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Greenhouse Pepper Growth and Yield Response to Copper Application

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Abstract. **Copper (electrolytically generated or from cupric sulfate) is increasingly used to control diseases and algae in the greenhouse industry. However, there is a shortage of information regarding appropriate management strategies for Cu^{2+} (Cu) in greenhouse hydroponic production. Three greenhouse studies were conducted to examine the growth and yield responses of sweet pepper (*Capsicum annuum* L., Triple 4, red) to the application of Cu in hydroponic production systems. In the first two experiments, plants were grown on rockwool and irrigated with nutrient solutions containing Cu at concentrations of 0.05, 0.55, 1.05, 1.55, and 2.05 mg·L^{-1}. Copper treatments were started either when plants were 32 days old and continued for 4 weeks, or when plants were 11 weeks old and continued for 18 weeks, respectively. In the third experiment, roots of solution cultured pepper seedlings were exposed to Cu (1.0, 1.5, and 2.0 mg·L^{-1}) containing nutrient solutions for 2 hours per day for 3 weeks. Higher Cu treatment initialized when plants were 32 days old significantly reduced plant leaf number, leaf area, leaf biomass, specific leaf area, stem length and shoot biomass. The calculated Cu toxicity threshold was 0.19 mg·L^{-1}. However, when treatment initialized at plants were 11 weeks old, Cu did not have significant effects on leaf chlorophyll content, leaf area or specific leaf area. Copper started to show significant negative effects on leaf biomass and shoot biomass at 1.05 mg·L^{-1} or higher levels. Copper treatments did not have any significant effect on fruit number, fresh weight or dry weight. Under all the Cu levels, fresh fruit copper contents were lower than 0.95 mg·kg^{-1} which is below the drinking water standard of 1.3 mg·kg^{-1}. Seedling growth was significantly reduced by exposing roots to Cu (≥1.0 mg·L^{-1}) containing solutions even for only 2 h·d^{-1}.**

Greenhouse conditions are favorable to plant growth and also creating an optimal environment for various plant pathogens and algae. Commercial greenhouse operations not only employ conventional control methods (e.g., pesticides spray and biological control agents), but actively explore new methods, such as electrolytically generated copper, ozone and UV to treat nutrient solutions, growth facilities and materials. Increasing numbers of growers have started using either electrolytically generated copper (Zheng et al., 2004), cupric sulfate (Alva et al., 1999; Hill et al., 2000), or some other copper containing fungicides and bactericides (Kaplan, 1999) to control diseases or pathogens both in the greenhouse and in field crop production. However, a question which urgently needs to be answered is the Cu^{2+} (Cu) concentration (possibly threshold) that can be used to control greenhouse diseases and algae without negatively affecting crops. Much of the research on Cu toxicity has been on the responses of plants to Cu polluted soil (Fernandes and Henriques, 1991; Panou-Filotheou et al., 2001). Zheng et al. (2004) recently reported on Cu toxicity on greenhouse ornamental crops; however, we are not aware of any study that has provided Cu toxicity data for greenhouse hydroponic vegetable production.

Sweet pepper is one of the three major greenhouse vegetable crops in both Europe and Northern America; and rockwool is the primary growth medium used in greenhouse pepper production. Using Cu to control pathogens in hydroponics raises concerns about potential phytotoxic effects and Cu accumulation in fruit.

The objectives of this study were to 1) determine the dose-response of hydroponically grown pepper plants to Cu; 2) determine Cu phytotoxicity in pepper plants at two different growth stages; 3) assess copper levels in fruit of pepper plants irrigated with high concentration Cu solutions; 4) evaluate the intermittent application of Cu in greenhouse pepper production as an alternative management strategies.

