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Micronutrient Toxicity in Seed Geranium (*Pelargonium × hortorum* Bailey)

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Abstract. **Seed geranium *(Pelargonium × hortorum)* micronutrient toxicity symptoms were induced by applying elevated levels of B, Cu, Fe, Mn, Mo, and Zn in fertilizer solution. Beginning at the 3-4 true leaf stage, seedling plants established in 11-cm (0.67-liter) pots containing peat-lite growing medium were fertilized at each irrigation for 5 weeks with solutions containing 0.25, 0.5, 1, 2, 3, 4, 5, and 6 mM plus the standard concentration of each micronutrient. The standard solution contained 20 μM B, 0.5 μM Cu, 10 μM Fe, 10 μM Mn, 0.5 μM Mo, and 4 μM Zn. All treatment solutions contained a fixed level of macronutrients. Visible foliar toxicity symptoms were produced when the nutrient solution contained 0.5 mM B, 0.5 mM Cu, 5 mM Fe, 1 mM Mn, 0.25 mM Mo, or 0.5 mM Zn. Reduction in dry matter yield was evident when 1 mM B, 2 mM Cu, 3 mM Fe, 2 mM Mn, 0.5 mM Mo, or 1 mM Zn was used in the fertilizer solution. Leaf chlorophyll contents decreased as Cu and Mn levels in the concentration range tested increased. Elevated levels of Fe increased tissue chlorophyll contents. The relationship between the nutrient solution and tissue concentrations of each of the six micronutrients was determined.**

A soil medium high in nutrient holding capacity does not usually require additional supply of micronutrients when used for short-term crops such as bedding plants. Micronutrient fertilization, however, is essential for bedding plants grown in the soil-less root media , which are now extensively used in greenhouse crop production. Growers formulate micronutrients based on the Hoagland solution (Hoagland and Arnon, 1950), or use commercial fertilizers containing both macro- and micronutrients. Since plants require only minute quantities of these elements, a nutrient imbalance may occur when commercial fertilizers are used with water containing high levels of some micronutrients. Micronutrient deficiencies and toxicities have been reported for boron in chrysanthemum (Gogue and Sanderson, 1973), Easter lily (Marousky, 1981) and begonia (Elliot and Nelson, 1981; Nelson et al., 1979), for molybdenum in poinsettia (Hammer and Bailey, 1987), for manganese in *Spathiphyllum* (Broschat and Donselman, 1986), and for iron in *Tolmiea* (Smith, 1985). Such investigations are largely lacking in bedding plants. The objective of this study was to induce and characterize micronutrient toxicity symptoms of seed geraniums grown in a peat-lite mix.

Materials and Methods

Seedlings of plug-grown 'Ringo Scarlet' seed geranium *(Pelargonium ×hortrum)* were transplanted at the 2 true leaf stage into 11-cm (0.67-liter) plastic pots containing a sphagnum peat-moss-perlite mix devoid of nutrient additions other than dolomitic limestone (Sunshine mix #2; Fisons Horticulture, Vancouver, B. C., Canada). Plants were watered for 2 weeks with a standard fertilizer solution containing macronutrients (in mM): 2.5 Ca^{+2}, 1 Mg^{+2}, 5 K^{+}, 2 NH_4^{+}, 10 NO_3^{-}, 2 H_2PO_4, 1 SO_4^{-2}. The micronutrient concentrations of the standard solution (a modification from Hoagland and Arnon, 1950) were (in μM): 20 B, 0.5 Cu, 10 Fe, 10 Mn, 0.5 Mo, and 4 Zn. Plants then were watered, as needed, for 5 weeks with the standard solution or with treatment solutions containing 0.25, 0.5, 1, 2, 3, 4, 5, and 6 mM of B, Cu, Fe, Mn, Mo, or Zn. The chemical sources of the minor elements used were: boric acid (H_3BO_3), copper sulfate ($CuSO_4 \cdot H_2O$), iron sulfate ($FeSO_4 \cdot 7H_2O$), manganese sulfate ($MnSO_4 \cdot 3H_2O$), sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$), and zinc sulfate ($ZnSO_4 \cdot 7H_2O$). All fertilizer treatment solutions contained the standard macro- and micronutrient levels except for the specific micronutrient being tested. All solutions were prepared using glass-distilled water. The pH of the solution was adjusted to 6.5 with 1 N HCl or KOH. For each micronutrient, five replications of nine treatments (concentrations) were completely randomized on a greenhouse bench. Plants were spaced 25 cm apart. A total of 270 plants (9 concentrations/micronutrient, five replications/concentration, six micronutrients) were used.

