Nutrient Uptake in Container-grown Impatiens and Petunia in Response to Root Substrate pH and Applied Micronutrient Concentration

Brandon R. Smith¹ and Paul. R. Fisher²

Department of Plant Biology, University of New Hampshire, Durham, NH 03824

William R. Argo

Blackmore Company, Belleville, MI 48111

Additional index words. iron, manganese, soilless medium, peat, chelate, ethylenedinitrilotetraacetic acid, EDTA, SPAD, chlorophyll, carotenoid, fertilizer, greenhouse, floriculture

Abstract. The objective was to quantify the effect of substrate pH and micronutrient concentration on tissue nutrient levels in *Petunia ×hybrida* Hort. Vilm.-Andr. and *Impatiens wallerana* Hook. F. Plants were grown in 10-cm-diameter pots for 4 weeks in a 70% peat : 30% perlite medium amended with five lime rates to achieve substrate pH values ranging from pH 4.4 to 7.0. Plants were irrigated with (in mg·L⁻¹) 210N–31P–235K–200Ca–49Mg. Micronutrients were applied as an EDTA (ethylenedinitrilotetraacetic acid) chelated micronutrient blend (C111), at 1×, 2×, and 4× concentrations of 0.50Fe–0.25Mn–0.025Zn–0.04Cu–0.075B–0.01Mo. Patterns of tissue concentrations across substrate pH differed from nutrient solubility in the medium, particularly with regard to Mn. Foliar N content decreased slightly as substrate pH increased, whereas foliar Ca, Mg, and S increased. Although foliar P and K varied with pH, there was no consistent trend between species. Foliar total Fe, ferrous Fe, and Cu decreased as substrate pH increased, whereas foliar Zn increased. Foliar Mn content decreased for both species as pH rose to 6.0, and then increased from pH 6.0 to 7.0. In contrast, Mn level in the substrate, measured in a saturated medium extract using deionized water as the extractant, decreased as pH increased from pH 4.4 to 7.0. Chlorophyll content decreased when the ratio of tissue Fe to Mn was <0.57 (impatiens) or <0.71 (petunia), or Fe was <106 (impatiens) or 112 (petunia) μg·g⁻¹. SPAD chlorophyll index also declined in petunia with foliar Mn >42 μg·g⁻¹. Increasing C111 increased foliar Cu, total Fe and ferrous Fe in both species, and B forimpatiens, and partly compensated for reduced nutrient solubility at high pH.

Substrate pH affects nutrient solubility in a peat-based medium, and the resulting uptake of nutrients into plant tissue (Nelson, 1998; Peterson, 1981). Solubility of P, Fe, Mn, Zn, Cu, and B decrease as pH increases in a highly organic medium, whereas Mo solubility increases with increasing pH (Biernbaum and Argo, 1995; Lucas and Davis, 1961; Peterson, 1981; Wright and Niemiera, 1987). For floricultural crop species grown in a soilless medium, the most common nutrient imbalances that occur when pH is outside the optimum range of 5.6 to 6.2 (Bailey and Nelson, 1998) are either toxic levels of Fe and Mn at low pH or Fe deficiency at high pH (Albano and Miller, 1998; Argo and Fisher, 2002; Biernbaum et al., 1988; Reed, 1996).

Received for publication 13 Jan. 2003. Accepted for publication 3 Nov. 2003. This is New Hampshire Agricultural Experiment Station scientific contribution 2160. We acknowledge the University of New Hampshire Agricultural Experiment Station, Anna and Raymond Tuttle Horticultural Endowment, D.S. Cole Growers, FIRST, Greencare Fertilizers Inc., Pleasant View Gardens, The Scotts Company Inc., and Sun Gro Horticulture Inc. for supporting this research. We thank John Biernbaum, Chris Neefus, Stuart Blanchard, Leland Jahnke, and Dean Kopsell for their advice.

¹Graduate research assistant.

²Associate professor and corresponding author.

The form and concentration of micronutrients applied in a water-soluble fertilizer (WSF) affect the available nutrient concentration in the soil solution across a range in pH. Solubility of sulfate and oxide forms of metal micronutrients in peat-based media is very high at a low media pH, but decreases rapidly as the media pH increases >5.5 (Peterson, 1981; Reed, 1996). Chelated metal micronutrients tend to remain soluble over a wider pH range, and the stability of Fe chelates at high pH (>6.5) is EDDHA (ethylenediaminobis(2-hydroxyphenyl)acetic acid) >> DTPA (diethylenetrinitriolpentaacetic acid) > EDTA > HEDTA (N-(2-hydroxyethyl)ethylenedinitrilotriacetic acid) >> citrate (Boxma, 1981; Lindsay, 1979; Reed, 1996).

A review of product labels from commercially available blended water-soluble fertilizers used in North America shows that common sulfate micronutrient forms are FeSO₄·7H₂O, MnSO₄·H₂O, ZnSO₄·H₂O and CuSO₄·5H₂O, and common chelated micronutrient forms are Fe-EDTA, Mn-EDTA, Zn-EDTA, and Cu-EDTA. Iron-DTPA and Fe-EDDHA are also commercially available, but are not commonly found in blended fertilizers. Common forms of B and Mo are H₃BO₃, Na₂B₄O₇, and Na₂MoO₄.

