17 or 14 replicate pots for *Calibrachoa* and *Pelargonium*, respectively. The leaf count for *Calibrachoa* was determined by counting the number of leaves on the 2.5 cm nearest the meristem, of the largest shoot, whereas all unfolded leaves were counted for *Pelargonium*.

SPAD and chlorophyll. The Minolta SPAD meter indirectly measures chlorophyll content without damaging plant tissues. SPAD

chlorophyll index values are determined by measuring the ratio of light transmitted by the leaf at a red wavelength (650 nm) and an infrared wavelength (940). SPAD measurements were taken on the 5 most recent leaves on *Pelargonium*, and on 5 random leaves from the newest 2.5 cm of growth for *Calibrachoa*, and then averaged.

Smith et al. (2004) found the correlation between SPAD vs. chlorophyll level in *Pelargonium* 'Ringo Deep Scarlet' to be SPAD = 14.96 + 37.30 × chlorophyll content (mg·g⁻¹ of dried tissue), $r^2 = 0.95$, $p < 0.001$.

To correlate SPAD to chlorophyll level for *Calibrachoa*, chlorophyll content was measured using the same protocol described by Smith et al. (2004) in a subsample of *Calibrachoa* at the end of the experiment. Chlorophyll content was measured on 44 leaves randomly selected from a range of plants with healthy and symptomatic tissue and correlated with SPAD index values (average of five measurements per plant) for the same leaves. Five discs per plant were selected using a 0.64-cm-diameter one-hole punch, and frozen until analysis. For analysis, discs were ground in a 13 × 100-mm pyrex tissue grinder (Corning, Corning, N.Y.) using a pinch of sand and 2 mL of 95% ethanol. The extract was diluted with an additional 3 mL of 95% ethanol, then 2 mL of final solution was centrifuged (Beckman Microfuge 11) for 120 s at 220 r/s and 11,600 g_n. Solution was measured for absorbance at 470, 649, and 664 nm using a Spectronic 21D spectrophotometer (Milton Roy, Ivyland, Pa.). Total chlorophyll was determined according to the method of Lichtenthaler (1987), based on the mean dry mass of five discs.

Using this method, the correlation between SPAD vs. chlorophyll level in *Calibrachoa* 'Trailing White' was found to be SPAD = 9.31 + 29.35 × chlorophyll content (mg·g⁻¹ of dried tissue), $r^2 = 0.91$, $p < 0.001$.

Tissue nutrients. Tissue was harvested from each pot and first rinsed in deionized water and then in a 0.5% nonionic, phosphate-free detergent (Aquet; Bel-Art, Pequannoc, N.J.) solution. This was followed by a rinse in a 0.1 M HCl solution, and then a final rinse in deionized water. The harvested tissue was dried in a 50 °C oven for a minimum of 7 d. After drying, petioles and stems were removed and leaves were ground to pass a 0.5-mm screen (Cyclotec sample mill model 1093; Tecator, Hogånäs, Sweden). Ten milliliters of HNO₃ was added to 0.3 g of ground tissue and microwave digested (MARS 5; CEM Corp., Matthews, N.C.) for 15 min while ramping to 170 °C, then for another 15 min at 170 °C. The sample was diluted to 40 mL with distilled, deionized water, and measured for nutrients using inductively coupled plasma (ICP) atomic emission spectrophotometry. Molybdenum in the tissues was below ICP detection limits and will not be reported.

Micronutrient source: In order to check our experimental procedures and identify potential sources of Fe or Mn other than the applied Fe solutions, we analyzed micronutrient content of substrate components, lime source, water, nutrient solutions, and equipment, with three

Table 1. ANOVA summary table showing the effects of Fe form and concentration on plant growth and media data for *Pelargonium* grown at low lime (substrate pH ranging from 4.1 to 4.9) after 30 d, with 14 replicate plants per treatment combination. There was no difference between the zero Fe control and other concentrations, and least-square means are therefore not reported for concentrations. Figure 2 shows Fe form $\times$ concentration least-square means for SPAD, shoot dry weight, and percent necrotic or chlorotic leaves from the same ANOVA analysis reported in this table.