Plants were grown in a fiberglass greenhouse with temperature set points of 17– 18C night and 21–24C day during April through May. The relative humidity ranged from 40% to 80% and day irradiance levels from 550 to 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, depending on the weather conditions. Although affected plants began to show foliar toxicity earlier, symptom descriptions and photographs were taken 5 weeks after the treatments began. Plants were harvested for biomass determinations after 5 weeks of nutrient treatments. For dry matter yield, plants were oven-dried at 75C for 48 h. For chlorophyll analysis, three replicate samples of five leaf discs (8-mn-diameter) were randomly taken from newly matured leaves of the plants treated with Cu, Fe, or Mn. The five leaf disc samples were placed in 5 ml of 80% N,N-dimethylformamide in 10-ml glass test tubes and stored in the dark at 4C for 7 days. Absorbance of the sample preparations were determined at 664.5 nm and 647 nm, using 1-cm quartz cuvettes and a double beam spectrophotometer (Series No. 634; Varian, Sunnyvale, Calif.). The estimations

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Table 1. Changes in plant height, biomass yield, tissue micronutrient contents of 'Ringo Scarlet' geranium as affected by elevated levels of micronutrients in the fertilizer solution.

Element	Solution concn (mM)	Solution concn ppm	Plant ht (cm)	Fresh wt (g)	Dry wt (g)	Final medium pH[z]	Tissue content (mg·kg^{-1})
B	0.02	0.22	16.3	71.1	8.81	6.7	34
	0.25	2.7	19.2	72.9	9.86	6.3	118
	0.5	5.4	19.3	62.2	8.52	6.4	337
	1	10.8	14.7	42.0	6.65	6.3	623
	2	21.6	11.7	19.4	4.00	6.5	1110
	3	32.4	11.8	10.6	2.60	6.6	1510
	4	43.2	8.2	5.3	2.03	6.3	1860
	5	54.0	7.6	4.5	1.90	6.5	2280
	6	64.8	7.7	3.4	1.73	6.3	2300
Linear			***	***	***	---[y]	***
Quadratic			***	***	***	---	**
LSD (0.05)			1.2	4.4	0.80	---	
Cu	0.0005	0.03	16.1	72.8	8.57	6.4	0.3
	0.25	15.9	17.1	81.1	10.60	6.1	43
	0.5	31.8	18.1	80.2	10.24	6.1	78
	1	63.5	16.8	65.6	8.41	5.4	227
	2	127.0	15.8	55.7	7.69	5.6	409
	3	190.5	15.6	51.3	7.75	5.4	478
	4	254.0	14.6	47.8	7.65	5.0	711
	5	317.5	13.9	32.0	4.80	4.6	1,110
	6	381.0	13.1	26.0	4.20	4.6	918
Linear			***	***	***	---[y]	***
Quadratic			NS	NS	NS	---	NS
LSD (0.05)			2.0	9.2	1.50	---	
Fe	0.01	0.56	15.8	73.7	8.74	6.6	82
	0.25	14.0	17.3	87.2	9.66	6.3	129
	0.5	27.9	18.0	85.8	10.81	6.3	129
	1	55.8	17.6	73.9	8.13	5.8	186
	2	111.6	15.7	59.7	7.71	5.6	350
	3	167.4	16.5	51.8	7.07	5.3	569
	4	223.2	16.0	37.4	5.58	5.2	618
	5	278.0	13.8	34.0	5.30	5.2	951
	6	334.8	12.5	21.4	4.00	4.8	889
Linear			***	***	***	---[y]	***
Quadratic			**	NS	NS	---	NS
LSD (0.05)			1.7	7.9	1.20	---	
Mn	0.01	0.55	19.2	70.7	8.52	6.8	118
	0.25	13.7	19.6	73.5	9.92	6.5	696
	0.5	27.5	19.2	68.0	8.93	6.2	1170
	1	54.9	17.9	62.5	8.01	6.0	2250
	2	109.8	15.6	41.3	5.97	5.7	4040
	3	164.7	14.2	30.5	4.62	5.5	5250
	4	219.6	12.3	23.1	3.39	5.7	7760
	5	274.5	12.7	20.8	3.26	5.5	9260
	6	329.4	10.4	10.7	1.98	5.7	10800
Linear			***	***	***	---[y]	***
Quadratic			NS	***	*	---	*
LSD (0.05)			1.8	7.9	1.36	---	
Mo	0.0005	0.05	15.8	69.8	8.58	6.8	34
	0.25	24.0	16.4	70.5	8.96	6.4	219
	0.5	48.0	14.3	59.5	7.31	6.2	485
	1	95.9	11.6	26.3	3.63	6.5	1140
	2	191.8	8.6	14.3	2.21	6.6	3050
	3	287.7	7.6	8.2	1.52	6.3	2940
	4	383.6	7.1	7.7	1.44	6.6	3630
	5	479.5	5.7	4.0	0.90	6.4	3800
	6	575.4	6.1	4.4	1.11	6.6	1810
Linear			***	***	***	---[y]	***
Quadratic			***	***	***	---	**
LSD (0.05)			1.4	7.1	1.20	---	

