Response to Copper Toxicity for Three Ornamental Crops in Solution Culture

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Abstract. Electrolytically generated copper is increasingly used to control diseases and algae in the greenhouse industry. However, there is a shortage of information regarding appropriate management strategies for copper in ornamental crop production. The objectives of this study were to characterize the response of three ornamental crops (*Dendranthema × grandiflorum* L. ‘Fina’, *Rosa × hybrida* L. ‘Lavlinger’, *Pelargonium × hortorum* L. ‘Evening Glow’) to different solution levels of Cu²⁺ (ranging from 0.4 to 40 µM) and to determine the critical levels above which toxic responses became apparent. The following measurements were used to assess the treatments: leaf chlorophyll fluorescence (F_v/F_m), leaf chlorophyll content, and visible injury of leaf and root. Excessive copper reduced plant root length, root dry weight, total dry weight, root to shoot ratio, leaf area, and specific leaf area in all three species. The critical solution level of Cu²⁺ that resulted in significantly reduced plant dry weight for chrysanthemum was 5 µM; for miniature rose, 2.4; and for geranium, 8 µM. Plant visible root injury was a more sensitive and reliable copper toxicity indicator than visible leaf injury, leaf chlorophyll content, F_v/F_m, or leaf and stem copper content. Generally, all the species exhibited some sensitivity to Cu²⁺ in solution culture, with chrysanthemum and miniature rose being most sensitive and geranium being least sensitive. Caution should be taken when applying copper in solution culture production systems.

Copper is one of the essential micronutrient elements for plants. However, excessive copper is toxic to living organisms, including plants. Because copper is a toxic heavy metal, it has been used in pesticides, bactericides, and fungicides in agriculture (Kaplan, 1999; Scheck and Pscheidt, 1998). Electrolytically generated copper and cupric sulphate have been used in greenhouses to control disease and algae. Recent survey data indicate that the usage of electrolytically generated copper to control disease and algae is increasing in the greenhouse industry in Ontario, Canada, and elsewhere, such as Greece. However, a question that urgently needs to be answered is the Cu²⁺ concentration that can be used in order to control greenhouse diseases and algae without negative effects on crops. Most of the research on Cu²⁺ toxicity was on the responses of plants to Cu²⁺-polluted soil (Borkert et al., 1998; Fernandes and Henriques, 1991; Panou-Filothou et al., 2001). However, very few studies have been carried out concerning the response of ornamental crops to Cu²⁺ application in hydroponic production systems.

Chrysanthemum, miniature rose, and geranium are three important ornamental crops in the North American greenhouse industry. Miniature rose was chosen to represent woody ornamental crops, and the other two species to represent herbaceous crops.

Maximum quantum efficiency of PSII (F_v/F_m) is used as a sensitive indicator of plant photosynthetic performance, with optimal values of around 0.83 measured for most plant species (Johnson et al., 1993). F_v/F_m is widely used as a reliable diagnostic indicator of plant environmental stresses (Maxwell and Johnson, 2000) and it is reported that excessive Cu²⁺ can affect PSII (Mohanty et al., 1989; Pätsikkä et al., 1998; Vavilin et al., 1995).

A SPAD meter is commonly used for measuring plant leaf chlorophyll content and thus is used as a diagnosis tool for some environmental stress or nutrient deficiency (Blackmer and Schepers, 1995).

The objectives of these experiments were to: characterize the response of three ornamental

crops (chrysanthemum, miniature rose, and geranium) to different Cu²⁺ levels in solution culture; and determine the critical level above which Cu²⁺ toxic effects were apparent. In addition, we were interested in determining the relative values of the following measurements: leaf chlorophyll fluorescence (F_v/F_m), leaf chlorophyll content (SPAD meter reading), and visible injury symptoms on leaf and root in the diagnosis of copper toxicity.

