Taro Responses to Excess Copper in Solution Culture

Steven A. Hill¹ and Susan C. Miyasaka²

University of Hawaii–Manoa, Hawaii Branch Station, 461 West Lanikaula Street, Hilo, HI 96720

Russell S. Yost³

Department of Agronomy and Soil Science, University of Hawaii–Manoa, 1910 East-West Road, Honolulu, HI 96822

Additional index words. *Colocasia esculenta*, copper toxicity, cupric sulfate, plant nutrition

Abstract. Cupric sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) has been proposed for use in Hawaii as a molluscicide to control golden apple snail (*Pomacea canaliculata* Lamarck) infestations of taro [*Colocasia esculenta* (L.) Schott]. Two hydroponic, greenhouse studies were conducted to determine the effects of solution Cu^{2+} levels on taro growth, the solution Cu^{2+} toxicity threshold, and useful diagnostic indicators of Cu toxicity. In the first experiment, taro cultivars Lehua maoli and Pololu were grown at nine levels of Cu^{2+} ranging from 0.5 to 25.0 μM . In the second experiment, 'Lehua maoli' was grown at six levels of Cu^{2+} ranging from 0.25 to 2.5 μM . Significant ($P \leq 0.05$) toxic effects included reduced dry matter production, leaf area, and root length : root dry weight ratio, and both impaired photosynthesis and a generalized reduction of cation accumulation in leaf blade tissue. The solution Cu^{2+} toxicity threshold (based on 90% of relative total dry weight) for young taro plants was 1.2 μM . Because Cu does not accumulate in leaf blade tissues with increasing solution Cu^{2+} levels, leaf Cu concentration cannot be used as a diagnostic indicator of Cu toxicity in taro.

Taro is an herbaceous, perennial plant of the family Araceae that can be grown under flooded, low oxygen conditions analogous to those used for rice (*Oryza sativa* L.) culture (Coll. of Trop. Agr. and Human Resources, 1997). The major commercial products are the corm and cormels (starchy underground stems). The corm is susceptible to damage from a number of sources (Ooka, 1994), collectively known as corm rot. Grazing of corms and cormels by snails leads to opportunistic infections and is a problem in flooded taro culture.

The golden apple snail, a native of South America (Halwart, 1994), is a major pest of

taro in Hawaii, driving some small growers out of business and threatening the survival of the flooded taro industry (Chang, 1993). Control measures in Hawaii range from hand-picking to use of Cayuga black ducks (*Anas platyrhynchos* Linn. *domesticus*) (College of Trop. Agr. and Human Resources, 1997) to application of cupric sulfate as a molluscicide (Chang, 1993). In 1994, cupric sulfate was registered temporarily by the Hawaii Dept. of Agriculture as a means of controlling golden apple snail infestations in wetland taro (Hawaii Dept. of Agr., 1994); however, its registration has lapsed recently (M. Isherwood, personal communications). Commonly used as a micronutrient fertilizer (Mabbett, 1992), cupric sulfate is widely available and inexpensive.

Little is known about the potential phytotoxic effects of solution Cu^{2+} on taro. In other crops, Cu toxicity has been induced through application of Cu-containing fungicides (Tiller and Merry, 1981), industrial Cu pollution (Lidon and Henriques, 1992, 1994), or the use of metal-contaminated sewage sludge as a soil amendment (Luo and Rimmer, 1995).

Copper is required as an essential micronutrient, and is a component of proteins involved in redox reactions and photosynthetic electron transport in plants (Marschner, 1995). Excess Cu reportedly reduces photosynthetic oxygen evolution in chloroplasts (Maksymiec and Baszynski, 1996), increases formation of reactive oxygen species, permeability of the plasma membrane and leakage of potassium (K) ions from roots (Weckx and Clijsters, 1996), and induces iron (Fe) deficiency (Taylor and Foy, 1985).

The objectives of these solution culture experiments were to characterize the response of taro to excess root-zone Cu^{2+} , and to determine the critical foliar Cu concentration and critical solution Cu^{2+} level associated with incipient Cu toxicity of taro.

Materials and Methods

In the first experiment, two common wetland taro cultivars, Lehua maoli and Pololu, were grown at nine levels of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$: 0.5 (control), 2.5, 5.0, 7.5, 10.0, 12.5, 15, 20, and 25 μM . Based on results of the first experiment, 'Lehua maoli' was grown in the second experiment at six treatment levels of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$: 0.25, 0.5, 0.75, 1.0, 1.5, and 2.5 μM . Both experiments contained four randomized complete-blocks.