Table 1. continued.

Element	Solution concn (mM)	Solution concn ppm	Plant ht (cm)	Fresh wt (g)	Dry wt (g)	Final medium pH[z]	Tissue content (mg·kg^{-1})
Zn	0.005	0.26	15.7	67.5	8.46	6.5	56
	0.25	16.4	13.7	63.8	7.75	6.2	323
	0.5	32.7	16.0	64.5	8.20	6.4	492
	1	65.4	15.3	50.0	6.81	6.0	660
	2	130.8	13.9	35.3	4.99	5.5	958
	3	196.2	12.6	28.8	3.92	5.2	1340
	4	261.6	11.6	21.3	3.50	5.8	1750
	5	327.0	10.5	15.7	2.56	5.7	2220
	6	392.4	7.6	10.0	1.65	5.7	3210
Linear			***	***	***	---[y]	***
Quadratic			NS	***	**	---	**
LSD (0.05)			1.5	7.7	1.06	---	

[z]Growing medium pH was determined after plants were harvested.
[y]Not determined.
[NS, *, **, ***]Nonsignificant or significant at P = 0.05, 0.01, and 0.001, respectively.

of chlorophyll-a, chlorophyll-b, and total chlorophyll were made by the equations described by Inskeep and Bloom (1985): chlorophyll- $a = 12.70_{A664.5}$-2.79_{A647};chlorophyll- $b = 20.70_{A647}$–$4.62_{A664.5}$; total chlorophyll = 19.90_{A647}+$8.08_{A664.5}$.

For the standard solution plants of the Cu, Fe, and Mn treatments, three replicate samples of five leaf discs were randomly taken from newly matured leaves of the plants grown with each standard solution and used for chlorophyll analysis.

For the determination of tissue micronutrient contents, the dried shoots (stems and leaves) were ground through a 20-mesh (0.84 mm) screen. One-gram tissue samples were digested in 10 ml concentrated HNO_3 overnight in a Taylor digestion tube (Baxter Scientific Product; North Kansas City, Me.). The volume of the solution was reduced to 5 ml by heating at 125C. The solution was then removed from the digestion tube and diluted to a 50-ml volume with deionized water by agitation. After the residue settled, mineral compositions of the solution were analyzed by ICP (inductively coupled plasma optical emission spectrometry, Model 975; Plasma Atomcomp by Thermo Jarrell Ash, Franklin, Mass.) following the procedures of Halvin and Soltanpour (1980).

Growing medium was carefully separated from the root system after plants were harvested. Media from all 5 replicate pots of each treatment were combined and air-dried. The pH was determined by saturated pastes prepared with deionized water as described by Workman et al. (1988).

Plant height, biomass yield, and tissue mineral and chlorophyll content data were analyzed using the step-wise regression model of the CoStat program (CoHort Software, Minneapolis).