Effect	Plant ht (cm)	SPAD chlorophyll index	Shoot dry wt (g/plant)	Percentage of leaves with necrotic lesions	Substrate pH	Substrate EC (dS·m ⁻¹)
ANOVA effects ^z						
Iron form	**	NS	*	NS	***	***
Concentration	NS	*	NS	*	***	***
Iron form $\times$ concentration	NS	*	NS	**	**	NS
Iron form						
Fe-EDDHA	6.53 a ^z	29.2 a	0.41 a	26.9 a	4.2 b	2.1 a
Fe-EDTA	6.20 b	29.8 a	0.36 ab	26.9 a	4.4 a	1.8 b
FeSO ₄	5.98 b	28.9 a	0.32 b	25.0 a	4.5 a	1.5 c

^zLetters after each least-square mean represent mean separation using Tukey's HSD at the $p = 0.05$ level. NS,*,**,**Nonsignificant or significant at $p = 0.05, 0.01, \text{ or } 0.001$, respectively.

Fig. 1. Effect of applied Fe form and concentration on (A) shoot dry mass, (B) SPAD chlorophyll index and (C) percent of all leaves with necrotic lesions, for *Pelargonium* (Fe-efficient) grown at low pH (4.1 to 4.9), or (D) shoot dry mass, (E) SPAD chlorophyll index and (F) percent of leaves in the top 2.5 cm of the shoot with necrotic lesions, for *Calibrachoa* (Fe-inefficient) grown at high pH (6.3 to 6.9). Symbols represent the means of 14 replications for (A–C) and 17 replications for (D–F) ± 1 standard error.

replications per source. Peat–perlite and lime samples were prepared using HNO₃ and microwave digestion.

Nutrients were also extracted from peat/perlite using the SME method with either 0.005 M diethylenetriaminepenta-acetic acid (DTPA) or

deionized water as extractants (Warncke, 1995). Warncke (1995) recommended modifying the SME method using 0.005 M DTPA for greenhouse media because extractable micronutrient levels are increased compared with water extraction (Berghage et al., 1987).

Data analysis. Data were analyzed separately by species, using analysis of variance (ANOVA) in the general linear models procedure (PROC GLM) of SAS (SAS Institute, 1999), where the main effects tested were Fe form and Fe concentration, along with their interaction. Least-square means for Fe form and Fe form $\times$ Fe concentration interactions were compared using Tukey's HSD mean comparison test, and concentration main effects were compared against control (no additional Fe) using Dunnett's test. There were 7 or 6 replications for tissue nutrient data (each replication from 3 combined plants), and 14 or 17 replications for pH, EC, and growth data for each combination of Fe form and concentration treatments for *Pelargonium* and *Calibrachoa*, respectively.

Results and Discussion

Pelargonium grown at low pH.

pH and EC. Initial substrate pH and EC at planting were 4.9 and 0.2 dS·m⁻¹. After planting, pH differed between measurement days ($p < 0.001$) with least-square mean pH levels of 4.4, 4.6, 4.1, and 4.4 on days 7, 14, 23, and 30, respectively.

On day 30, the substrate of plants receiving Fe-EDDHA was 0.2 to 0.3 pH units lower than that of plants receiving either Fe-EDTA or FeSO₄ (Table 1). Substrate pH tended to increase with increasing Fe concentration, ranging from pH 4.2 for the 0 Fe control to 4.6 for 2 to 4 mg·L⁻¹ Fe.

Substrate EC was higher on day 30 than on day 0, with a final EC of 1.5 to 2.1 dS·m⁻¹ depending on the treatment (Table 1). Although final EC differed between Fe forms, EC for all treatments was within the normal range for potted plants of 1.2 to 2.5 dS·m⁻¹ (Argo and Fisher, 2002). Using Dunnett's test, the least-square mean EC levels for 0.5 to 4 mg·L⁻¹ Fe treatments were not different from the control (1.81 dS·m⁻¹).

Plant growth. Plants that received Fe-EDDHA had the greatest height (Table 1) and dry mass (Table 1, Fig. 1), followed by plants receiving Fe-EDTA or Fe-sulfate. Fe concentration did not affect plant height or dry mass.