Tissue-cultured plantlets were purchased from a commercial laboratory (Paradise Propagations, Hilo, Hawaii), transplanted into blocks of an inert, porous media (Oasis blocks; Smithers-Oasis, Kent, Ohio) and acclimatized for ≈ 2 weeks under intermittent mist and 80% shade. The plantlets were grown in open containers containing complete nutrient solution for ≈ 1 month in a greenhouse. They were then transferred to the experimental apparatus, supported on racks located directly above 10 L of aerated nutrient solution in polyethylene buckets, following the method of Miyasaka et al. (1993).

Macronutrients in the nutrient solution were (in mM): 0.3 $\text{NH}_4\text{-N}$, 2.7 $\text{NO}_3\text{-N}$, 0.1 phosphorus (P), 1.2 K, 1.0 Ca, 0.4 Mg, 0.7 S, 0.04 Na, and 0.08 Cl. Micronutrients were (in μM): 2 Mn, 6 B, 1 Zn, 0.1 Mo, and 10 Fe as Fe-ethylene-bis-[2-(o-hydroxyphenyl-glycine)] (EDDHA). Solution pH was adjusted weekly to maintain a range of 4.5–5.5 in order to maintain a constant level of free Cu^{2+} activity. Previous experiments showed that levels of pH over 5.5 appeared to enhance the toxic effect of Cu^{2+} in solution culture experiments (Lexmond and van der Vorm, 1981).

Nutrient solutions were changed after 3 weeks to restore depleted nutrients and water levels. Plants were observed weekly for diseases, pests, and toxicity symptoms during the experimental period. Occasional infestations of aphids were controlled by wiping leaf blades with water-soaked polyester fiberfill.

Greenhouse facilities were located at the Univ. of Hawaii's Waikeke Experiment Station, near Hilo (lat. 19° 38' N, long. 155° 5' E). The mean daily maximum and minimum temperatures (ranges in parentheses) over the course of the experiments were 39 °C (29 to 44 °C) and 21 °C (18 to 23 °C). Ambient temperatures >40 °C may be excessive, but did not affect taro growth in solution culture. Plants were grown with Cu^{2+} treatments for 26 d during July to Aug. 1996 in the first experiment, and 26 d during Oct. 1996 in the second.

Leaf blade chlorophyll was measured before harvest with a chlorophyll meter (Minolta SPAD-502; Osaka, Japan) (Blackmer and Schepers, 1995). In addition, net leaf blade photosynthesis was measured with a LI-6200

Received for publication 9 Aug. 1999. Accepted for publication 15 Feb. 2000. This research was funded in part by the Hawaii Department of Agriculture, Plant Pest Control Branch. Journal Series No. 4464, College of Tropical Agriculture and Human Resources, Univ. of Hawaii, Honolulu. We thank Leslie S. Kodani and Dennis T. Matsuyama of the Beaumont Research Station in Hilo for their assistance in collection of data. Mention of a product or trade name does not constitute a guarantee or warranty of the product by the University of Hawaii, nor an endorsement over similar products not mentioned. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

¹Former graduate student. Current address: Tinian Agricultural Research and Extension Service, Northern Marianas College, P.O. Box 134, Tinian, Commonwealth of the Northern Marianas Islands, 96952.

²Associate Professor. To whom reprint requests should be addressed. E-mail address: miyasaka@hawaii.edu

³Professor.

portable photosynthesis system (LI-COR, Lincoln, Nebr.) prior to harvest in the first experiment.

At harvest, plants were separated into leaf blade, petiole, sucker, corm and roots; and fresh weights of each component were determined. Areas of leaf blades and lengths of roots were measured using a digital image analysis system (DIAS) (Decagon Devices, Pullman, Wash.). Plant parts were subsequently dried in a forced air oven at 65 °C to constant weight. Total dry weight was calculated as the sum of leaf blade, petiole, roots, and suckers. Corm dry weight was not included in our calculations because its quantity did not change greatly over 26 d. The root length : root dry weight ratio was calculated by dividing root length (m) by root dry weight (g) for each plant.

In the first experiment, dried leaf blades and roots from two randomly selected blocks were ground separately to pass through a 1-mm mesh. Subsamples were ashed in a muffle furnace at 600 °C, dissolved in 5 M HCl, then diluted to 1 M HCl, and analyzed for K, P, Ca, Mg, Fe, Mn, Zn, and Cu using an inductively-coupled plasma spectrometer (model 6500; Perkin-Elmer, Norwalk, Conn.) at the Univ. of Hawaii's Agricultural Diagnostic Center (Isaac and Jones, 1972). Boron was analyzed using the azomethine-H method (Wolf, 1974). In the second experiment, leaf blades and roots from four blocks of two Cu²⁺ levels (0.5 and 2.5 µM) were analyzed for P, K, Ca, Mg, Fe, Mn, Zn, Cu, and B as described earlier. In addition, leaf blades and roots from four blocks of two additional Cu²⁺ levels (1.0 and 1.5 µM) in Expt. 2 were analyzed for Cu.