Another factor affecting micronutrient nutrition is the concentration applied to the

crop. Our review of commercially available blended water-soluble greenhouse fertilizers used in North America (Smith et al. [companion article a]) found that median micronutrient concentrations were 0.5 mg·L⁻¹ Fe (range: 0.1 to 6 mg·L⁻¹), 0.2 mg·L⁻¹ Mn (range: <0.01 to 1), 0.05 mg·L⁻¹ Cu (range: <0.01 to 0.5), 0.1 mg·L⁻¹ Zn (range: <0.01 to 0.55 mg·L⁻¹), 0.05 mg·L⁻¹ B (range: <0.01 to 1.0), and 0.03 mg·L⁻¹ Mo (range: <0.001 to 0.5 mg·L⁻¹) for every 100 mg·L⁻¹ N. The concentration of nitrogen applied to a crop is therefore likely to affect whether the micronutrient concentrations are adequate for plant growth, or are insufficient (deficiency) or excessive (toxicity) at a particular substrate pH.

Nutrient uptake can differ greatly from nutrient solubility because of a variety of biological factors in the rhizosphere, such as active transport mechanisms by roots, root exudation of chelating, reducing, and acidifying agents, competition and antagonisms between nutrients and other salts, and competition with soil microorganisms (Marschner, 1995). For example, Lucas and Davis (1961) and Peterson (1981) concluded that the optimal pH for P nutrition was 5.5 in media without soil, because above this pH water-soluble P concentration in the medium began to decrease. However, Adams et al. (1978) found that the P content of lettuce leaves was unaffected by medium pH up to 6.5, even though the concentration of water-soluble P measured in the medium was 38% of that measured at pH 5.5. Argo and Biernbaum (1996) also found that although the concentration of water-soluble P in the root medium decreased with increasing pH, the effect onimpatiens shoot-tissue P was minimal until pH was above 7.2.

Our goal was to quantify the effect of substrate pH and micronutrient concentration on tissue nutrient levels for two floricultural crop species—hybrid petunia (*Petunia ×hybrida* Hort. Vilm.-Andr.) and hybridimpatiens (*Impatiens wallerana* Hook. F.). We hypothesized that the primary effect of increasing substrate pH would be a reduction in tissue concentrations of micronutrients (especially Fe), based on existing research (Fisher et al., 2003; Miller et al., 1984; Peterson, 1981). We further hypothesized that increased micronutrient concentration in fertilizer would increase leaf chlorophyll level by increasing tissue levels of these nutrients and compensating for lower solubility at high substrate pH. Specific objectives were to 1) quantify the effect of substrate pH on tissue macronutrients, 2) quantify the effect of substrate pH on tissue micronutrients, particularly with regard to Fe and Mn, and 3) to correlate tissue nutrient levels with pigment concentration.

Materials and methods

Design. Two species, *Petunia ×hybrida* ‘Priscilla’ and *Impatiens wallerana* ‘Rosebud Purple Magic’, were grown in a 70% peat : 30% perlite medium at five preplant lime incorporation rates, and irrigated using a complete WSF that contained one of 3 different micronutrient concentrations in a factorial design (five lime

rates $\times$ three micronutrient concentrations). The experiment was a randomized, complete-block design that took place on four benches in the same greenhouse compartment, where each bench represented a block. Combinations of micronutrient concentration and lime rate were randomized within each block, and each combination contained 10 replicate pots per block. Data were analyzed using analysis of variance (ANOVA) and Tukey's mean comparison in PROC GLM of SAS (SAS Institute, Cary, N.C.). In addition, we performed linear regression analysis on medium Mn versus tissue Mn; medium Fe versus tissue Fe (total); medium Fe versus tissue Fe²⁺; and tissue Fe (total) versus tissue Fe²⁺.

Growing conditions. Rooted cuttings were planted in 450-cm³ containers (10-cm standard round pots), spaced 13.5 cm apart on center. The experiment took place on four 1.2 $\times$ 4.6-m hard plastic benches in a 6.1 $\times$ 9.1-m glasshouse for 4 weeks. Greenhouse air and soil temperature was measured by a Campbell CR10X datalogger (Campbell Scientific Inc., Logan, Utah) using type-T thermocouples. The air sensor was aspirated and shielded, and mean daily air temperature ($\pm$ standard deviation) was 21.0 $^{\circ}$ C $\pm$ 0.4. Soil temperature was 20.2 $^{\circ}$ C $\pm$ 1.0. When ambient PAR (photosynthetically active radiation) light level fell below 600 μ mol-m⁻²-s⁻¹ between 06:30 to 17:30, we provided supplemental lighting with high-pressure sodium lamps at 46 μ mol-m⁻²-s⁻¹.

Growing medium. Both species were grown in a 70% (by volume) Canadian sphagnum peat (Fisons professional black bale peat, Sun Gro Horticulture, Bellevue, Wash.) and 30% super coarse perlite (Whittemore Co., Lawrence, Mass.). At mixing, 0.9 kg-m⁻³ of a 6N-4P-7.5K-10Ca-1Mg-0.3Fe-0.04Mn-0.02Zn-0.036Cu-0.049B-0.0097Mo preplant nutrient charge fertilizer (GreenCare Fertilizers, Inc, Chicago, Ill.) was added. Preplant nutrients were derived from KNO₃, Ca(NO₃)₂, triple superphosphate (0N-19.8P-0K), CaSO₄, MgSO₄, Fe₂O₃, MnO₂, CuO, H₃BO₃, and (NH₄)₆Mo₇O₂₄.