Materials and Methods

Three common greenhouse ornamental crop species, chrysanthemum, miniature rose, and zonal geranium, were grown in solution culture with different Cu²⁺ levels in the greenhouse of Univ. of Guelph, Ont., Canada (lat. 43°34'N) from 2000 to 2002. Rockwool rooted cuttings were first transplanted in 2-L containers containing aerated one-quarter strength modified Hoagland solution (EC 0.58 dS·m⁻¹, pH 5.5), and the solution changed to full strength (EC 2.3 dS·m⁻¹, pH 5.5) after ≈3–5 d. The full strength nutrient solution contained macronutrients (in mM): 11.8 NO₃⁻-N, 4.0 NH₄⁺-N, 1.5 P, 3.6 Ca, 5.0 K, 2.0 S, and 1.0 Mg; micronutrients (in µM): 5.0 Mn, 3.5 Zn, 20 B, 0.5 Mo, 0.4–0.8 Cu²⁺, and 25 Fe as FeCl₃. Copper was supplied with reagent-grade CuSO₄·5H₂O. The copper treatments (Table 1) were initialized 7–14 d after the transplanting, when roots were 5–10 cm long. The nutrient solution was changed twice a week to ensure sufficient nutrient for plant growth and to keep solution Cu²⁺ levels close to the targeted levels.

Visible root injury was checked and recorded several times within the first 24 h after the start of the Cu²⁺ treatment. Root and leaf visible Cu²⁺ toxicity symptoms were then checked and recorded once a day thereafter. Plants were harvested 4 weeks after the start of the treatments. At harvest, plants were washed with distilled water and separated into leaf, stem, and root, and dried in a forced air oven at 65 °C to a constant weight. The longest root length was measured and leaf area was measured by a LI-3100 area meter (LI-COR, Lincoln, Neb.). Total dry weight was calculated as the sum of leaf, stem, and roots. Root to shoot ratio was calculated by dividing root dry weight by the sum of leaf and stem dry weights. Specific leaf area (SLA) was calculated by dividing leaf area by leaf dry weight of each plant (cm²·g⁻¹).

Leaf chlorophyll content was measured

Table 1. Solution Cu²⁺ concentration levels, replicate number per treatment, experimental period, greenhouse temperature, and relative humidity (RH) during the experiments.

Experiment	Solution Cu ²⁺ levels (µM)	Replication no./treatment	Time	Temp (°C) day/night	RH (%) day/night
Chrysanthemum Expt. I	0.4, 5.0, 10.0, 16.0, 20.0, 30.0, 40.0	6	Nov. 2001–Jan. 2002	21/18	50/60
Chrysanthemum Expt. II	0.5, 1.5, 3.0, 6.0, 8.0	6	April–June 2002	25/19	70/80
Miniature rose	0.8, 2.4, 4.7, 9.4, 15.7	6	Dec. 2001–Mar. 2002	21/18	50/60
Geranium	0.5, 1.0, 2.0, 4.0, 8.0, 12.0, 15.0, 20.0	5	Oct. 2001–Jan. 2002	21/18	50/60

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with a chlorophyll meter (Minolta SPAD-501, Osaka, Japan). Leaf chlorophyll fluorescence measurements were made on the last fully expanded leaf with a Fluorescence Monitoring System FMS 2 (Hansatech Instruments, Norfolk, England) between 14:00 and 16:00 HR. The minimal level of fluorescence (F_0) was obtained under modulated red light ($2 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, frequency 20 kHz) and maximal fluorescence yield (F_m) was recorded following exposure to a saturating light pulse (0.8 s) of $8000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, provided by an 8-V/25-W halogen lamp (Bellaphot, Osram). Fluorescence signals were analyzed according to Genty et al. (1989). The maximum (or potential) quantum efficiency of PSII photochemistry:

$$F_v/F_m = (F_m - F_0)/F_m$$

was measured after a 40-min period of dark-adaptation.