In our experiments, tissue-cultured plantlets of taro were used to minimize disease incidence and maximize response uniformity. The choice of treatment levels for the first experiment was based on results of an earlier experiment (J. Liu and X. Huang, unpublished data), in which taro was grown from vegetative propagating materials consisting of lower petiole and upper corm ("hulis"). Tissue-cultured plantlets may be more sensitive to excess Cu²⁺, since most levels of solution Cu²⁺ were too high in Expt. 1, falling above the toxicity threshold. As a result, only data from the lowest two Cu²⁺ treatments (0.5 and 2.5 µM) were used for a comparison of control and toxic levels of root-zone Cu²⁺ in Expt. 1.

Analysis of variance (ANOVA) procedures were conducted using Statistix V. 4.0 (Statistix, St. Paul, Minn.) software. In Expt. 1, effects of solution Cu²⁺ and cultivar were calculated along with their interaction. In Expt. 2, linear (Cu) and quadratic (Cu²) effects of solution Cu²⁺ were calculated for total dry weight, leaf area, and chlorophyll content. To compare results with Expt. 1, concentrations of nutrients other than Cu in tissues were compared at two solution Cu²⁺ levels (0.5 and 2.5 µM) in Expt. 2, and only linear Cu treatment effects were calculated. Copper concentrations in roots and in shoots were regressed against solution Cu²⁺ levels.

Relative dry weight was calculated by dividing total dry weight of a plant by the mean of control plants for that particular cultivar and

experiment. Using the method of Hill et al. (1998), the zone of tolerance to Cu²⁺ was considered to be below 1.0 µM and the zone of non-linear response phase was considered to be above 3.1 µM. Linear regression was conducted for relative total dry weights of experimental treatments between 1–3 µM-Cu²⁺.

Nutrient contents were calculated by multiplying concentrations (on a dry weight basis) by plant dry weight. Any factor affecting plant growth will also indirectly affect nutrient accumulation. The null hypothesis (Jarrell and Beverly, 1986) tested was that an increase of Cu toxicity would either have no effect on or increase nutrient concentrations, and either have no effect on or decrease total nutrient contents because of reduced dry matter production.

Results and Discussion

Visual symptoms. An early response of taro to toxic levels of solution Cu²⁺ was an olive-green coloration starting from the leaf tip. For plants at intermediate Cu²⁺ levels, this coloration covered only the leaf tip to perhaps one quarter of the leaf. For plants at higher levels, entire leaf blades were often affected. The olive-green coloration was present on all leaves, but most severe symptoms were noted on the older ones. This coloration gradually disappeared after 2 weeks of exposure to toxic Cu²⁺ levels. The absence of yellow or white coloration as a symptom of Cu toxicity in taro contrasts with the chlorosis found in other plant species because of either excess Cu-induced Fe deficiency (Taylor and Foy, 1985) or destruction of membranes caused by lipid peroxidation (Marschner, 1995).

Taro plants grown in the higher Cu²⁺ levels also exhibited a pattern of tip necrosis in older leaves, covering up to one-quarter of the most severely affected leaves. This visual symptom also disappeared over the course of the experiment, as necrotic leaves senesced and were replaced by less necrotic ones. Given the transient nature of visible Cu toxicity symptoms in taro, and their presence only at very high solution Cu²⁺ levels, they are not likely to be useful in field diagnosis.

Total dry matter accumulation. Total plant dry weight of taro in the first experiment significantly decreased as levels of solution Cu²⁺

increased from 0.5 to 2.5 µM (Table 1). Similar results were found for dry weights of leaf blades, petioles, and roots (data not shown). Total plant dry weight of 'Pololu' was significantly greater than that of 'Lehua maoli' at both treatment levels. The lower dry weight of 'Lehua maoli' than of 'Pololu' could be a result of genetic differences between cultivars, or of dasheen mosaic virus (DMV) infection (Hu et al., 1995) observed only in 'Lehua maoli'.

Total plant dry weight decreased significantly as solution Cu²⁺ levels increased in Expt. 2 (Table 2). Dry weight of 'Lehua maoli' plants grown at 2.5 µM Cu²⁺ was significantly lower than that of control plants.