All treatments showed evidence of nutrient toxicity symptoms including marginal or leaf-spot chlorosis and necrosis. Plant stress (chlorosis and necrosis) was apparent in most plants after 28 d, with leaf chlorosis progressing to necrotic lesions and eventual leaf death in older leaves (Fig. 2). Although the percent necrotic foliage ranged from 13% to 46%, depending on the treatment (Fig. 1C), none of the treatments resulted in commercially acceptable plants, including the control given the high aesthetic quality required for ornamental plants.

Although there was a statistically significant interaction between Fe concentration and form on SPAD chlorophyll index (Table 1), there

were no clear trends among treatments (Fig. 1B). Leaf count per plant was not affected by Fe form or concentration (data not shown). At the highest Fe concentration, plants receiving Fe-EDDHA and Fe-EDTA had a higher proportion of necrotic leaves than those receiving FeSO_4 (Table 1, Fig. 1C).

Foliar nutrients. Foliar Fe concentrations were above the sufficiency range of 100 to 580 $\mu\text{g}\cdot\text{g}^{-1}$ reported for *Pelargonium × hortorum* by Mills and Jones (1996). Even the zero Fe con-

trol had foliar Fe concentrations of 874 $\mu\text{g}\cdot\text{g}^{-1}$. Maximum Fe level (1239 $\mu\text{g}\cdot\text{g}^{-1}$) occurred with 2 $\text{mg}\cdot\text{L}^{-1}$ Fe from Fe-EDTA (Fig. 3G).

Foliar Mn concentrations ranged from 413 to 609 $\mu\text{g}\cdot\text{g}^{-1}$ (Fig. 3H) and were also above the suggested sufficiency of 40 to 325 $\mu\text{g}\cdot\text{g}^{-1}$ for *Pelargonium × hortorum* (Mills and Jones, 1996). The ratio between Fe and Mn ranged from 1.4 to 2.2, and tended to be lower for plants receiving FeSO_4 than those receiving the other Fe forms (Fig. 3I).

Chlorosis and necrosis in *Pelargonium* was probably caused by toxic levels of several micronutrients. Iron and Mn have been the primary micronutrients reported to cause toxicity symptoms in *Pelargonium* and *Tagetes* grown at low pH (Albano et al., 1996; Hulme and Ferry, 1999). In certain treatments (FeSO_4 treatments at the 0.5 and 1 $\text{mg}\cdot\text{L}^{-1}$ Fe levels, Fig. 3F, K), high foliar concentrations of Cu and Zn could have also contributed to the necrosis, reduced chlorophyll content, and lack of vigor.

Fig. 2. (left) Representative plants showing the effect of applied Fe form and concentration on *Pelargonium* (Fe-efficient) grown at low pH (4.1 to 4.9), on day 28, and (right) an example of the nutrient toxicity symptoms including chlorosis progressing to necrotic lesions.

Fig. 3. Effect of applied Fe form and concentration on tissue nutrient concentrations ($\mu\text{g}\cdot\text{g}^{-1}$ of dry mass) for *Pelargonium* (Fe-efficient) grown at low pH (4.1 to 4.9). Symbols represent the means of 7 replicate samples $\pm$ one standard error. Sufficiency ranges are taken from Mills and Jones (1996).

Table 2. A nutrient budget for Fe and Mn potentially provided by fungicide, medium, and lime compared with 1 mg Fe/L supplied from Fe-EDTA. Means represent three replicate samples from ICP analysis. Acid digestion and saturated medium extracts (SME) were prepared for peat/perlite, and lime was analysed using acid digestion only.