Dried plant tissues (leaf, stem, and root) were ground separately into 1-mm size for macro- and micronutrient analysis. Sub-samples were ashed in a muffle furnace at a temperature of 500°C for 4 h, then dissolved in 1.0 M HCl solution and analyzed for P, K, Ca, Mg, Cu, Zn, Mn, B, and Fe using an atomic absorption spectrometer (Varian, SpectraAA-300, Victoria, Australia) at the Laboratory Service Division, Univ. of Guelph. There were very obvious negative plant growth effects at higher Cu^{2+} concentrations (Table 2); therefore, we only analyzed plant tissue chemical compositions of the three lowest copper treatments for chrysanthemum (Expt. I) and miniature rose. Due to the limited scope of this study and economic considerations, we did not analyze tissue

chemical compositions of the chrysanthemum (Expt. II) and geranium plants.

All the experiments had a completely random design. Treatments (solution Cu^{2+} levels), replicate numbers of each treatment, experiment time, and the greenhouse environment conditions of these experiments are listed in Table 1. Solution Cu^{2+} levels in chrysanthemum Expt. II were based on the results of chrysanthemum Expt. I. Analysis of variance (ANOVA) was conducted using SAS (version 8; SAS Institute, Cary, N.C., 1999). Multiple comparisons among the treatments of each experiment were conducted using Tukey's Studentized range (HSD) test.

Results and Discussion

Visible injury. For both chrysanthemum (Expts. I and II) and geranium, roots started to show brown tips within 24 h from the start of the treatment in the higher Cu^{2+} solutions; however, it took 1 week for roots of miniature rose roots to show Cu^{2+} toxicity symptoms (e.g., brown root tips and enhanced lateral root formation). Copper-enhanced lateral root formation in lettuce seedlings was reported by Savage et al. (1981) as well. In contrast, Taylor and Foy (1985) found that copper inhibited lateral root formation in *Triticum aestivum*. For chrysanthemum, the lowest solution Cu^{2+} concentration at which roots showed visible Cu^{2+} injury within 24 h was $5 \mu\text{M}$ in Expt. I and $6 \mu\text{M}$ in Expt. II. Roots did not show any visible injury below these levels, even at the end of these experiments. Geranium roots started to show visible injury within 24 h when Cu^{2+} level

was at $8 \mu\text{M}$ or higher. However, roots in solution with Cu^{2+} concentration of $4 \mu\text{M}$ started to show Cu^{2+} toxicity symptoms after 3 d from the start of treatment. Miniature rose showed root Cu^{2+} toxicity symptoms $\geq 4.7 \mu\text{M}$. Visible root tip injury under excessive Cu^{2+} solution was also found on birches (Utriainen et al., 1997). For all species, the extent of Cu^{2+} visible root injury increased as solution Cu^{2+} concentration increased. Extent of root injury and number of injured roots increased gradually, until at latter stages of the experiments, a full dark brown root system and obvious root growth depression was observed in the higher Cu^{2+} concentrations. Inhibited root elongation has also been reported in taro (Hill et al., 2000), rice (Lidon and Henriques, 1992), corn (Ouzounidou et al., 1995), and other plant species (Ouzounidou, 1994).

Different from roots, visible leaf injury was only manifested in some experiments (geranium and chrysanthemum Expt. II) and not in the others (miniature rose and chrysanthemum Expt. I). Even when there was visible leaf injury, the injury appeared much later than root injury. For geranium, some leaves of plants grown in Cu^{2+} at or above $8 \mu\text{M}$ solution started to show chlorosis 1 week after the start of Cu^{2+} treatment. However, plants grown in solution with Cu^{2+} concentration lower than $8 \mu\text{M}$ did not show any visible symptom, even at the end of the experiment. In chrysanthemum Expt. II, some plants grown in solution with $\text{Cu}^{2+} \geq 6 \mu\text{M}$ started to show leaf chlorosis several days after the initiation of the copper treatments, and chlorotic leaf number increased gradually. Leaf chlorosis was seen

Table 2. Effects of increasing Cu^{2+} concentrations in the nutrient solution on the length of the longest root, root dry weight, total dry weight, root : shoot dry weight ratio, leaf area, and specific leaf area (SLA) of chrysanthemum, geranium, and miniature rose in solution culture. Within species (experiment) and tissue, data bearing the letter are not significantly different at $P \leq 0.05$.