Relative total dry weights of plants grown between 1 and 3 µM Cu²⁺ from both experiments were regressed against solution Cu²⁺. The toxicity threshold (based on 90% of relative dry weight) of taro to solution Cu²⁺ was 1.2 µM (Fig. 1). Similar results were found in corn (*Zea mays* L.), a Cu-sensitive species (Marschner, 1995) that had a tolerance threshold (calculated as 90% of maximum root fresh weight) of ≈1 µM Cu²⁺ (Ouzounidou et al., 1995). In contrast, *Silene compacta* Fisch., a Cu-tolerant species, had a tolerance threshold (calculated as 90% of maximum root length) of ≈14 µM Cu²⁺ (Ouzounidou, 1994). Tissue-cultured plantlets of taro may be more sensitive to excess Cu than plants grown from "hulis"; however, this information is needed, because many farmers now grow tissue-cultured plantlets when multiplying selected cultivars.

Root morphology. The root length : root dry weight ratio of taro was significantly less when grown in 2.5 µM Cu²⁺ than in 0.5 µM Cu²⁺ in Expt. 1. (Table 1). Total root length also significantly decreased with increased Cu²⁺ level (data not shown). These results implied a shift toward shorter, thicker roots as a response to excessive root zone Cu²⁺. In rice (Lidon and Henriques, 1992), corn (Ouzounidou et al., 1995), and other plant species (Ouzounidou, 1994), toxic root-zone Cu²⁺ levels also inhibited root elongation.

Responses of shoots. Apparent chlorophyll content of taro leaf blades was not affected by solution Cu²⁺ in Expt. 1 (Table 1). Chlorophyll content was significantly higher for 'Pololu' than for 'Lehua maoli' (Table 1). In Expt. 2, chlorophyll content increased and

Table 1. Effects of solution Cu²⁺ levels on total per plant dry weight (leaf blade, petiole, roots, and suckers), root length:root dry weight ratio, chlorophyll content, and net photosynthesis of two taro cultivars grown hydroponically in Expt. 1.

Cultivar	Soln. Cu ²⁺ (µM)	Total dry wt (g)	Root length/dry wt (m·g ⁻¹)	Chlorophyll content (SPAD units)	Net photosynthesis (µmol·m ⁻² ·s ⁻¹)
Lehua	0.5	8.87(1.29) ^a	43.4(4.5)	46.5(1.9)	11.9(1.3)
	2.5	2.54(0.45)	28.6(6.0)	45.8(1.1)	10.4(1.7)
Pololu	0.5	13.60(0.91)	26.8(1.5)	59.5(2.2)	15.3(0.8)
	2.5	6.22(2.38)	22.4(1.4)	57.7(3.5)	11.6(1.8)
<i>Significance</i>					
Cu		*	*	NS	*
Cultivar		*	*	*	*
Cu × Cultivar		NS	NS	NS	NS

^aMean for four replicates, with standard error in parentheses.

NS, *Nonsignificant or significant at $P \leq 0.05$.

Table 2. Effects of increasing Cu^{2+} levels on total dry weight per plant (leaf blade, petiole, roots, and suckers), leaf area, and chlorophyll content of leaf blades of 'Lehua maoli' taro grown hydroponically in Expt. 2.

Soln. Cu^{2+} (μM)	Total dry wt (g)	Leaf area (cm^2)	Chlorophyll content (SPAD units)
0.25	10.24 (1.17) ^y	959 (89)	61.5 (1.5)
0.50	10.08 (0.66)	872 (102)	63.5 (0.3)
0.75	9.49 (0.75)	790 (124)	64.0 (1.9)
1.00	10.74 (0.88)	960 (182)	65.0 (1.5)
1.50	8.45 (0.49)	820 (61)	65.0 (2.2)
2.50	3.48 (1.20)	334 (97)	60.2 (2.2)

Cu	*	*	NS
Cu^2	*	NS	*

^yMean for four replicates, with standard error in parentheses.

NS, *Nonsignificant or significant at $P \leq 0.05$.

then decreased with increasing solution Cu levels, resulting in a significant quadratic effect (Table 2).

Contrasting reports exist for the effect of excess Cu on chlorophyll content. Copper toxicity reduced chlorophyll content of *Silene compacta* (Ouzounidou, 1994); in contrast, excess Cu increased chlorophyll content and reduced leaf area in runner beans (*Phaseolus coccineus* L.) at the early growth stage (Maksymiec and Baszynski, 1996). Apparently, Cu toxicity can either increase or decrease chlorophyll content, depending on its effect on leaf expansion.

The significant cultivar effect in Expt. 1 could be a result of genetic differences in leaf blade 'greenness' of the two cultivars. Alternatively, it might have been caused by DMV infection observed only in 'Lehua maoli', because the virus is expressed with numerous

pale green areas in a characteristic feathery pattern across leaf-blades.