Results

Boron. Two out of the five plants grown with 0.5 mM (5.4 mg·liter^{-1}) B developed a slight leaf chlorosis symptom 2 weeks after the treatment began. The leaf tissue B content at this treatment was 337 µg·g^{-1} dry weight (Table 1). The chlorosis symptoms developed when plants were grown with 1 mM or higher solution B concentrations and corresponding tissue B levels were 623 mg·kg^{-1} or higher. As B concentrations in the nutrient solution increased beyond 2 mM (22 mg·liter^{-1}), plants became stunted with reduced leaf sizes and marginal necrosis (Fig. 1). The severity of leaf chlorosis and marginal necrosis became more pronounced as the nutrient B concentration and treatment duration increased. Plant heights, as well as fresh and dry weights, decreased when the fertilizer B concentration was 1 mM or higher (Table 1). Variation in boron concentration in the fertilizer solution had little effect on the pH of the growing medium (Table 1).

Copper. Some of the plants grown with Cu concentrations as low as 0.25 mM (16 mg·liter^{-1}) developed a slight chlorosis. Interveinal chlorosis was common when plants were grown with 0.5 mM (28 mg·liter^{-1}) or higher Cu concentrations (Fig. 1). The leaf Cu contents in the plants grown with 0.25 mM and 0.5 mM copper in the fertilizer solution were 43 µg·g^{-1} and 78 µg·g^{-1}, respectively (Table 1). Increasing Cu levels in the nutrient solution did not affect leaf sizes: leaves of plants grown with 6 mM (381 mg·liter^{-1}) Cu were as large as those of the control plants (Fig. 1). Plants treated with 5 mM(318 mg·liter^{-1})and 6 mM(381 mg·liter^{-1}) Cu showed some necrotic margins on old leaves. Plant heights were not reduced with nutrient Cu concentrations below 4 mM (254 mg·liter^{-1}). However, fresh and dry weights were reduced when Cu in the fertilizer exceeded 2 mM (127 mg·liter^{-1}). As Cu concentrations in the fertilizer solution were increased to 6 mM, the pH of the growing medium dropped from 6.4 (control) to 4.6 (Table 1). Leaf chlorophyll contents decreased as Cu levels in the fertilizer solution increased (Fig. 2).

Iron. As Fe concentration in the fertilizer solution increased, green leaf pigmentation progressively intensified (Fig. 1). Plant growth was not adversely affected until Fe levels in the solution exceeded 4 mM (223 mg·liter^{-1}). Solution Fe concentrations higher than 5 mM (278 mg·liter^{-1}) reduced leaf sizes and produced large purplish-black spots in some leaves. These leaf spots may have been caused by foliage contacts with the fertilizer solutions high in iron sulfate during irrigation. Plant heights were not affected by Fe until the concentration exceeded 5 mM (278 mg·liter^{-1}). Fresh and dry weights of plants remained unaffected until solution Fe concentrations exceeded 3 mM (167 mg·liter^{-1}). The pH of the growing medium became lower as Fe concentration in the fertilizer solution increased (Table 1). Leaf chlorophyll-a and chlorophyll-b as well as total chlorophyll contents increased as Fe levels in the nutrient solution increased (Fig. 2). chlorophyll-b levels increased at a greater ratio (85%) than chlorophyll-a (24%) up to 4 mM Fe.

Manganese. Manganese toxicity was characterized by reduced plant and leaf sizes, foliar chlorosis, and leaf spots (Fig. 1). Affected plants produced numerous black spots less than 0.5 mm in diameter on newly formed leaves. These spots became larger and often coalesced when plants were fertilized with 4 mM (220 mg·liter^{-1}) or higher levels of Mn. Foliar toxicity symptoms developed when plants were exposed to 1 mM (56 mg·liter^{-1}) or higher Mn concentrations and when tissue Mn levels were 2.25