Solution Cu^{2+} (μM)	Longest root length (cm)	Root dry wt (g/plant)	Total dry wt (g/plant)	Root to shoot ratio	Leaf area (cm^2/plant)	SLA ($\text{cm}^2\cdot\text{g}^{-1}$)
Chrysanthemum Expt. I						
0.4	18.5 ± 0.59 a	0.35 ± 0.026 a	2.11 ± 0.191 a	0.20 ± 0.007 a	242 ± 24 a	177 ± 5.6 a
5	4.2 ± 0.33 b	0.05 ± 0.005 b	1.05 ± 0.153 b	0.06 ± 0.009 b	63 ± 9 b	97 ± 6.1 b
10	3.8 ± 0.56 b	0.03 ± 0.002 bc	1.00 ± 0.071 b	0.03 ± 0.003 c	59 ± 4 b	96 ± 4.9 b
16	3.3 ± 0.72 b	0.02 ± 0.003 bc	0.96 ± 0.051 b	0.02 ± 0.003 c	55 ± 3 b	97 ± 4.2 b
20	3.1 ± 0.42 b	0.03 ± 0.004 bc	1.11 ± 0.128 b	0.02 ± 0.002 c	65 ± 1 b	97 ± 5.3 b
30	3.7 ± 0.70 b	0.02 ± 0.003 c	0.70 ± 0.088 b	0.02 ± 0.005 c	40 ± 6 b	92 ± 5.7 b
40	4.4 ± 0.24 b	0.02 ± 0.002 bc	0.73 ± 0.073 b	0.03 ± 0.004 c	44 ± 5 b	99 ± 3.9 b
Chrysanthemum Expt. II						
0.5	43.5 ± 4.79 a	1.18 ± 0.073 a	8.75 ± 0.414 a	0.16 ± 0.009 a	964 ± 44.7 ab	206 ± 4.5 a
1.5	38.5 ± 3.57 a	1.33 ± 0.128 a	10.28 ± 1.333 a	0.15 ± 0.014 a	1124 ± 159.5 a	201 ± 6.3 a
3	36.3 ± 3.40 ab	1.32 ± 0.031 a	10.49 ± 0.378 a	0.14 ± 0.005 a	1158 ± 73.5 a	206 ± 7.3 a
6	22.1 ± 0.43 bc	0.42 ± 0.050 b	4.13 ± 0.845 b	0.12 ± 0.011 ab	414 ± 115.3 c	174 ± 15.4 a
8	20.7 ± 0.51 c	0.52 ± 0.065 b	5.41 ± 0.449 b	0.10 ± 0.007 b	622 ± 32.1 bc	198 ± 6.2 a
Miniature Rose						
0.8	21.3 ± 0.84 a	0.44 ± 0.022 a	2.97 ± 0.200 a	0.18 ± 0.012 a	290 ± 30 a	192 ± 12.2 ab
2.4	19.2 ± 0.95 a	0.33 ± 0.055 b	1.99 ± 0.250 bc	0.19 ± 0.011 a	217 ± 36 ab	205 ± 11.9 a
4.7	14.8 ± 1.02 b	0.30 ± 0.042 b	1.96 ± 0.220 b	0.21 ± 0.062 a	162 ± 28 bc	162 ± 15.2 b
9.4	13.1 ± 1.55 b	0.22 ± 0.048 bc	1.54 ± 0.282 c	0.17 ± 0.013 ab	141 ± 35 bc	184 ± 20.9 ab
15.7	9.3 ± 0.51 c	0.26 ± 0.107 c	1.54 ± 0.100 c	0.24 ± 0.120 b	120 ± 14 c	197 ± 17.8 ab
Geranium						
0.5	30.2 ± 1.96 a	1.75 ± 0.086 a	10.46 ± 0.811 a	0.20 ± 0.012 a	1585 ± 146 a	240 ± 5.7 a
1	24.8 ± 1.23 a	1.58 ± 0.199 ab	10.25 ± 0.941 a	0.18 ± 0.012 a	1540 ± 134 a	234 ± 1.9 ab
2	18.3 ± 1.41 b	1.22 ± 0.122 bc	8.77 ± 1.013 ab	0.16 ± 0.010 ab	1339 ± 167 ab	233 ± 6.2 ab
4	11.1 ± 0.60 c	0.88 ± 0.091 dc	8.88 ± 0.150 ab	0.11 ± 0.014 c	1227 ± 25 abc	210 ± 3.6 bc
8	8.1 ± 0.68 c	0.62 ± 0.048 d	6.15 ± 0.395 bc	0.11 ± 0.010 bc	811 ± 49 dc	209 ± 3.0 bc
12	7.4 ± 1.29 c	0.56 ± 0.084 d	6.01 ± 0.749 bc	0.10 ± 0.010 c	784 ± 107 dc	207 ± 6.0 c
15	9.7 ± 0.97 c	0.64 ± 0.091 d	6.72 ± 0.481 bc	0.10 ± 0.009 c	865 ± 97 dc	215 ± 8.0 abc
20	6.3 ± 1.02 c	0.47 ± 0.063 d	5.23 ± 0.278 c	0.10 ± 0.010 c	595 ± 58 d	203 ± 8.0 c