Net photosynthesis was significantly greater in plants from the 0.5- μM than from the 2.5- μM Cu^{2+} treatment in Expt. 1 (Table 1). It was also higher for 'Pololu' than for 'Lehua maoli' (Table 1). The inhibitory effect of excess root-zone Cu^{2+} on photosynthesis has been observed in many plant species; however, the exact mechanisms involved are still debated (Droppa and Horvath, 1990). The greater net photosynthesis in 'Pololu' may be a result of genetic differences or of a detrimental effect of DMV on photosynthesis in 'Lehua maoli'.

Leaf area significantly decreased with increasing solution Cu^{2+} in Expt. 2 (Table 2). Also, in Expt. 1, plants grown at 2.5 μM Cu^{2+} had a significantly smaller average leaf area than did control plants (data not shown). Thus, in addition to reduced net photosynthetic rates,

Fig. 1. Effect of increasing solution Cu^{2+} on relative total dry weight of taro grown hydroponically in both experiments. Lehua I refers to 'Lehua maoli' grown in Expt. 1 and Lehua II refers to 'Lehua maoli' grown in Expt. 2. Plotted points are means of four samples, and error bars represent standard errors of the mean. Between 1 and 3 μM Cu, the linear regression equation is: $y = 143 - 43x$; $r^2 = 0.67$.

Fig. 2. Effect of increasing solution Cu^{2+} on tissue Cu concentrations in roots and shoots of taro 'Lehua maoli' grown hydroponically in Expt. 2. For leaf blade Cu: $y = 13.3 + 0.84x$; $r^2 = 0.09$. For root Cu: $y = 71 + 163x$; $r^2 = 0.95$.

Table 3. Effects of solution Cu^{2+} levels on nutrient concentrations in leaf blades and roots (dry weight basis) of two taro cultivars grown hydroponically in Expt. 1.

Tissue	Cultivar	Soln.	K	Ca	Mg	Fe	Mn	Zn	Cu
		μM	$\text{g}\cdot\text{kg}^{-1}$						
			$\text{mg}\cdot\text{kg}^{-1}$						
Leaf	Lehua	0.5	53.8 (0.2) ^z	19.2 (0.45)	3.05 (0.05)	120 (10)	294 (32)	56 (2)	16 (1)
		2.5	44.3 (0.9)	14.6 (1.40)	2.85 (0.05)	76 (1)	82 (12)	21 (3)	14 (1)
	Pololu	0.5	56.9 (3.9)	17.3 (0.45)	3.25 (0.05)	130 (1)	219 (23)	63 (11)	18 (2)
		2.5	34.3 (6.6)	15.1 (0.80)	2.85 (0.15)	91 (1)	132 (12)	33 (6)	18 (4)
Root	Lehua	0.5	69.4 (2.0)	6.2 (0.25)	3.05 (0.15)	146 (35)	62 (24)	103 (16)	136 (4)
		2.5	55.4 (5.5)	5.7 (0.10)	3.80 (0.30)	454 (272)	38 (4)	48 (2)	472 (12)
	Pololu	0.5	47.5 (9.3)	7.4 (0.40)	7.30 (2.40)	150 (18)	20 (6)	86 (2)	91 (17)
		2.5	53.6 (5.4)	6.4 (0.05)	3.50 (0.30)	328 (200)	54 (26)	52 (10)	431 (27)
Significance in Leaf									
Cu			*	*	*	*	*	*	NS
Cultivar			NS	NS	NS	NS	NS	NS	NS
Cu × Cultivar			NS	NS	NS	NS	NS	NS	NS
Significance in Root									
Cu			NS	NS	NS	NS	NS	*	*
Cultivar			NS	*	NS	NS	NS	NS	*
Cu × Cultivar			NS	NS	NS	NS	NS	NS	NS

^yMean for two replicates, with standard error in parentheses.

NS, *Nonsignificant or significant at $P \leq 0.05$.

leaf area available for photosynthesis also decreased in response to Cu^{2+} toxicity. A reduction in leaf area with excess Cu has also been reported for runner beans (Maksymiec and Baszynski, 1996).

Copper concentration. The concentrations of Cu in leaf blades were not affected by solution Cu^{2+} from 0.5 to 2.5 μM in either experiment, but the concentrations in roots increased dramatically with solution Cu^{2+} level (Tables 3, 4). In Expt. 1, the concentrations were higher in roots of 'Lehua maoli' than in those of 'Pololu' (Table 3).