Type N microfine dolomitic hydrated lime (63.2% Ca(OH)₂, 33.8% MgO, 0.8% SiO₂, 0.4% H₂O, 0.2% Al₂O₃, 0.1% Fe₂O₃, 0.03% S; 92% of which passed through a 45- μ m screen, National Lime and Stone, Findlay, Ohio) was incorporated at 1.1, 1.5, 1.9, 2.4, or 3.1 kg-m⁻³ to give initial substrate pH values of (SE $\pm$ 0.01) 4.5, 4.8, 5.3, 6.1, and 7.2, and with initial electroconductivity (EC) of (SE $\pm$ 0.01) 1.1, 1.2, 1.3, and 1.3 dS-m⁻¹, respectively. A deionized water solution containing 1.4 mL-L⁻¹ of a wetting agent (Psi Matric, Aquatrols, Cherry Hill, N.J.) was added to all medium at 0.14 L-m⁻³, and the medium was allowed to equilibrate for 5 d before planting.

Fertilization. Plants were fertilized with a deionized water macronutrient solution consisting of 210N-31P-235K-200Ca-49Mg from 5 mM Ca(NO₃)₂, 5 mM KNO₃, 2 mM MgSO₄, and 1 mM KH₂PO₄. Micronutrients were supplied by Compound 111 (abbreviated to C111 in this text, Scotts Co., Marysville, Ohio) EDTA-chelated micronutrient complex [0.74% Mg (MgSO₄), 0.232% B (H₃BO₃), 0.1136% Cu (Cu-EDTA), 1.5% Fe

(Fe-EDTA), 0.74% Mn (Mn-EDTA), 0.0242% Mo (Na₂MoO₄), 0.075% Zn (Zn-EDTA)]. C111 was added to the macronutrient solution at 0.033, 0.067, or 0.134 g-L⁻¹. Using Fe as an example, C111 supplied 0.5, 1.0 or 2.0 mg-L⁻¹ Fe, respectively.

Tensiometers (Mini "LT", Irrrometer, Riverside, Calif.) were randomly placed in three pots per species. All plants of an individual species were top-watered with treatment solutions when medium dryness exceeded -5 kPa. Each pot received $\approx$ 110 mL of solution at each irrigation, with a mean leaching rate of <8%.

Data collection. To measure pH and EC, a saturated paste was prepared from the media using deionized water as the extractant (Warnke, 1995). Substrate pH was measured directly in the slurry, and electroconductivity was measured on the filtered extract 0, 7, 14, and 21 d after planting from two destructively sampled replicates per block. Final pH and EC were collected 28 d after planting on all replicates. Substrate pH was measured using a solid-state pH probe (model 6165 Sure-Flow pHuture Probe; Orion Technologies, Beverly, Mass.) with a pH meter (model 620; Orion), and EC was measured with an electroconductivity meter (model 130; Orion).

Medium and tissue nutrient concentrations were quantified using inductively coupled plasma (ICP) atomic emission spectrophotometry. Samples were collected 28 d after planting, and the four final replications per pH $\times$ C111 combination within each block were combined for ICP analysis of the root medium and plant tissue.

Tissue nutrients. Tissue harvested from each pot was rinsed in a distilled water solution containing 0.5% nonionic, phosphate-free detergent (Aquet, Bel-Art, Pequannoc, N.J.). Plant leaves were separated from petioles and stems, and dried in a 50 $^{\circ}$ C oven for 7 d. Dried tissue was ground in a Thomas-Wiley grinding mill in order to pass through a 40-mesh screen. Ten milliliters of HNO₃ was added to 0.3 g of ground tissue and microwave digested for 15 min while ramping to 170 $^{\circ}$ C, and then for another 15 min at 170 $^{\circ}$ C. The sample was diluted to 50 mL with distilled, deionized water, and nutrient concentrations were determined using the ICP. Molybdenum levels were not measured with adequate precision, and are not reported.

Active Fe. Oserkowsky (1933) and Bennett (1945) found that extracting the physiologically active ferrous iron (Fe²⁺) with dilute HCl provided a more accurate diagnosis of Fe nutritional status in leaf tissue than total Fe. Ferrous Fe was determined according to the method of Oserkowsky (1933), which was modified by Llorente et al. (1976). We added 10 mL of 1 N HCl to 0.5 g of dried tissue in a 15-mL centrifuge tube placed horizontally on an orbital shaker (VWRbrand, So. Plainfield, N.J.) and shaken for 4 h at 2.0 r-s⁻¹. Samples were centrifuged at 167 r-s⁻¹ for 30 min, then the upper phase of the solution was filtered twice through filter paper (no. 1, Whatman paper, Maidstone, Kent, U.K.) using a buchner funnel under vacuum extraction. The Fe level in the filtrate was analyzed by ICP.

Comparison between SPAD and tissue nutrient levels. The Minolta SPAD meter (Soil Plant Analysis Development, Ramsey, N.J.) indirectly measures chlorophyll content in a nondestructive manner. SPAD measurements were taken on 10 random leaves per plant and were reported in detail by Smith et al. ([companion article a]). To correlate tissue nutrient levels and chlorosis, we compared the levels of tissue nutrients with measured SPAD values. The nonlinear procedure in SAS (SAS Institute, Cary, N.C.) was used to fit an empirical model to data for each species using equations based on Anderson and Nelson (1975). We fit two alternative linear models to each nutrient, where *Nutr* represented the foliar concentration of the nutrient:

1. **One phase:** There was a linear relationship ($a + b_1 \text{Nutr}$) between the nutrient and SPAD index, fitted using linear regression to estimate *a* and *b*₁.