Materials and Methods

Experiment I. Nine-week-old *Capsicum annuum* L. (sweet pepper, cultivar Triple 4, red) seedlings, grown on rockwool blocks [10 × 10 × 8 cm (L × W × H)], were transplanted to rockwool slabs [15 × 91.5 × 7.5 cm (W × L × H)] in a greenhouse vegetable production system at the University of Guelph on 28 Feb. 2003. Plants were irrigated as needed with the nutrient solution containing (mg·L^{-1}) 180 N, 50 P, 378 K, 190 Ca, 75 Mg, 120 S, 0.8 Fe, 0.55 Mn, 0.33 Zn, 0.5 B, 0.05 Cu, and 0.05 Mo. In all the nutrient solutions used in the present study, the Fe was supplied as $FeCl_3$, instead of chelated Fe. The nutrient solution pH was 5.8. On 13 Mar. 2003, Cu^{2+} (Cu) treatments were initialized by irrigating plants with the above nutrient solution containing a final Cu concentration of 0.05, 0.55, 1.05, 1.55, or 2.05 mg·L^{-1}. Thereafter, plants were irrigated with different nutrient solutions at different development stages following the recommendation of Ontario Ministry of Agriculture and Food (OMAF, 2001) for greenhouse pepper production, while continuing the Cu treatments. During the vegetative stage, the set point of the temperature and the relative humidity (RH) in the greenhouse were 23/21 °C (day/night) and 70% (day and night), respectively; during the harvest stage, the set point for temperature was 24/21 °C (day/night) and for the RH was 70% (day and night). All other operations were similar to common commercial greenhouse practices.

Leaf chlorophyll content index (CCI) was measured on the youngest fully expanded leaf 1 week after the initialization of Cu treatment, and this leaf was marked (referred to as old leaf hereafter) and measured again once a week thereafter. Also, once a week, the CCI of the youngest fully expanded leaf of the day (referred to as young leaf hereafter) was measured. The CCI was measured with a chlorophyll content meter (CCM-200; Opti-Sciences, Tyngsboro, Mass.).

Fruit were harvested when they reached maturity. The final harvest of the plant and fruit were conducted on 18 July 2002. At the final harvest, plants were separated into leaves, stems, and green and ripe fruit, and fresh weights were determined. Leaf area of each plant was measured using a leaf area meter (LI-3100; LI-COR, Inc., Lincoln, Neb.). All plant tissues were dried separately in a forced air oven at 65 °C until dry weights remained constant. Specific leaf area (SLA, cm^2·g^{-1}) was calculated by dividing leaf area by leaf biomass of each plant. Shoot biomass was the sum of the aerial vegetative plant parts including flowers.

Dried ripe fruit were ground into 1 mm particles for copper content analysis. Sub-samples (1 gram each) were digested using a dry ash method (at 475 °C) and then dissolved in 1.0 M HCl solution. Copper concentrations of the above solutions were analyzed by an atomic absorption spectrometer (SpectraAA-300; Varian, Victoria, Australia) at the Laboratory Service Division, University of Guelph.

The experiment was a randomized complete block design with five Cu concentrations in three blocks. There were four plants in each plot.

Experiment II. Pepper seeds (Triple 4, red) were sown in rockwool cubes and placed on a mist bench on 17 July 2002. After germination, seedlings were moved to a production greenhouse at the University of Guelph and watered with the same nutrient solution as used in the early stage in Experiment I. Plants were transplanted to rockwool slabs in a commercial greenhouse production setting on August 27, 2002 and Cu treatments were started one day later (when plants were 32 d old). Subsequently, plants were irrigated as needed with the above nutrient solution containing 0.05, 0.55, 1.05, 1.55, and 2.05 mg·L^{-1} Cu. The experiment was

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a randomized complete block design with five Cu treatments in three blocks. There were four plants in each plot. The environmental condition of the greenhouse and all the management practices were kept the same as those during the vegetative stage in Experiment I.

Leaf visible injury was assessed every day. Plants were harvested for growth analysis after 4 weeks of Cu treatment. The growth analysis methods used were the same as those used in Experiment I. The stem length was measured from the substrate surface to the top of the plant.

Experiment III. Pepper seeds (same cultivar as used in the above two experiments) were sown in rockwool cubes in a greenhouse. Seedlings (32 d old) were transplanted to a 2-L plastic pot containing a nutrient solution with 0.05 $mg \cdot L^{-1}$ Cu. When plants were 46 d old, Cu treatments were initialized by exposing plant roots to the above nutrient solution containing 0.05, 1.0, 1.5, and 2.0 $mg \cdot L^{-1}$ Cu for 2 $h \cdot d^{-1}$, and then returned to their original nutrient solutions.

After 21 d of Cu treatment, the CCI of the youngest fully expanded leaves were measured, and then the plants were harvested for growth analysis following the methods used in Experiment I. In addition, the roots were washed with deionized water until clean and dried in a forced air oven at 65 °C until weight constant. Root to shoot ratio was calculated by dividing the root biomass by the biomass sum of the aerial parts of each plant.

This experiment was a completely randomized design with 10 replications in each treatment.