Copper concentrations in leaf blades also did not increase with increasing root-zone Cu^{2+} in Expt. 2 (Fig. 2). As a result, use of leaf tissue Cu as a toxicity index, as advocated by Gupta (1979), is not possible in taro. Copper concentrations in root tissues, however, linearly increased with solution Cu^{2+} (Fig. 2). Similar results in which roots retained Cu were found for corn (Doncheva et al., 1996) and winter wheat (*Triticum aestivum* L.) (Jensen and Adalsteinsson, 1989). Also, Cu deposition in roots was observed in rice (Lidon and Henriques, 1994). Retention and sequestration of Cu in roots could be a mechanism of resistance to Cu toxicity.

Concentrations of other nutrients. Nutrient concentrations of K, Ca, Mg, Fe, and Mn in

leaf blades decreased in both experiments as solution Cu^{2+} levels increased from 0.5 to 2.5 μM (Tables 3, 4), and concentrations of Zn decreased significantly in Expt. 1 (Table 3). However, despite reduced levels of these cations at excess solution Cu^{2+} , nutrient concentrations appeared to be within the adequate ranges for taro (Ares et al., 1996; Austin et al., 1994; Miyasaka and Bartholomew, 1982; O'Sullivan et al., 1996). Nutrient deficiencies induced by excess Cu^{2+} do not appear to have been a major factor involved in Cu toxicity of taro grown in these solution culture studies. However, under field conditions of limited nutrient availability, excess Cu^{2+} could possibly induce deficiencies of these cations.

Nutrient concentrations of P and B in leaf blades and roots were not significantly affected by solution Cu. The average P concentrations in leaf blades in Expts. 1 and 2 were $4.2 (\pm 0.3)$ $\text{g}\cdot\text{kg}^{-1}$ and $4.7 (\pm 0.4)$ $\text{g}\cdot\text{kg}^{-1}$ (dry weight basis), respectively (standard errors of mean in parentheses). The average B concentrations in leaf blades in Expts. 1 and 2 were $57 (\pm 5)$ $\text{mg}\cdot\text{kg}^{-1}$ and $53 (\pm 8)$ $\text{mg}\cdot\text{kg}^{-1}$, respectively. These foliar concentrations of P and B appeared to be within the adequate ranges of these nutrients for taro (O'Sullivan et al., 1996).

Accumulation of other nutrients. To determine whether a change in nutrient accumula-

tion in tissues was due simply to depressed dry matter production (e.g., reverse of growth dilution effect; see Jarrell and Beverly, 1986), we compared ANOVA of nutrient concentrations with those of total contents (Table 5). Both concentration and total content of nutrients had to be reduced before we considered accumulation of that nutrient to be depressed by excess Cu. In taro leaf blades in both experiments, both nutrient concentrations and contents of K, Fe, and Mn were lower in the 2.5 μM Cu^{2+} treatment than in the 0.5 μM Cu^{2+} treatment (Table 5; data not shown for Expt. 1). Accumulation of Ca and Mg was also depressed in leaf blades in Expt. 2 (Table 5), whereas, Zn accumulation was depressed in leaf blades in Expt. 1 (data not shown). In roots in Expt. 2, only K accumulation was depressed (Table 5); in contrast, in Expt. 1, nutrient accumulation was not depressed in roots (data not shown).

The reduced accumulation of K in both leaf blades and roots of taro is in agreement with results for winter wheat, in which excess Cu reduced active Rb^+ (analog for K) influx, and decreased K concentrations in both roots and shoots (Jensen and Adalsteinsson, 1989). For other cations, mineral transport from root to shoot tissues appeared to be constrained at excess Cu^{2+} levels. Similar results in which concentrations in shoots were depressed relative to those in roots with increasing Cu levels were found for Mn and Fe in rice (Lidon and Henriques, 1992), and for Ca, K, and Fe in *Silene compacta* (Ouzounidou, 1994).

Practical implications. Copper is adsorbed rapidly by clay minerals and organic matter found in soils that are characteristic of flooded taro-growing areas in Hawaii (Hue et al., 1997). However, continuous cupric sulfate application, based on the maximum amount allowed (Hawaii Dept. of Agriculture, 1994), could build up soil Cu^{2+} concentrations over a century or two to levels that are toxic to taro (Hue et al., 1997). Alternatively, Cu is a required plant nutrient that is absorbed by taro, and removal of plant biomass from fields will reduce the level of soil Cu. Results of a field study examining the effects of cupric sulfate addition on taro will be reported in a later paper.

Table 4. Effects of solution Cu^{2+} levels on nutrient concentrations in leaf blades and roots (dry weight basis) of taro cv. Lehua maoli grown hydroponically in Expt. 2.