2. **Two phase:** a) Below a critical nutrient level *Nutr*_{crit}, a positive linear relationship ($a + b_1 \text{Nutr}$) described the effect of the nutrient on SPAD index up to a critical SPAD value (*C*_{crit}), and b) above *Nutr*_{crit}, the slope changed to a linear relationship with a different gradient ($C_{crit} + b_2(\text{Nutr} - \text{Nutr}_{crit})$) with nutrient concentration, possibly because of nutrient saturation or toxicity. Four parameters (*a*, *b*₁, *b*₂, and *Nutr*_{crit}) were estimated by the nonlinear procedure. *C*_{crit} was calculated from $a + b_1 \text{Nutr}_{crit}$.

We reported one model for each nutrient. If a unique statistical solution existed for all parameters for both model 1 and model 2, as estimated by SAS, we selected the model with 1) the highest adjusted *r*² or 2) the fewest parameters (i.e., model 1) if adjusted *r*² was equal for more than one model.

Results and Discussion

pH and electroconductivity. Substrate pH and EC data for this experiment were presented by Smith et al. ([companion article a]). Petunia tended to have a slightly higher substrate pH (by up to 0.2 units at a given lime rate) than impatiens, however C111 did not affect substrate pH. For petunia, the least-square mean pH values of the lime treatments over time (SE $\pm$ 0.02) were 4.6, 4.9, 5.3, 6.1, and 7.0 pH units, whereas for impatiens the mean pH values were 4.4, 4.7, 5.1, 6.0, and 7.0. Substrate EC remained within acceptable ranges (Warnke, 1995) throughout the experiment, and increased from an average 1.2 dS-m⁻¹ at planting to 2.29, 2.45, and 2.57 dS-m⁻¹ at the end of the experiment for the 0.033, 0.067, and 0.134 g-L⁻¹ C111 treatments, respectively.

Foliar macronutrients. Substrate pH affected all foliar macronutrient concentrations (Table 1). Foliar N content decreased slightly as substrate pH increased (by <10% from the lowest to highest pH level), whereas foliar Ca, Mg and S tended to increase. The increase in foliar Ca and Mg with increasing substrate pH probably resulted from additional dolomitic lime used to achieve the higher substrate pH treatments. Although foliar P and K varied with pH, there was no consistent trend between species. Increasing C111 concentration decreased

P overall with petunia, but in impatiens there was a significant interaction, whereby increasing C111 resulted in a decline in P at low pH and an increase in P at high pH. Given the number of nutrients statistically analyzed for this experiment, however, the interaction for P may not be horticulturally significant.

Foliar macronutrients were generally within the acceptable ranges for bedding plant species used by testing laboratories (including the survey range from Mills and Jones (1996) in Table 1, and ranges reported by Vetanovetz (1996)). Differences between observed levels and suggested nutrient ranges (for example high observed N content for impatiens) may indicate that the published ranges may not accurately reflect toxic or deficient levels for these particular cultivars. Differences between our results and other ranges may have

resulted from our tissue sampling method, however, because all foliage was included in the analysis whereas commercial testing laboratories generally sample recently mature leaves (Vetanovetz, 1996).

Foliar micronutrients. Substrate pH affected all foliar micronutrient levels (Table 2). In both species, total Fe and ferrous Fe consistently decreased as substrate pH increased. Ferrous Fe in tissue was positively correlated with total Fe in tissue for both species ($p < 0.001$, data not shown). For petunia, foliar contents of Zn and B increased as pH rose, whereas Cu content decreased. Zn increased in impatiens as substrate pH increased.

Foliar Mn did not change significantly as pH increased to 6 (Table 1), but when substrate pH increased from 6 to 7, the mean Mn content increased for both petunia (by 80% compared

with pH 6) and impatiens (37%). Biernbaum and Argo (1995) reported a similar trend for impatiens grown at a wide pH range and irrigated with 3 WSFs differing in NH_4 to NO_3 ratio, Ca, Mg, and S content, but containing the same micronutrients concentrations. Foliar Mn in the Biernbaum and Argo (1995) study decreased from pH 4.0 to 6.0, then increased as medium pH rose from 6.0 to 8.5. The ratio of Fe to Mn in plant tissue, two nutrients that can have antagonistic effects on uptake (Bunt, 1988; Mills and Jones, 1996), decreased with increasing substrate pH in both species.

Increasing the concentration of C111 led to a higher foliar content of total Fe (impatiens only), ferrous Fe, and Cu in both species (Table 2), and increased the ratio of Fe to Mn. Higher concentrations of C111 decreased foliar Mn in petunia, but impatiens foliar Mn was not

Table 1. Summary ANOVA table showing the effects of Compound 111 EDTA-chelated micronutrient complex (C111) and substrate pH (least-square mean over time) on tissue macronutrient content ($\mu\text{g}\cdot\text{g}^{-1}$ dry tissue) for petunia and impatiens. Species were analyzed separately. Tissue was collected 28 d after planting from four replicate samples, with each sample combining tissue from four plants.