Fig. 1. Leaf F_v/F_m of chrysanthemum (Expt. I) and miniature rose grown in solution with different Cu^{2+} concentrations. The values are measured on the last fully expanded leaves on day 23 from the start of Cu^{2+} treatment for chrysanthemum and on day 21 for miniature rose. Values are means of six replicates $\pm$ standard error. Means with a common letter are not different ($P \leq 0.05$) by Tukey's studentized range (HSD) test.

in chrysanthemum Expt. II, but not in Expt. I. This may be due to the faster growth of plants during spring (Expt. II) than winter (Expt. I) (see plant total dry weight in Table 2). Fast-growing plants need adequate nutrient supply, and when there is a root injury, plant leaves may show nutrient deficiency symptoms (Rey and Tsujita, 1987). Fast-growing plants also may take up more Cu^{2+} , which can cause more toxicity and root injury. The mechanisms that underlie the leaf chlorosis symptom need more research. The leaf chlorosis that occurred in Expt. II and the geranium experiment agreed with the findings of Lee et al. (1996) on *Pelargonium xhortorum* and Taylor and Foy (1985) on *Triticum aestivum*. However, we did not see any olive-green coloration on the leaf in any of our experiments as observed by Hill et al. (2000) on taro or leaf necrosis on both taro (Hill et al., 2000) and *Triticum aestivum* (Taylor and Foy, 1985) under excessive Cu^{2+} concentration. Leaf copper toxicity visible symptoms may differ, depending on the plant species and environmental conditions.

Leaf chlorophyll fluorescence. Leaf chlorophyll fluorescence (F_v/F_m) was measured on day 9 and 23 from the start of Cu^{2+} treatments in chrysanthemum Expt. I. Treatment effects were evident on day 23 (Fig. 1), but not on day 9 (data not shown). For miniature rose, F_v/F_m was measured every 2–4 d after 1 week from the start of Cu^{2+} treatment. There was no

treatment effect until 3 weeks after the start of the treatment (Fig. 1). Only the highest Cu^{2+} concentration (15.7 μM) treatment showed significantly ($P < 0.05$) lower F_v/F_m than the control (0.8 μM). Päsikkä et al. (1998) did see a decrease of F_v/F_m in the leaf of *Phaseolus vulgaris* grown in excessive Cu^{2+} nutrient solution; however, the decrease was measured 2 weeks after the start of the treatment. As a diagnostic tool to detect Cu^{2+} toxicity for chrysanthemum and miniature rose, the change of leaf F_v/F_m was slow and less reliable than visible root injury.