Statistical analysis. Analysis of variance was performed to detect whether there were treatment effects. When the treatment effect was significant ($P < 0.05$), a multiple comparison of means was conducted using Tukey's HSD, or a linear regression analysis was performed after log-transforming of the dependant variable. If there was no significant treatment effect, then data were presented as the average of all the treatments. Statistical analysis was conducted using SAS (version 9.1, SAS Institute Inc., Cary, N.C.).

Results

Experiment I. No visible symptoms of Cu toxicity or nutrient deficiency were observed on leaves of any of the plants during the entire experimental period. Sequential CCI measurements made on both the old leaves and young leaves also did not show any significant Cu treatment effect. The average CCI for the youngest expanded leaves was 64 ± 0.3 (mean ± standard error) and for the old leaves was 65 ± 0.3. There were no significant Cu treatment effects on leaf area (8226 ± 352 cm^2/plant) or specific leaf area (184 ± 3 $cm^2 \cdot g^{-1}$). No significant ($P > 0.05$) differences in leaf and shoot biomass between Cu concentrations ($mg \cdot L^{-1}$) of 0.05 and 0.55 were observed. However, both leaf and shoot biomass were significantly reduced at Cu ≥1.05 $mg \cdot L^{-1}$ (Fig. 1). Copper treatment did not have any significant ($P > 0.05$) effect on ripe fruit number (2.5 ± 0.3 fruit /plant), ripe fruit fresh weight (342 ± 40 gram/plant) or dry weight (32.4 ± 4.7), green fruit number (3.3 ± 0.4), green fruit fresh (366 ± 49) or dry weight (26.8 ± 3.6), total fruit fresh weight (708 ± 58), or dry weight (59.2 ± 5.0). Tissue analysis showed that fruit copper contents of all the treatments were below 10 $mg \cdot kg^{-1}$ dry mass, which was the detection limit of the method used.

Experiment II. Within 2 weeks after the start of the Cu treatments, young leaves of several plants irrigated with Cu concentration ≥1.05 $mg \cdot L^{-1}$ started to show chlorosis. At the end of the experiment, all the plants under 1.05 $mg \cdot L^{-1}$ or higher Cu concentrations had young leaves showing chlorotic symptoms. Final harvest data showed that leaf number, leaf area, leaf biomass, specific leaf area (SLA), stem length and shoot biomass of the seedlings were significantly ($P < 0.001$) reduced by higher Cu concentrations (Fig. 2). There was a significant ($P < 0.0001$, $r^2 = 0.9441$) linear relationship [$Log(Y) = -0.3735X + 0.9707$] between log transformed seedling shoot biomass (Y, g/plant) and nutrient solution Cu concentration (X, $mg \cdot L^{-1}$). According to this equation, the toxicity threshold (based on 10% reduction in shoot biomass) of rockwool grown pepper seedlings to nutrient solution Cu was 0.19 $mg \cdot L^{-1}$.

Experiment III. Roots started to show brown tips after the first 2-hour exposure to 1.5 and 2.0 $mg \cdot L^{-1}$ Cu solutions. All the plants started to show brown roots after 3 consecutive days of 2-h exposure periods to 1.0, 1.5, and 2 $mg \cdot L^{-1}$ Cu solutions. On day 12, all the plants in the 1.5 and 2.0 $mg \cdot L^{-1}$ Cu solutions started to show chlorosis on young leaves. Chlorophyll content index measured on the youngest fully expanded leaves on day 21 showed that there was a significant ($P = 0.005$) negative linear relationship ($Y = 38.5 - 0.27X$) between CCI (Y) and solution copper concentration (X). Growth analysis after 21 d of copper treatment showed that leaf area, leaf biomass, stem length, stem biomass, the longest root length, root biomass,

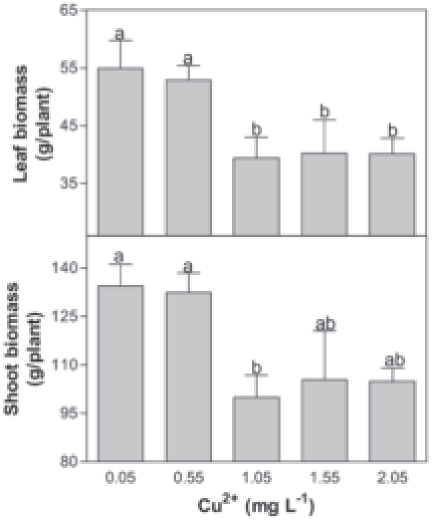

Fig. 1. Leaf and shoot biomasses of pepper plants (Triple 4, red) irrigated with Cu solution (started at week 11). Data are the mean ± SE of three replications (four subsamples in each replication). Bars bearing the same letter are not significantly different at the 5% level (by Tukey's HSD).