Solution	Concn (mg·L ⁻¹)		Concn (mg/pot)		Notes
	Fe	Mn	Fe	Mn	
Deionized water	0.001	0.016	0.00	0.01	100 mL/pot, 7 irrigations = 700 mL/pot
EDTA fertilizer solution ²	0.990	0.480	0.69	0.34	1 mg·L ⁻¹ Fe, 100 mL/pot, 7 irrigations
Fungicidal drench ²	1.780	0.014	0.18	0.00	0.6 g Banrot/L, 100 mL/pot, 1 application
Peat-perlite (unamended with lime)	Concn (μg·g ⁻¹)		Concn (mg/pot)		
	Fe	Mn	Fe	Mn	
Acid digestion	749.333	16.500	33.653	0.741	99.8 g·L ⁻¹ bulk density, 450 mL/pot
SME DTPA extraction ²	14.300	0.300	4.421	0.093	450 mL media/pot, 687 mL extractant/L media
SME deionized water extraction	0.300	0.040	0.093	0.012	450 mL media/pot, 687 mL extractant/L media
Lime amendments (acid digestion)	Concn (μg·g ⁻¹)		Concn (mg/pot)		
	Fe	Mn	Fe	Mn	
Calcitic lime	473.440	41.420	0.256	0.022	Only applied to highest lime treatment (at 1200 g·m ⁻³), 450 mL/pot = 0.54 g/pot
Hydrated lime ²	754.517	47.977	0.306	0.019	900 g·m ⁻³ for lowest lime treatment, 450 mL/pot = 0.405 g/pot
Flowable lime ²	1897.360	217.043	0.985	0.113	10 mL·L ⁻¹ × 100 mL/pot × 1 appn = 1 mL flowable lime/pot. 51.9% solid in original solution = 0.519 g/pot
Total load			Fe	Mn	
Total load of Fe and Mn for lowest lime treatment			6.582	0.562	1 mg·L ⁻¹ solution, including DTPA-extractable nutrients from peat-perlite, lowest lime treatment, assumes complete dissociation of hydrated and flowable lime
Total load from fertilizer solution (%)			11	60	

²Included in calculation of total load of Fe and Mn for the lowest lime treatment. Fertilizer solution and fungicidal drench Fe and Mn concentrations include deionized water.

Table 3. ANOVA summary table showing effects of Fe form and concentration on plant growth and media data for *Calibrachoa* grown at media pH 6.3 to 6.9 after 28 d, with 17 replicate plants per combination of Fe form and concentration. There was no effect of Fe form or concentration on media pH and data are therefore not reported. Figure 2 shows Fe form × concentration least-square means for SPAD, shoot dry weight, and percent necrotic or chlorotic leaves from the same ANOVA analysis reported in this table.

Effect	Ht (cm)	SPAD chlorophyll index	Leaf count	Shoot dry wt (g/plant)	Percentage of leaves with necrotic lesions	Media EC (dS·m ⁻¹)
ANOVA effects ²						
Iron form	***	***	***	***	NS	*
Concentration	***	***	***	***	***	**
Iron form × concentration	***	***	NS	**	**	NS
Fe form						
EDDHA	7.9 a ²	24.2 a	11.4 c	0.48 a	9.5 a	2.3 b
EDTA	7.2 ab	18.5 b	12.3 b	0.41 b	8.2 a	2.5 a
FeSO ₄	6.8 bc	15.9 b	14.2 a	0.32 c	12.8 a	2.5 a
Fe concentration ³						
0.0	6.1	12.0	15.4	0.30	30.88	2.2
0.5	6.8	18.9*	12.5*	0.35*	8.7*	2.3
1.0	7.5*	20.2*	11.9*	0.44*	4.0*	2.5*
2.0	7.8*	21.9*	11.9*	0.47*	2.2*	2.6*
4.0	8.3*	24.5*	11.5*	0.47*	5.0*	2.5

²Letters after each least-square mean represent mean separation using Tukey's HSD at the $p = 0.05$ level.

³Comparison of each Fe concentration against the 0 Fe control using Dunnett's test at the $p = 0.05$ level.

NS, *, **, *** Nonsignificant or significant at $p = 0.05$, 0.01, or 0.001, respectively.

In this experiment, all *Pelargonium* treatments showed nutritional stress (necrotic lesions and overall lack of vigor) regardless of the form and concentration of Fe applied. It is probable that at the low substrate pH used in the experiment (pH 4.1 to 4.9), all three forms of Fe were highly soluble, and thus available to the plant (Boxma, 1981; Lindsay, 1979). For example, research by de Krijg et al. (1996) with chrysanthemums found that Fe uptake was not affected by iron form (either Fe-EDTA or FeSO₄) at a low pH range from 4.8 to 6.0.