SPAD meter measurement. In chrysanthemum Expt. II, SPAD meter measurements were conducted on the last fully expanded leaf on day 3, after the start of the copper treatment and then once a week on the same day on the same leaf (old leaf) and the last fully expanded leaf (new leaf) on the day of measurement. Chlorophyll contents in the new leaves of the plants treated with higher Cu^{2+} concentrations (6 and 8 μM) were consistently lower than those treated with lower Cu^{2+} concentrations (3 μM and lower) starting from day 9 (data not shown). Statistical analysis showed that there was no significant difference among 0.4, 1.5, and 3 μM Cu^{2+} concentrations, and no difference between 6 and 8 μM . However, there was a significant treatment effect between the two groups at the 5% level. For the chlorophyll content in the old leaf, the trend was the same

(data not shown). For geranium, SPAD meter measurements were conducted on the last fully expanded leaf from day 5 after the initiation of the copper treatment; then once a week on the same day of the week on the last fully expanded leaf of the day of measurement. After 10 d of treatment, only Cu^{2+} concentrations at 12 μM or higher had significantly lower chlorophyll contents than the 0.5 μM level. Gradually, leaves of the 8 μM Cu^{2+} treatment started to show lower chlorophyll content compared to leaves of plants in solution with Cu^{2+} at 4 μM or lower (data not shown). Similar to the yellowish leaf symptom, chlorophyll content did not show any difference between treatments 5 d after the initiation of Cu^{2+} treatment, even though roots were visibly injured within 24 h. In addition, there is no standard SPAD meter reading growers can compare to in order to decide whether their crop is healthy or not, and an optimum SPAD reading may differ between cultivars or species. SPAD measurement was not as indicative as visible root injury in diagnosing copper toxicity for these plant species. Furthermore, based on their own results of Cu^{2+} toxicity on taro and other researchers' results on other species, Hill et al. (2000) concluded that "Cu toxicity can either increase or decrease chlorophyll content, depending on its effect on leaf expansion."

Growth. Excessive copper in the nutrient solution reduced root length, root dry weight, plant total dry weight, root : shoot ratio, leaf area, and specific leaf area ratio in all three species (Table 2). There are other reports on excessive Cu^{2+} depressing root (Hill et al., 2000; Lidon et al., 1992; Ouzounidou et al., 1994, 1995) and leaf (Hill et al., 2000; Maksymiec and Baszynski, 1996) growth in other species. The decreased root : shoot ratio indicates that plant roots are more sensitive to Cu^{2+} toxicity than shoots in these species. This is in agreement with our observations of visible root injury. For these ornamental crops, plant leaf and aboveground plant size are important plant parameters. If we take the copper concentration at which total plant dry weight and leaf area started to show significant ($P > 5\%$) reductions as the critical Cu^{2+} level, then the critical level for chrysanthemum in solution culture was 5 μM ; for miniature rose, 2.4–4.7 μM ; and for geranium, 8 μM . These critical levels are similar to those at which roots started to show brown tips within 24 h (for chrysanthemum and geranium) or within 1 week (for miniature rose). Previous studies reported that in solution culture the critical Cu^{2+} level for taro is 1.2 μM (Hill et al., 2000, based on 90% of relative dry weight), and for corn is $\approx 1 \mu\text{M}$ in the study by Ouzounidou et al. (1995), calculated as 90% of maximum root fresh weight, but a significant decrease in root and leaf biomass was only found at 10 μM Cu^{2+} in the experiment of Kaplan (1999) and Mocquot et al. (1996); for *Silene compacta* Fisch. is $\approx 14 \mu\text{M}$ (Ouzounidou, 1994), calculated as 90% of maximum root length. It is clear that different species or cultivars, populations, or clones of the same species may have different Cu^{2+} sensitivities (Ouzounidou et al., 1994; Utriainen et al., 1997). Among the