Substrate	Petunia							Impatiens					
	N	P	K	Ca	Mg	S		N	P	K	Ca	Mg	S
C111	NS	*	**	NS	*	NS		NS	NS	NS	NS	NS	NS
Substrate pH	***	*	***	**	***	***		***	**	**	**	***	***
C111 × substrate pH	NS	NS	NS	NS	NS	NS		NS	*	NS	NS	NS	NS
Substrate pH	Least-square means						Substrate pH	Least-square means					
4.6	60910 a	7152 ab	52100 bc	17901 ab	10746 bc	3276 bc	4.4	66200 a	6142 ab	21925 a	19186 b	12531 c	4948 c
4.9	60524 a	7097 b	50662 bc	16908 b	11273 bc	3061 c	4.7	66400 a	6486 ab	21440 a	22216 a	13639 bc	6172 bc
5.3	59150 ab	7679 ab	48966 c	16491 b	11793 c	3062 c	5.1	66000 a	5872 b	19999 ab	21456 ab	13634 bc	5234 bc
6.1	59000 ab	8023 a	53595 ab	17394 ab	13690 ab	3570 b	6.0	63310 b	6405 ab	19064 b	21761 ab	14120 b	6228 b
7.0	57170 b	7305 ab	57594 a	19267 a	15262 a	4104 a	7.0	64280 b	6796 a	20851 ab	22958 a	16941 a	8809 a
C111 concn (g·L ⁻¹)	Least-square means						C111 concn (g·L ⁻¹)	Least-square means					
0.033	59660 a	7800 a	54206 a	18166 a	12764 ab	3561 a	0.033	65600 a	6471 a	21304 a	21704 a	14158 a	6378 a
0.067	59515 a	7500 ab	50523 b	17399 a	12997 a	3374 a	0.067	64690 a	6224 a	20211 a	21598 a	14274 a	6181 a
0.134	59070 a	7100 b	53021 ab	17211 a	11897 b	3309 a	0.134	65485 a	6327 a	20453 a	21505 a	14087 a	6276 a
Survey range min ²	38500	4700	31300	12000	3600	3300		36400	7700	13700	17500	8900	8300
Survey range max	76000	9300	66500	28100	13700	8000		58300	9200	23500	24000	36400	8700

²Minimum and maximum values reported by Mills and Jones (1996) for each species.

NS,*,**,***Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively. Letters after each treatment mean represent mean separation using Tukey's HSD at $p = 0.05$.

Table 2. Summary ANOVA table showing the effects of Compound 111 EDTA-chelated micronutrient complex (C111) and substrate pH (least-square mean over time) on tissue micronutrient content ($\mu\text{g}\cdot\text{g}^{-1}$ dried tissue) for petunia and impatiens. Species were analyzed separately. Tissue was collected 28 d after planting from four replicate samples, with each sample combining tissue from 4 plants.

	Petunia								Impatiens							
Parameter	Fe	Fe ²⁺	Mn	Fe:Mn ²	Cu	Zn	B		Fe	Fe ²⁺	Mn	Fe:Mn	Cu	Zn	B	
C111	*	**	*	***	***	NS	NS		***	***	NS	***	***	NS	**	
Substrate pH	***	***	***	***	***	***	***		***	***	***	***	**	*	*	
C111 × substrate pH	NS	NS	NS	NS	NS	NS	NS		NS	NS	NS	NS	NS	NS	NS	
Substrate pH	Least-square means							Substrate pH	Least-square means							
4.6	159 a	81 a	155 b	1.02 a	14 a	75 c	36 abc	4.4	146 a	97 a	218 b	0.67 a	9 ab	22 b	39 a	
4.9	130b	69 ab	153 b	0.85 b	13 a	83 bc	29 c	4.7	138 a	91 a	226 b	0.61 ab	11 a	25 ab	39 a	
5.3	120bc	62 b	152 b	0.76 bc	12 a	105 ab	31 bc	5.1	118 b	81 ab	208 b	0.57 bc	9 b	25 ab	37 ab	
6.1	104cd	47 c	143 b	0.72 c	8 b	115 a	38 ab	6.0	101 b	64 bc	194 b	0.52 c	9ab	33 ab	31 ab	
7.0	91d	38 c	257 a	0.34 d	8 b	127 a	43 a	7.0	100 b	58 c	265 a	0.38 d	8 b	46 a	26 b	
C111 concn (g·L ⁻¹)	Least-square means							C111 concn (g·L ⁻¹)	Least-square means							
0.033	113 a	51 b	185 a	0.65 b	10 b	98 a	37 a	0.033	103 b	67 b	224 a	0.46 c	8 b	24 a	28 b	
0.067	123 a	63 a	173 ab	0.75 a	10 b	111 a	35 a	0.067	123 b	74 b	223 a	0.56 b	9 b	31a	34 ab	
0.134	126 a	65 a	171 b	0.82 a	13 a	94 a	35 a	0.134	136 a	92 a	219 a	0.63 a	11 a	36 a	40 a	
Survey range min ^y	84	N/A ^x	44	N/A	3	25	18		107	N/A	329	N/A ^x	20	57	23	
Survey range max	168	N/A	177	N/A	19	115	43		130	N/A	419	N/A	37	67	25	

³Ratio between total Fe and total Mn in the leaf tissue.

⁴Minimum and maximum values reported by Mills and Jones (1996) for each species. Survey data not available for ferrous iron or Fe:Mn.

⁵N/A = not applicable.

NS,*,**,***Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively. Letters after each treatment mean represent mean separation using Tukey's HSD at $p = 0.05$.

Fig. 1. The effect of substrate pH and fertilizer micronutrient concentration (C111) on the concentration of Fe and Mn in the medium 28 d after transplant, measured in a saturated paste prepared from the medium using deionized water as the extractant: (A) Fe and (B) Mn, for petunia and impatiens 28 d after planting. Solid lines represent least-square means for each pH. Symbols represent least-square means for each C111 treatment (four replications) $\pm$ 95% confidence intervals ($\circ$ 0.033 g·L⁻¹, $\blacksquare$ 0.067 g·L⁻¹, and $\blacktriangle$ 0.134 g·L⁻¹).

affected by C111. Higher concentrations of C111 led to higher foliar B in impatiens, but did not affect petunia foliar B or Zn.