Further investigation ruled out contamina-

tion from the tanks, water, or nutrient solution as the source of micronutrients for toxicity symptoms in the 0 mg·L⁻¹ Fe treatment. However, several additional sources of Fe and Mn were identified (Table 2). For example, the peat-perlite substrate, Banrot fungicide, hydrated lime, and Limestone-F were all found to contain significant levels of Fe and Mn that probably also contributed to toxicity.

To investigate whether Fe and Mn levels were similar in other peat sources, acid digestion and ICP analysis of seven commercial peat sources (Baccto Peat; Michigan Peat Co.,

Houston, Texas; Fisons Grower Grade Peat; Fine Peat, and Retail Fine Peat; Sun Gro Horticulture, Seba Beach, AB; Majestic and Trump Ace, Fafard, Agawam, Mass.) in a subsequent experiment found total Fe and Mn content to be 824 ± 446 and 48 ± 14 μg·g⁻¹, respectively. In comparison, total Fe and Mn measured in the peat source used in this experiment (with no perlite) was 948 and 31 μg·g⁻¹.

It is probable that Fe or Mn associated with the peat is only available to the plant through decomposition or, in the case of micronutrients, loosely bound to exchange sites. Berghage et al. (1987) found that DTPA-extractable Fe correlated well with plant available Fe. Therefore, based on a DTPA extraction from peat (Table 2), at low substrate pH the peat could be a significant (4.42 mg/pot) source of Fe (but not Mn), potentially exceeding that supplied by seven irrigations of 1 mg·L⁻¹ Fe nutrient solution (0.7 mg/pot, Table 2).

Other contaminant sources of micronutrients may also have supplied Fe and Mn. For example, Fe in the hydrated lime or flowable lime was probably Fe oxide, which has increased solubility as pH decreases (Lindsay, 1979). Both types of lime used in our experiment have been shown to be highly reactive, with little residual left once the reaction is complete (Argo and Biernbaum, 1996; Bishko et al., 2002). The fungicide supplied 0.18 g Fe/pot or 26% of that supplied by seven irrigations of 1 mg·L⁻¹ Fe nutrient solution (Table 2). The fungicide and lime contaminant sources may have delivered a pulse of Fe and Mn at their time of application.

Calibrachoa grown at high pH.

Initial substrate pH and EC at planting were

6.9 and 0.4 dS·m⁻¹. After planting, pH differed between measurement days ($p < 0.001$) with least-square mean pH levels of 6.5, 6.8, 6.3, and 6.4 on days 7, 14, 23, and 30, respectively. Substrate EC increased over time ($p < 0.001$) in an approximately linear fashion to 2.46 dS·m⁻¹ by day 28.

Plant growth. All plant growth variables were affected by the interaction of applied Fe form and concentration, with the exception of mean leaf count in which Fe form and concentration had main effects only (Table 3).

We observed that treatments with a high leaf count were associated with chlorosis, and that these shoots produced multiple small leaves with short internodes, similar to B deficiency symptoms reported by Mills and Jones (1996). Plants grown with FeSO₄, which had the lowest SPAD chlorophyll index, also had the highest leaf count (Table 3). As the applied Fe concentration was increased, the mean leaf count decreased (Table 3).

Plants receiving Fe-EDDHA were tallest and had the highest shoot dry mass and SPAD

chlorophyll index, followed by those receiving Fe-EDTA and then FeSO₄ (Table 3). The mean shoot dry mass and SPAD of the Fe-EDDHA plants increased from 0.5 to 1 mg Fe/L and then did not change from 1 to 4 mg Fe/L (Fig. 1D and E). Dry mass and SPAD index were above the control level with the 2 and 4 mg·L⁻¹ Fe-EDTA treatments only. The dry mass and SPAD for plants receiving FeSO₄ plants did not increase significantly above the control at any concentration. All treatments significantly reduced the percent of necrotic leaves

Fig. 4. (left) Representative plants showing the effect of applied Fe form and concentration for *Calibrachoa* (Fe-inefficient) grown at high pH (6.3 to 6.9) on day 28, and (right) an example of the nutrient deficiency symptoms. New leaves developed an overall chlorosis, progressing to necrotic lesions, flower bud abortion, and death of the meristem. Leaf number was increased and leaf size decreased in deficient foliage.