Table 3. Effects of solution Cu^{2+} levels on tissue copper and other nutrient compositions of chrysanthemum and miniature rose grown in solution culture for 4 weeks. Data are means of three samples, except data in chrysanthemum root sections (solution Cu^{2+} level 5 and 10 μM), which are from one single analysis of six combined samples due to small root system. Due to the small plant size, two samples from the same treatment were combined as one sample for analysis. Within species and tissue, data bearing the letter are not significantly different at $P \leq 0.05$.

	Solution	P	K	Ca	Mg	Cu	Zn	Mn	B	Fe
Tissue	μM	mg·g ⁻¹				μg·g ⁻¹				
Chrysanthemum Expt. I										
Leaf	0.4	8.4 a	73.0 a	33.9 a	4.0 a	32.4 a	336.0 a	203.2 a	41.7 a	86.2 a
	5	7.1 b	38.1 b	29.5 a	4.7 a	81.5 b	109.3 b	44.3 b	83.2 b	62.5 b\
	10	7.2 b	41.1 b	28.5 a	4.7 a	171.1 c	111.5 b	44.3 b	92.3 b	65.6 b
Stem	0.4	6.3 a	39.4 a	7.4 a	1.0 a	27.5 a	122.0 a	45.2 a	40.4 a	56.7 a
	5	3.0 b	14.0 b	3.9 b	0.6 b	73.1 b	26.4 b	5.1 b	28.1 a	46.9 a
	10	2.7 b	13.8 b	3.5 b	0.7 b	145.4 c	22.2 b	<5	29.3 a	52.5 a
Root	0.4	9.1	68.0	0.8	2.0	367.7	118.2	155.5	43.1	2085.9
	5	6.0	34.4	0.5	1.2	1496.0	58.5	<25	72.5	957.5
	10	3.2	20.8	0.7	0.8	4466.0	77.5	<50	98.9	1399.3
Miniature Rose										
Leaf	0.8	5.7 a	32.1 a	14.5 a	1.7 a	38.4 a	55.8 a	141.9 a	46.7 a	105.0 a
	2.4	6.3 a	29.4 b	14.9 a	1.7 a	39.6 a	55.4 a	161.6 a	51.4 ab	82.8 a
	4.7	6.3 a	29.0 b	14.7 a	1.9 b	42.8 a	59.4 a	180.7 a	57.8 b	98.3 a
Stem	0.8	5.8 a	26.6 a	11.2 a	2.2 a	28.1 a	84.8 a	65.3 a	30.6 a	84.1 a
	2.4	5.9 a	25.6 ab	11.0 a	2.3 a	31.8 a	89.7 a	71.3 a	37.4 b	67.8 b
	4.7	5.8 a	23.2 b	9.6 a	2.5 a	34.1 a	92.7 a	81.0 a	36.0 b	65.1 b
Root	0.8	7.3 a	60.0 a	10.7 a	4.7 a	329.5 a	482.4 a	295.1 a	45.9 a	2978.0 a
	2.4	7.0 a	54.2 ab	8.2 b	4.9 a	519.7 b	430.7 a	230.6 a	47.2 a	2088.0 b
	4.7	6.8 a	48.6 b	7.3 b	4.0 a	867.6 c	532.7 a	151.1 a	56.7 b	1820.2 b

three ornamental crops studied here, geranium was the most Cu^{2+} -resistant species.