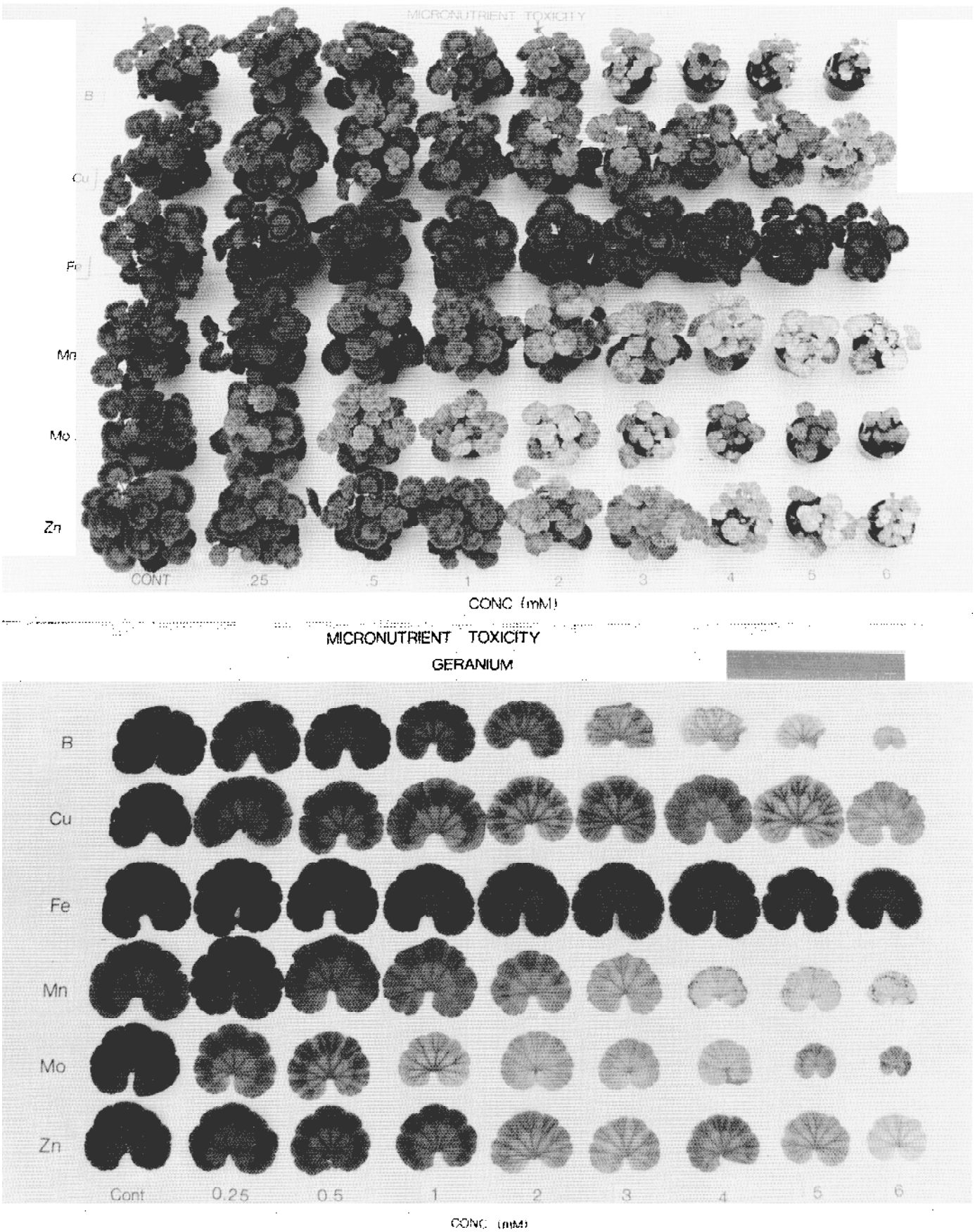

Fig. 1. Changes in plant growth **(A)** and leaf pigmentation **(B)** in 'Ringo Scarlet' seed geranium as influenced by increasing levels (0.25, 0.5,1,2,3,4,5, and 6 mM) of B, Cu, Fe, Mn, Mo, and Zn in the fertilizer solution.