Medium Fe and Mn. Substrate pH, C111, and their interaction affected the total Fe and Mn concentration in the root medium in both species, except for Mn in impatiens where there was no interaction. Soluble Fe in the growing medium decreased as substrate pH increased (Fig. 1A and B), consistent with published research on nutrient solubility in a soilless medium (Peterson, 1981). The lower the substrate pH, the greater the effect of C111 on increasing Fe in the medium (Fig. 1A and B), and at pH 7.0 C111 concentration did not affect medium Fe. Medium Fe and tissue Fe (both total Fe and ferrous Fe) were positively linearly correlated for both species ($p < 0.001$, data not shown).

Medium Mn also decreased as substrate pH increased (Fig. 1C and D). In contrast with medium Fe, however, the high (0.134 g·L⁻¹) C111 treatment resulted in increased medium Mn across the entire pH range. Mn in the medium and tissue were not correlated for either impatiens or petunia.

One aspect of EDTA-chelated micronutrients (as used in C111) is that Fe-EDTA becomes unstable in the medium solution above pH 6.5 (Lindsay, 1979). Evidence for this instability was found in the reduced Fe levels in the root medium at high substrate pH (Fig. 1). Problems exist, however, in correlating soil chemical equilibrium relationships with the measured nutrient levels in medium and tissue. The nutrient concentration in the root medium of a growing crop is the result of both solubility and uptake by plant roots. In addition, correlations between the medium and tissue nutrients may be poor because the medium nutrient content is a measure of availability at one point in time, whereas the tissue content is the result of nutrient accumulation over an extended growing period.

The pattern of foliar Mn concentration may have been caused by a combination of soil chemistry (for example, Mn-EDTA is more stable than Fe-EDTA at pH 7 (Norvell, 1991)) and an antagonistic interaction between uptake of Fe and Mn (Marschner, 1995). We have seen a similar tissue Mn increase at high substrate pH consistently in other unpublished experiments with petunia, which have also used the EDTA-chelated form of micronutrients, and the high Mn content in tissue at substrate pH above 6.5 is consistently associated with low tissue Fe content. Furthermore, we have observed that the tissue Mn content at high pH can decline following a corrective soil drench

Table 3. Regression of foliar nutrient concentration and SPAD chlorophyll index. Two models were fit for each nutrient for each species: either a single straight line ($a + b_i \text{Nutr}$, where Nutr represented the nutrient concentration in $\mu\text{g}\cdot\text{g}^{-1}$), or a two-line model (for $\text{Nutr} \leq \text{Nutr}_{\text{crit}}$, $a + b_1 \text{Nutr}$; for $\text{Nutr} > \text{Nutr}_{\text{crit}}$, $a + b_1 \text{Nutr}_{\text{crit}} + b_2 (\text{Nutr} - \text{Nutr}_{\text{crit}})$). Adjusted r^2 was higher for the two-line model than the one-line model for B, Fe, Fe²⁺, Mg, Mn, and Fe to Mn ratio for petunia, and B, Fe, Fe²⁺, Mg, and Fe to Mn ratio for impatiens. Data were collected 28 d after transplanting, and the model was fit to combined data for each species from all pH and C111 levels ($n = 60$).

Species	r^2	Parameter estimates			$Nutr_{crit}$ (critical nutrient level)
		a (intercept)	b_l (1 st gradient)	B_2 (2 nd gradient)	
Petunia (μg·g ⁻¹)					
N	0.25	−43.18	0.001437	---	---
P	NS	---	---	---	---
K	0.34	83.45	0.000784	---	---
Ca	0.22	71.98	0.00169	---	---
Mg	0.48	47.46	0.000216	0.00355	13065
S	0.47	70.27	−0.00822	---	---
Fe	0.51	4.38	0.353	0.042	112.1
Fe ²⁺	0.46	25.06	0.319	0.041	64.0
Mn	0.69	42.30	0.019	−0.14	170.0
Zn	0.15	50.46	−0.082	---	---
Cu	0.37	29.95	1.10	---	---
B	0.28	−13.27	2.73	−0.42	22.4
Fe:Mn (unitless)	0.83	17.94	38.43	2.69	0.71
Impatiens (μg·g ⁻¹)					
N	0.13	−26.99	0.001197	---	---
P	0.09	70.21	−0.00301	---	---
K	NS	---	---	---	---
Ca	NS	---	---	---	---
Mg	0.25	49.14	0.000267	−0.00340	14821
S	0.17	61.83	−0.00172	---	---
Fe	0.44	16.41	0.33	0.061	106.0
Fe ²⁺	0.42	19.75	0.52	0.09	58.3
Mn	NS	---	---	---	---
Zn	NS	---	---	---	---
Cu	0.10	42.94	0.875	---	---
B	0.21	28.72	0.85	−0.037	28.5
Fe:Mn (unitless)	0.54	25.15	50.87	6.33	0.57

NS Nonsignificant.

Fig. 2. Regression analysis of the SPAD chlorophyll index versus the total tissue-Fe (A and B), or Mn (C and D), and the Fe to Mn ratio (E and F) in leaf tissue for petunia and impatiens 28 d after transplant. The solid line was fit using a two-line model where below a critical nutrient level $Nutr_{crit}$, a positive linear relationship ($a + b_1 Nutr$) described the effect of the nutrient on SPAD chlorophyll index to a critical SPAD value (C_{crit}), and above $Nutr_{crit}$, the slope changed in a linear relationship with a different gradient ($C_{max} + b_2(Nutr - Nutr_{crit})$) with nutrient concentration, presumably because of saturation. Data were collected 28 d after transplanting, and the model was fit to combined data from all pH and C111 treatments for each species ($n = 60$).

with Fe-EDDHA or Fe-DTPA, suggesting an antagonism with Fe is occurring.