Tissue	Soln. Cu^{2+} — μM —	K	Ca	Mg	Fe	Mn	Zn	Cu
		g·kg ⁻¹			mg·kg ⁻¹			
Leaf blade	0.5	48.5 (1.8) ^z	12.2 (0.4)	5.5 (0.3)	147 (4)	264 (30)	30 (3)	14 (2)
	2.5	41.7 (1.8)	10.6 (0.4)	4.4 (0.1)	116 (5)	135 (11)	28 (3)	16 (1)
Root	0.5	61.2 (2.5)	4.8 (0.4)	3.0 (0.1)	204 (19)	22 (4)	90 (6)	147 (21)
	2.5	46.8 (3.4)	5.4 (0.4)	3.4 (0.4)	388 (175)	27 (6)	66 (10)	476 (19)
<i>Significance in Leaf</i>								
Cu		*	*	*	*	*	NS	NS
<i>Significance in Root</i>								
Cu		*	NS	NS	NS	NS	NS	*

^zMean for four replicates, with standard error in parentheses.

NS, *Nonsignificant or significant at $P \leq 0.05$.

Table 5. Summary of analysis of variance for effects of increased solution Cu^{2+} on nutrient concentrations and total contents in leaf blades and roots of 'Lehua maoli' grown hydroponically in Expt. 2.

Tissue	Nutrient	Concn	Content	Interpretation
Leaf, Root	null result	+, 0 ^z	0, —	No effect
Leaf	PO_4^{2-}	0	---	No effect
	K^+	---	---	Accumulation depressed
	Ca^{2+}	---	---	Accumulation depressed
	Mg^{2+}	---	---	Accumulation depressed
	Fe^{3+}	---	---	Accumulation depressed
	Mn^{2+}	---	---	Accumulation depressed
	Zn^{2+}	0	---	No effect
	Cu^{2+}	0	---	No effect
	BO_3^{2-}	0	0	No effect
	PO_4^{2-}	0	---	No effect
Root	K^+	---	---	Accumulation depressed
	Ca^{2+}	0	---	No effect
	Mg^{2+}	0	---	No effect
	Fe^{3+}	0	0	No effect
	Mn^{2+}	0	0	No effect
	Zn^{2+}	0	---	No effect
	Cu^{2+}	+	0	No effect
	BO_3^{2-}	0	0	No effect

^z+, —, 0, concentrations or total contents increased significantly, decreased significantly, or did not change significantly (at $P \leq 0.05$) as solution Cu increased from 0.5–2.5 μM .

Literature Cited

- Ares, A., S.G. Hwang, and S.C. Miyasaka. 1996. Taro response to different iron levels in hydroponic solution. *J. Plant Nutr.* 19:281–292.
- Austin, M.T., M. Constantinides, and S.C. Miyasaka. 1994. Effect of magnesium on early taro growth. *Commun. Soil Sci. Plant Analysis* 25:2159–2169.
- Blackmer, T.M. and J.S. Schepers. 1995. Use of a chlorophyll meter to monitor nitrogen status and schedule fertigation for corn. *J. Production Agr.* 8:56–60.
- Chang, L. 1993. Snails threaten to devour taro fields on Kauai: Isle farmers take measures to slow the advancing army. *Honolulu Star Bul.* A:1, May 14.
- College of Trop. Agr. and Human Resources. 1997. Taro: Mauka to makai. Coll. of Trop. Agr. and Human Resources, Univ. of Hawaii, Honolulu.
- Doncheva, S., B. Nikolov, and V. Ogneva. 1996. Effect of copper excess on the morphology of