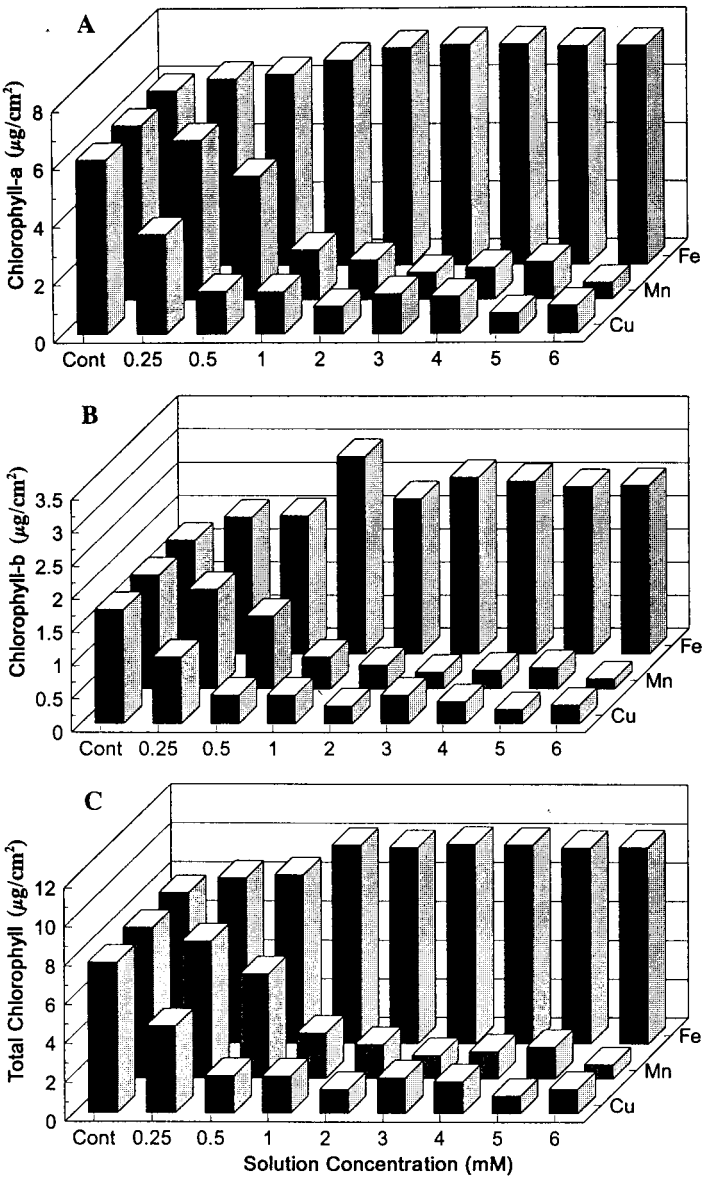

Fig. 2. Changes in leaf chlorophyll-a (A), chlorophyll-b (B) and total chlorophyll content (C) of 'Ringo Scarlet' seed geranium plants grown with various concentrations of Cu, Fe and Mn.

g·kg^{-1}or higher (Table 1). Plant heights and biomass production were reduced when the fertilizer solution contained 2 mM (110 mg·liter^{-1}) or higher Mn and when the tissue Mn contents were 4.04 g·kg^{-1} or greater (Table 1). Leaf chlorophyll-a, chlorophyll-b, and total chlorophyll contents were progressively reduced as Mn concentration in fertilizer solution increased (Fig. 2).

Molybdenum. Seed geranium plants were highly vulnerable to elevated levels of Mo. Fertilizer Mo concentrations as low as 0.25 mM (24 mg·liter^{-1}) caused leaf yellowing after 2 weeks in treatment. Leaves of affected plants developed severe chlorosis. Leaves of plants exposed to 2 mM (192 mg·liter^{-1}) or higher Mo concentrations developed edge burns and light-brown or orange color pigmentation (Fig. 1). Plants exposed to elevated Mo levels were stunted. Fresh and dry matter yields were significantly reduced when plants were fertilized with 0.5 mM (48 mg·liter^{-1}) or higher Mo concentrations or when the tissue Mo contents were 485 mg·kg^{-1} or greater (Table 1).