Nutrient level versus SPAD. In a companion article (Smith et al. [companion article a]), chlorosis (defined as a visually obvious decrease in green color of leaves, and measured by decline in SPAD chlorophyll index below 40 in petunia and 50 in impatiens) was reported in both species at pH 6.1 and 7.0. Chlorosis did not occur in the pH range 4.4 to 5.3 using a micronutrient concentration similar to that of the micronutrient concentration for the median fertilizer described above at 100 mg·L⁻¹

N. The chlorosis diminished at pH 6.1 or 7.0 when the applied micronutrient concentration was increased.

From regression analysis of the foliar concentration of each nutrient against SPAD chlorophyll index (Table 3), the highest r^2 for petunia occurred for the Fe:Mn ratio, Mn, and total Fe. Correlation coefficients were lower overall for impatiens compared with petunia. For impatiens, the highest r^2 occurred with Fe to Mn ratio, total Fe, and Fe²⁺ and there was no significant correlation with Mn. For both species, SPAD was positively correlated with

tissue total Fe, up to $Nutr_{crit}$ (112 µg·g⁻¹ for petunia and 106 µg·g⁻¹ for impatiens, Table 3, Fig. 2A and B) and above $Nutr_{crit}$ SPAD values changed only slightly as Fe level increased. The nonlinear regression curve therefore indicated that when total Fe level in tissue was below 112 µg·g⁻¹ for petunia, or 106 µg·g⁻¹ for impatiens, Fe was limiting and there was a decrease in SPAD with the corresponding increase in chlorosis. Petunia SPAD values were stable (i.e., the gradient parameter, b_1 had a low value at 0.02) as foliar Mn increased up to a $Nutr_{crit}$ of 170 µg·g⁻¹, and above $Nutr_{crit}$ foliar Mn was negatively correlated with SPAD (Fig. 2C). There was no significant relationship between SPAD and foliar Mn for impatiens (Fig. 2D). Tissue Fe:Mn ratio and SPAD were strongly correlated for both species (Figure 2E, F), with a positive trend up to $Nutr_{crit}$ and a leveling of as the Fe to Mn ratio increased above $Nutr_{crit}$. Nonlinear regression analysis estimated that there was a decrease in SPAD when the Fe to Mn ratio was below 0.71 for petunia and 0.57 for impatiens.

Because substrate pH affected all nutrients, and because C111 included all micronutrients, nutrient correlations should not be interpreted as directly causing chlorosis. In fact, it is likely that there were a combination of factors interacting to affect the observed chlorosis. Based on our analysis, however, Fe and Mn interactions appeared to have the greatest influence on chlorophyll content.

Conclusions

Research on nutrient solubility in a soil-less medium in an experiment without plants by Peterson (1981) showed a strong decline in P, Mn, Fe, B, and Zn as pH increased from 4.3 to 7.8, whereas extractable Ca and Mg increased with increasing pH. In contrast, patterns of tissue nutrient concentration of B in petunia, and P, Mn, and Zn in both species differed from those nutrient solubility patterns. This experiment therefore emphasized that the effect of substrate pH on tissue nutrient levels for plants grown in a soilless medium cannot be predicted based solely on nutrient solubility. Although Fe and Cu decreased in tissue with increasing pH, in line with nutrient solubility in the medium (Peterson, 1981), foliar Zn increased with increasing substrate pH. Mn levels also suggested a bimodal pattern with substrate pH, and Mn levels were highest at pH 7. Trends in foliar B and P content differed between species (Tables 1 and 2), further illustrating the complexity of plant–rhizosphere interactions.

As hypothesized, increasing micronutrient level in the applied fertilizer did affect tissue levels, but only by increasing Fe and Cu in petunia, and Fe, Cu, and B in impatiens. Mn level actually decreased in petunia with the increasing micronutrient concentration, and was unaffected in impatiens. An optimum range in substrate pH for plant growth is based on the goal to allow “micronutrient cations to be soluble enough to satisfy plant needs without becoming so soluble as to be toxic” (Brady and Weil, 2000). Given that micronutrient levels in

the media and tissue can increase as applied micronutrient fertilizer increases, the optimum range in substrate pH for any crop may vary depending on the fertilizer strategy.

Uptake of Fe and Mn has often been shown to be mutually antagonistic (Warden and Reisenauer, 1991), and excess soluble Fe or Mn can suppress uptake of the less soluble of the two nutrients (Knezek and Greinert, 1971). The Fe to Mn ratio in water-soluble fertilizers is important in determining uptake and foliar concentration of the two nutrients, and the Fe:Mn ratio in the root medium is recommended to be between 1:1 and 2:1 (Hulme and Ferry, 1999). However, Reisenauer (1994) cautions that, "although the interaction of Mn and Fe appear to be a mutual antagonism, quantitatively the relationship is much more complex and its many aspects not well understood", and factors such as chelation, levels of other nutrients, and rhizosphere pH influence this interaction. The importance of Fe, Mn, and their ratio on observed chlorosis in our experiment indicates the need to explore the potential to utilize highly soluble forms of Fe (e.g., Fe-EDDHA) in commercial greenhouse production, and varying the ratio between Fe and Mn in fertilizer, in order to reduce susceptibility to chlorosis at high substrate pH.