- the nucleus in maize root meristem cells. *Physiol. Plant.* 96:118–122.
- Droppa, M. and G. Horvath. 1990. The role of copper in photosynthesis. *CRC Critical Rev. Plant Sci.* 9:111–123.
- Gupta, U. 1979. Copper in agricultural crops, p. 255–283. In: J.O. Nriagu (ed.). *Copper in the environment. Part I: Ecological cycling*. Wiley, New York.
- Halwart, M. 1994. The golden apple snail *Pomacea canaliculata* in Asian rice farming systems: present impact and future threat. *Intl. J. Pest Mgt.* 40(2):199–206.
- Hawaii Dept. of Agriculture. 1994. Copper sulfate snow crystals, EPA Reg. No. 1109–21. Hawaii Dept. of Agr., Lihue.
- Hill, S., R. Abaidoo, and S. Miyasaka. 1998. Sodium chloride concentration affects early growth and nutrient accumulation in taro. *HortScience* 33:1153–1156.
- Hu, J.S., S. Meleisea, M. Wang, M.A. Shaarawy, and F.W. Zettler. 1995. Dasheen mosaic potyvirus in Hawaiian taro. *Australasian Plant Pathol.* 24:112–117.
- Hue, N.V., F. Guo, G. Zhang, R.S. Yost, and S.C. Miyasaka. 1997. Reactions of copper sulfate with wetland soils of Hawaii. *Commun. Soil Sci. Plant Analysis* 28(11&12):849–862.
- Isaac, R.A. and J.B. Jones, Jr. 1972. Effects of various dry ashing temperatures on the determination of thirteen nutrients in five plant tissues. *Commun. Soil Sci. Plant Analysis* 3:261–269.
- Jarrell, W.M. and R.B. Beverly. 1986. The dilution effect in plant nutrition studies. *Adv. Agron.* 34:197–224.
- Jensen, P. and S. Adalsteinsson. 1989. Effects of copper on active and passive Rb^+ influx in roots of winter wheat. *Physiol. Plant.* 75:195–200.
- Lexmond, M. and P.D.J. van der Vorm. 1981. The effect of pH on copper toxicity to hydroponically grown maize. *Netherlands J. Agr. Sci.* 29:209–230.
- Lidon, F.C. and F.S. Henriques. 1992. Copper toxicity in rice: Diagnostic criteria and effect on tissue Mn and Fe. *Soil Sci.* 154:130–135.
- Lidon, F.C. and F.S. Henriques. 1994. Subcellular localization of copper and partial isolation of copper proteins in roots from rice plants exposed to excess copper. *Austral. J. Plant Physiol.* 21:427–36.
- Luo, Y. and D.L. Rimmer. 1995. Zinc-copper interaction affecting plant growth on a metal-contaminated soil. *Environ. Pollution* 88:79–83.
- Mabbett, T. 1992. Copper for growth and protection. *Far Eastern Agr.*, November/December.
- Maksymiec, W. and T. Baszynski. 1996. Different susceptibility of runner bean plants to excess copper as a function of the growth stages of primary leaves. *J. Plant Physiol.* 149: 217–221.
- Marschner, H. 1995. *Mineral nutrition of higher plants*, 2nd ed. Academic Press, San Diego.
- Miyasaka, S.C. and D.P. Bartholomew. 1982. Calcium nutrition of taro (*Colocasia esculenta* (L.) Schott.), p. 665–669. In: E.H. Belen and M. Villanueva (eds.). *Intl. Symp. on Trop. Root and Tuber Crops*, Philippines, Sept. 17–21, 1979. Philippine Council for Agr. and Resources Res., Los Banos, Philippines.
- Miyasaka, S.C., C.M. Webster, and N.V. Hue. 1993. Differential response of two taro cultivars to aluminum: 1. plant growth. *Commun. Soil Sci. Plant Analysis* 24:1197–1211.
- Ooka, J.J. 1994. Taro diseases: A guide for field identification. HITAH Research Extension Series 148. College of Trop. Agr. and Human Resources, Univ. of Hawaii. Honolulu.
- O'Sullivan, J.N., C.J. Asher, and F.P.C. Blamey. 1996. Diagnostic criteria for nutrition disorders of taro, p. 83–90. In: E.T. Craswell, C.J. Asher, and J.N. O'Sullivan (eds.). *Mineral nutrient disorders of root crops in the South Pacific*, Proc. Wkshp., Nuku'alofa, Kingdom of Tonga, 17–20 April 1995. Austr. Ctr. for Intl. Agr. Res., Canberra, Australia.
- Ouzounidou, G. 1994. Root growth and pigment composition in relationship to element uptake in *Silene compacta* plants treated with copper. *J. Plant Nutrition* 17:933–943.
- Ouzounidou, G., M. Ciamporova, M. Moustakas, and S. Karataglis. 1995. Responses of maize (*Zeamays* L.) plants to copper stress—I. Growth, mineral content, and ultrastructure of roots. *Envir. Expt. Bot.* 35:167–176.
- Taylor, G.J. and C. D. Foy. 1985. Differential uptake and toxicity of ionic and chelated copper in *Triticum aestivum*. *Can. J. Bot.* 63:1271–1275.
- Tiller, K.G. and R.H. Merry. 1981. Copper pollution of agricultural soils, p. 119–137. In: J.F. Loneragan, A.D. Robson, and R.D. Graham (eds.). *Copper in soil and plants*. Academic Press, New York.
- Weckx, J.E.J. and H.M.M. Clijsters. 1996. Oxidative damage and defense mechanisms in primary leaves of *Phaseolus vulgaris* as a result of root assimilation of toxic amounts of copper. *Physiol. Plant.* 96:506–512.
- Wolf, B. 1974. Improvements in the azomethine-H method for the determination of boron. *Commun. Soil Sci. Plant Analysis* 5:39–44.