Fig. 5. Effect of applied Fe form and concentration on tissue nutrient concentrations ($\mu\text{g}\cdot\text{g}^{-1}$ of dry mass) for *Calibrachoa* (Fe-inefficient) grown at high pH (6.3 to 6.9). Symbols represent the means of 6 replicate samples $\pm$ one standard error. Sufficiency ranges based on general ornamental crops are taken from Vetanovetz (1996), with the exception of S (Mills and Jones, 1996).

compared with the control, with no lesions in any of the Fe-EDDHA treatments from 0.5 to 4 mg Fe/L (Fig. 1F).

Foliar nutrients. Control plants receiving no Fe in the fertilizer solutions were the first to show the symptoms of nutrient deficiency, with chlorosis obvious by day 7, followed by plants receiving the lowest two concentrations of FeSO₄. Figure 4A shows *Calibrachoa* from each experimental treatment after 28 days, with necrosis and chlorosis symptoms (Fig. 4B) identical to those for Fe deficiency reported for *Calibrachoa* by Fisher et al. (2003).

Application of Fe-EDDHA at 1 mg·L⁻¹ Fe or higher resulted in vigorous, horticulturally-acceptable plants, even though they were grown at a substrate pH significantly higher than that recommended for iron-inefficient plants (Argo and Fisher, 2002). In terms of plant appearance, the only other treatment that resulted in growth comparable with plants receiving Fe-EDDHA was Fe-EDTA at 4 mg·L⁻¹ Fe.

Foliar Fe concentration for plants grown with Fe-EDDHA was greater than from other Fe sources. The stability of Fe-EDDHA is 10⁴ to 10⁶ times higher than other available chelates such as Fe-EDTA and Fe-DTPA (Kroll, 1957) and the stability of Fe-EDDHA does not decline in a peat-based substrate as pH increases from 4.35 to 7.85 (Boxma, 1981). The lower stability of Fe-EDTA is caused by displacement of Fe from the chelate by other elements such as Ca and Zn (Álvarez-Fernández et al., 1996). FeSO₄ is the least soluble of the three Fe forms at high pH, and inorganic Fe decreases 1000-fold in solubility for each unit increase in pH (Lindsay, 1979).

Although foliar-Fe concentration was greatest for Fe-EDDHA plants, foliar Fe concentrations were near or below the sufficiency range in all treatments. Foliar-Fe was also not well correlated with SPAD chlorophyll level, percent necrotic leaves, shoot dry mass, or leaf count. However, when measured separately, chlorophyll levels of the Fe-EDDHA treatments were the highest, followed by Fe-EDTA and FeSO₄, respectively (data not shown). Total Fe in plant tissue is often poorly related with Fe deficiency symptoms, because not all foliar Fe is physiologically active (Bennett, 1945; Marschner, 1995; Oserkowsky, 1933). The reported sufficiency ranges for total Fe should therefore be interpreted with caution.

Other foliar micronutrient measurements, including the Fe to Mn ratio and total Mn, were affected by Fe treatments and were more closely correlated with plant growth variables than Fe. The Fe to Mn ratio was found by Smith et al. (2005b) to be a good indicator of micronutrient deficiency at high medium pH for *Petunia* and *Impatiens* compared with other nutrient levels including total and ferrous Fe. Fe:Mn was also well correlated ($r^2 = 0.83$) with SPAD chlorophyll index in this trial. The Fe to Mn ratios above 0.6 occurred with Fe-EDDHA treatments and the highest concentration of Fe-EDTA, and foliage on these plants had a deep green color and the maximum SPAD level (Figs. 1E and 4A). In comparison, Smith et al. (2005b) estimated that minimum acceptable Fe:Mn ratios (below

which SPAD dropped) were 0.57 for *Impatiens* or 0.71 for *Petunia*.