Literature Cited

- Adams, P., C.J. Graves, and G.W. Winsor. 1978. Some responses of lettuce, grown in beds of peat, to nitrogen, potassium, magnesium, and molybdenum. *J. Hort. Sci.* 53:275–281.
- Albano, J.P. and W.B. Miller. 1998. Marigold cultivars vary in susceptibility to iron toxicity. *HortScience* 33:1180–1182.
- Anderson, R.L. and L.A. Nelson. 1975. A family of models involving intersecting straight lines and concomitant experimental designs useful in evaluating response to fertilizer nutrients. *Biometrics* 31:303–318.
- Argo, W.R. and J.A. Biernbaum. 1996. The effect of lime, irrigation-water source, and water-soluble fertilizer on the pH and macronutrient management of container root-media with impatiens. *J. Amer. Soc. Hort. Sci.* 121(3):442–452.
- Argo, W.R. and P.R. Fisher. 2002. Understanding pH management for container-grown crops. Meister Publ., Willoughby, Ohio.
- Bailey, D.A. and P.V. Nelson. 1998. Managing micronutrients in the greenhouse. N.C. State Univ. Coop. Ext. Hort. Info. Lft. 553.
- Bennett, J.P. 1945. Iron in leaves. *Soil Sci.* 60:91–105.
- Biernbaum, J.A. and W.R. Argo. 1995. Effect of root-media pH on impatiens shoot micronutrient concentrations. *HortSci.* 30:858 (abstr.).
- Biernbaum, J.A., W.H. Carlson, C.A. Shoemaker, and R. Heins. 1988. Low pH causes iron and manganese toxicity. *Greenhouse Grower* 6(3):92–93, 96–97.
- Boxma, R. 1981. Effect of pH on the behavior of various iron chelates in sphagnum (moss) Peat. Comm. In *Soil Sci. and Plant Anal.*, 12:755–63.
- Brady, N.C. and R.R. Weil. 2000. Elements of the nature and properties of soils. Prentice Hall, Upper Saddle River, N.J.
- Bunt, A.C. 1988. Media and mixes for container-grown Plants. Unwin Hyman, London.
- Fisher, P.R., R. Wik, B.R. Smith, C.C. Pasian, M. Kmetz-González, and W.R. Argo. 2003. Correcting iron deficiency in *Calibrachoa xhybrida* Cerv. grown at high pH. *HortTechnology* 13(2):308–313.
- Hulme, F. and S. Ferry. 1999. How to avoid micronutrient toxicity syndrome. *GM Pro* 19(12):25–31.
- Knezek, B.D. and H. Greinert. Influence of soil Fe and Mn-EDTA interactions upon the Fe and Mn nutrition of bean plants. *Agron. J.* 63:617–619.
- Lindsay, W.L. 1979. Chemical equilibria in soils. 1st ed. Blackburn Press, Caldwell, N.J.
- Llorente, S., A. Leon, A. Torrecillas, and C. Alcaraz. 1976. Leaf iron fractions and their relation with iron chlorosis in citrus. *Agrochimica* 20:204–212.
- Lucas, R.E. and J.F. Davis. 1961. Relationships between pH values of organic soils and availabilities of 12 plant nutrients. *Soil Sci.* 92:177–182.
- Marschner, H. 1995. Mineral nutrition of higher plants. 2nd ed. Academic Press, San Diego, Calif.
- Miller, G.W., J.C. Pushnik, and G.W. Welkie. 1984. Iron chlorosis, a world wide problem, the relation of chlorophyll biosynthesis to iron. *J. Plant Nutr.* 7:1–22.
- Mills, H.A. and J.B. Jones. 1996. Plant analysis handbook II. MicroMacro Publ., Inc.
- Nelson, P.V. 1998. Greenhouse operations and management. 5th ed. Prentice Hall, Englewood Cliffs, N.J.
- Norvell, W. A. 1991. Reactions of metal chelates in soils and nutrient solutions, p. 187–223. In: J.J. Mortvedt, F.R. Cox, L.M. Shuman, and R.M. Welch (eds.). *Micronutrients in agriculture*. 2nd ed. Soil Sci. Soc. Amer., Madison, Wis.
- Oserkowsky, J. 1933. Quantitative relation between chlorophyll and iron in green and chlorotic leaves. *Plant Physiol.* 8:449–468.
- Peterson, J.C. 1981. Modify your pH perspective. *Florists Rev.* 169:34–36.
- Reed, D. M. 1996. Micronutrient nutrition. Ch. 9. p. 171–195. In: D.M. Reed (ed.). *Water, media, and nutrition for greenhouse crops*. Ball Publ., Batavia, Ill.
- Reisenauer, H.M. 1994. The interactions of manganese and iron, p.147–164. In: J.A. Manthey, D.E. Crowley, and D.G. Luster (eds.). *Biochemistry of metal micronutrients in the rhizosphere*. CRC Press, Boca Raton, Fla.
- Smith, B.R., P.R. Fisher, and W.R. Argo. 2004. Growth and pigment content of container-grown impatiens and petunia in relation to root substrate pH and applied micronutrient concentration. *HortScience* 39(6):1423–1427.
- Vetanovetz, R.P. 1996. Tissue analysis and interpretation, p. 197–220. In: D.W. Reed (ed.). *Water, media, and nutrition for greenhouse crops*. Ball Publ., Batavia, Ill.
- Warden, B.T. and H.M. Reisenauer. 1991. Manganese-iron interactions in the plant-soil system. *J. Plant Nutr.* 14:7–30.
- Warncke, D.D. 1995. Recommended test procedures for greenhouse growth media. Ch. 11. In: *Recommended soil testing procedures for the northeastern United States*. 2nd ed. Univ. Del. Agr. Expt. Sta. Bul 493, Dec. 1995.
- Wright, R.D. and A.X. Niemiera. 1987. Nutrition of container-grown woody nursery crops. *Hort. Rev.* 9:75–101.