Copper concentrations in plant tissues. Higher copper concentrations in the solution culture resulted in significantly increased copper concentrations in leaf, stem, and root for chrysanthemum. However, higher Cu^{2+} concentration only increased root copper concentration in miniature rose but did not increase copper concentrations in the leaf or stem tissues when solution Cu^{2+} concentration was as high as 4.7 μM (Table 3). In comparison with controls, chrysanthemum roots grown in 5 μM Cu^{2+} had >4 times the copper concentration, and roots grown in 10 μM Cu^{2+} had >12 times the copper concentration. Copper concentration in the root system was much higher than in the stem and leaf tissues. For example, chrysanthemum root tissue copper concentration when grown in Cu^{2+} of 10 μM was >26 times higher than that in the leaf tissue and >30 times higher than that in the stem tissue; miniature rose root tissue copper concentration when grown in Cu^{2+} of 4.7 μM was 20 times higher than that in the leaf tissue and >25 times higher than that in the stem tissue (Table 3). Much higher copper retention in roots than in leaves was reported in other studies as well (Alva et al., 1999; Hill et al., 2000). The issue of surface contamination should be considered when interpreting the results of root Cu^{2+} content analysis. A number of rinsing techniques have been reported in the literature to mitigate this possibility. In our study, the roots were washed only with distilled water at harvesting. However, the chrysanthemum root Cu^{2+} contents were comparable to the Cu^{2+} contents of one of the birch clone roots, which was washed with CaCl_2 (Utriainen et al., 1997) and citrus seedling roots washed with distilled water (Alva et al., 1999). The Cu^{2+} concentrations in miniature rose roots were in a similar range as the taro root Cu^{2+} concentrations in the copper

study conducted by Hill et al. (2000). It seems likely that our hydroponically grown plants were adequately rinsed with distilled water alone since the range of levels exhibited by the plants in our study was comparable to that in other studies employing more elaborate rinsing techniques. Our results and those of Hill et al. (2000) and Utriainen et al. (1997) clearly show that leaf tissue Cu content should not be used as a toxicity index, which is in disagreement with Gupta (1979).

Responses of other nutrients. Generally, for chrysanthemum, higher solution Cu^{2+} concentration decreased plant tissue P, K, Ca, Mg, Zn, Mn, and Fe levels (Table 3). However, solution Cu^{2+} concentration up to 4.7 μM did not have any effect on miniature rose plant tissue P, Mg, Zn, and Mn levels, but did decrease plant tissue K, Ca, and Fe levels (Table 3). Interestingly, for both chrysanthemum and miniature rose, higher solution Cu^{2+} significantly increased plant tissue B levels (Table 3). Despite the reduced nutrient levels in the plant tissues under higher solution Cu^{2+} concentration, up to 10 μM for chrysanthemum and 4.7 μM for miniature rose, these nutrient elements were still within the adequate levels for both of these species (Hanan, 1998). Taylor and Foy (1985) and Hill et al. (2000) found similar results in *Triticum aestivum* and taro. Therefore, the reduced plant growth in this study may be due to direct Cu^{2+} toxicity. However, the mechanism of the growth reductions under excessive Cu^{2+} concentrations need further research.

In conclusion, for these three species under solution culture conditions, plant visible root injury emerged as the best indicator of copper toxicity compared to visible leaf injury, leaf chlorophyll content, leaf chlorophyll fluorescence (F/F_m) measurement, and leaf or stem copper content. In all cases where roots showed visible injury within 24 h (for chrysanthemum and geranium) or 1 week (for miniature rose),

plant growth was eventually retarded. Critical levels of copper that induced visible root injury were the same for plant growth for all three species. Plants were sensitive to Cu^{2+} in solution culture, and caution is advisable to avoid overapplication of Cu^{2+} in solution culture. When plants are grown in substrates, especially organic ones, the absorption of Cu^{2+} by roots from the solution is reduced when Cu^{2+} is complexed with soluble organic compounds (Rey and Tsujita, 1987). Therefore, more research is needed to determine the critical levels of Cu^{2+} in nutrient solution for different growth media and different plant species in the greenhouse industry.

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