The levels of all macronutrients, as well as B, Mn, and Zn were lowest under the high concentration Fe-EDDHA treatments, regardless of micronutrient ionic charge or tendency to form metal-EDDHA bonds (Fig. 5). Because we only measured total tissue nutrient concentrations it is not possible to identify exactly how Fe-EDDHA affected uptake of other nutrients. There are several potential mechanisms. One mechanism is competition with Fe for binding sites on the root surface. High concentrations of one metal ion can inhibit uptake of another metal, with inhibitions recorded for the pairs Fe–Mn, Cu–Mn, Cu–Zn, Ca–Zn, Ca–Mn, Ca–Fe, and Mg–Zn (Laurie and Manthey (1994). A second could be a dilution effect, which can occur where plants grew more rapidly with Fe-EDDHA and although foliar concentration of other nutrients was decreased, total content (concentration × dry mass) may have been equal or greater. Finally, competitive inhibition of ion transport across the root membrane could have reduced micronutrient uptake (Kroll, 1957; Marschner, 1995). For example, Wallace et al. (1957) and others noted that Mn uptake may be reduced in plants following application of EDDHA or Fe-EDDHA in calcareous soils, and we also observed decreased Mn with increasing Fe-EDDHA (Fig. 5H).

Conclusions

For Fe-efficient plants grown at low pH, the form of iron is less important than at high pH, because as pH decreases both inorganic and chelated forms of Fe are soluble. The potential for Fe toxicity at low pH depends on the total quantity of Fe supplied by fertilizer and a number of other potential sources. The most important conclusion regarding Fe form and concentration for Fe-efficient species regards the importance of maintaining media pH at an adequately high level (above 6, Argo and Fisher (2002)) to ensure that Fe (as well as Mn, Cu, and Zn) from fertilizer and other sources is not accumulated to toxic levels.

In the production of Fe-inefficient plants grown at high pH, the quality of the iron source is very important. Most contaminant sources of Fe and Mn that may have contributed to toxicity at low pH appear to have been unavailable to the plant at high pH, leaving only Fe-EDDHA, Fe-EDTA, or FeSO₄ nutrient solutions as important Fe sources.

Argo and Fisher (2002) concluded that the acceptable pH range for growing iron-inefficient crops, such as *Calibrachoa*, is pH 5.4 to 6.2. However, this conclusion was based on the use of Fe-EDTA as the iron source, because it is the most common source found in commercially prepared water-soluble fertilizers. It is probable that the acceptable pH range would have been different if other sources of iron were used. For example, Fe-EDDHA may have expanded the acceptable pH range up to 6.4 or higher because its solubility is not affected by substrate pH up to 7.85 (Boxma, 1981). If the Fe source were FeSO₄, then the

acceptable pH range may be lower (below 6.0) because low substrate pH would be needed to increase Fe solubility and make Fe available to the plant.

Iron concentration in the water-soluble fertilizer also plays a key role in determining acceptable pH ranges. In our experiments, increasing the concentration of Fe-EDTA up to 4 mg·L⁻¹ Fe resulted in similar plant growth compared with using Fe-EDDHA applied at 1 mg·L⁻¹ Fe (Fig. 1). In comparison, chlorophyll level in plants grown with FeSO₄ plateaued at half the level of the Fe-EDDHA-grown plants. Thus, simply increasing the applied concentration with FeSO₄ may not be sufficient to overcome deficiency problems. Furthermore, the greater efficacy of Fe-EDDHA at low concentration compared with other iron forms may outweigh the additional cost of Fe-EDDHA as an Fe source for horticulture producers. Fe-EDDHA would have the greatest cost to benefit ratio for high-value crops grown at high pH.

Although Fe plays a pivotal role in determining acceptable pH ranges for Fe-efficient and Fe-inefficient crops (Albano and Miller, 1996; Argo and Fisher, 2002; Römhild, 1987), it is not the only nutrient affected by substrate pH (Peterson, 1981). In this study, the uptake of B, Cu, Mn, and Zn was also affected by substrate pH, especially at high pH (Fig. 5). A longer term study may have shown some limitations with using Fe-EDDHA for extending the upper level of the acceptable pH range for iron-inefficient crops.

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