Zinc. Zinc toxicity symptoms were characterized by yellowing of leaves with interveinal chlorosis. These chlorotic symptoms were similar to toxicity symptoms caused by Mn. However, the dark leaf speckles characteristic of Mn toxicity were not observed on the Zn-affected leaves (Fig. 1). Plants grown with 1 mM (65 mg·liter^{-1}) or higher concentrations of Zn showed a reduction in biomass yield (Table 1). Plants that showed biomass yield reduction contained 660 mg·kg^{-1} or higher Zn concentrations in the tissue (Table 1). 'Ringo Scarlet' seed geraniums should not be grown with a fertilizer solution containing 0.5 mM (33 mg·liter^{-1}) or higher concentrations of Zn.

Discussion

Among the six micronutrients tested, B and Mo most effectively induced foliar toxicity symptoms in 'Ringo Scarlet' seed geranium. Nutrient solution contents as low as 0.5 mM B and Mo damaged plants. The actual threshold concentrations that cause toxicity could be lower than 337 mg·liter^{-1} B and 485 mg·liter^{-1} Mo. Boron influence on seed geranium appeared to be similar to that on *Begonia* × *hiemalis* , which developed acute toxicity at tissue concentrations of 125 to 258 mg·kg^{-1} B (Elliot and Nelson, 1981).

Copper and Zn also caused damage to plants at 0.5 mM in the nutrient solution. Iron was found to be the most forgiving as far as toxicity is concerned; only the plants treated with 5 mM (278 mg·liter^{-1}) or higher concentrations of Fe showed a reduction in dry matter yield. It is uncertain if Fe was present as an available form at these high concentrations, since some iron oxides may have formed in the root zone area.

Plants treated with elevated levels of Cu and Mn developed a severe leaf yellowing symptom with reduced chlorophyll contents. The reduction in *chl* contents in plants treated with Cu or Mn may have been due to the competition between these micronutrients and magnesium for the same absorption site. Plants treated with elevated levels of Fe up to 6 mM increased in both chlorophyll-a and chlorophyll-b contents (Fig. 2). chlorophyll-b levels increased at a greater rate than chlorophyll-a suggesting that the light harvesting chlorophyll protein (LHCP) complex accumulates in chloroplasts under this treatment. The greater pigment levels enhanced by extremely high Fe concentrations did not improve dry matter production. However, supplementation of Fe at lower levels (0.0 1–0.5 mM) increased both pigment and dry matter production.

In all treatments, tissue concentrations of macronutrients were unaffected by increasing levels of micronutrients except when certain compound fertilizers containing both micro- and macronutrients were used (i.e., increased S when iron sulfate was used as a source of Fe).

The relationships between the fertilizer solution concentrations and tissue levels of the six micronutrients tested were linear, with variation in slopes. The tissue microelement contents of the plants may be predicted by the following equations on a dry weight basis:

Boron:	$y = 0.17 + 0.40x$	(r^2= 0.97)
Copper:	$y = 0.17 + 0.18x$	(r^2= 0.94)
Iron:	$y = 0.70 + 0.10x$	(r^2= 0.97)
Manganese:	$y = 0.28 + 1.78x$	(r^2= 0.99)
Molybdenum:	$y = 0.47 + 0.69x$	(r^2= 0.86)
Zinc:	$y = 0.12 + 0.46x$	(r^2= 0.97)

where, y = tissue micronutrient content in g·kg^{-1} dry weight, and x = mM concentration of microelement in the nutrient solution.

The slopes of these equations would have been different if tissue microelement levels were expressed as a function of the mg·liter^{-1} (ppm) solution concentrations of micronutrients, which differ from each other in atomic weights.

It is possible that lowered medium pH (<5.5) might have

influenced the development of micronutrient toxicity in plants treated with elevated levels (>3 mM) of Cu and Fe (Table 1). Elevated levels of B and Mo did not alter the final medium pH. However, the pH of the 6 mM Mn and Zn treatments slightly decreased from 6.5 to 5.7 at the time of plant harvest.

It is worth noting that biomass yield was greater when micronutrient concentration increased from the standard level to 0.25 mM or 0.5 mM for B, Cu, Fe, and Mn (Table 1). Elevated levels of Fe, for example, positively influenced green pigmentation in the foliage for the concentration range tested (Fig. 1). Further studies may be needed to fine tune the optimum concentrations of these micronutrients for growing seed geranium.

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