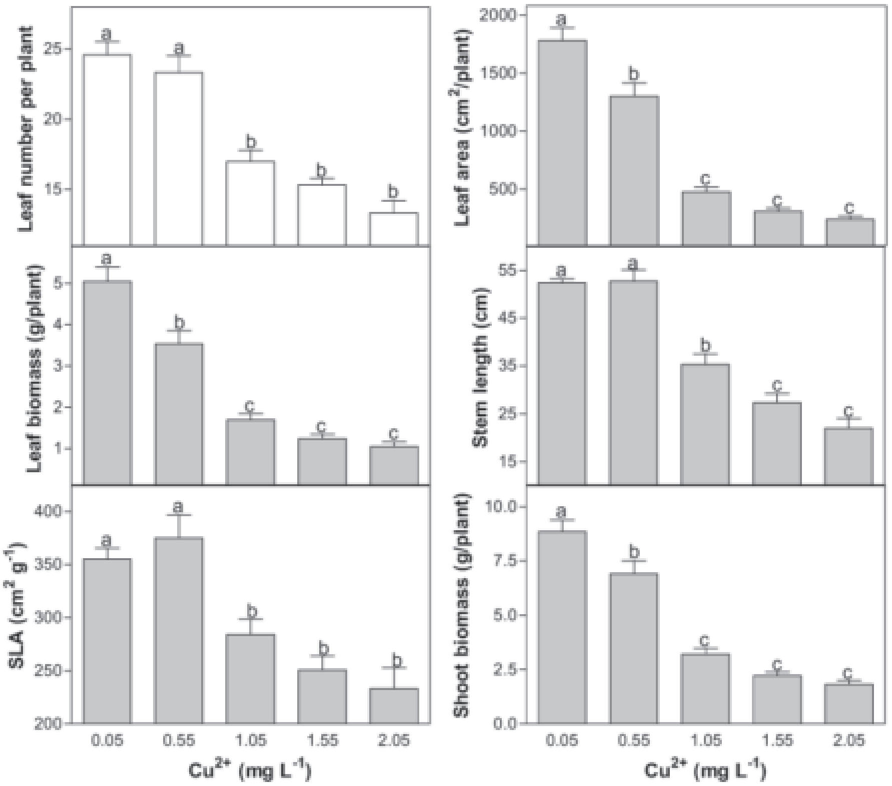

Fig. 2. Leaf number, leaf area, leaf biomass, specific leaf area (SLA), stem length and shoot biomass of pepper plants (Triple 4, red) irrigated with Cu solution at 4 to 5 weeks. Data are the mean ± SE of three replications (four subsamples in each replication). Bars bearing the same letter are not significantly different at 5% level (by Tukey's HSD).

Table 1. Leaf area, leaf biomass, stem length, stem biomass, the longest root length, root biomass, total biomass and root to shoot ratio of pepper (Triple 4, red) plants grown in different concentrations of Cu nutrient solutions for 2 $h \cdot d^{-1}$ for 21 d.[z]

Sln Cu ($mg \cdot L^{-1}$)	Leaf area (cm^2)	Leaf biomass (g/plant)	Stem length (cm)	Stem biomass (g/plant)	Root length (cm)	Root biomass (g/plant)	Total biomass (g/plant)	Root to shoot ratio
0.05	1076 a	3.3 a	30.0 a	1.6 a	40.5 a	1.3 a	6.3 a	0.26 a
1.0	493 b	1.9 b	23.8 b	1.3 b	20.8 b	0.5 b	3.7 b	0.15 b
1.5	538 b	1.9 b	22.3 bc	1.2 bc	17.4 b	0.4 b	3.5 b	0.13 b
2.0	414 b	1.6 b	20.4 c	1.0 c	16.7 b	0.3 b	2.9 b	0.13 b

[z] Copper treatments were started when plants were 46 d old. Data are the means of 10 replications. Data bearing the same letter are not significantly different at 5% level (by Tukey's HSD).

total biomass, and root to shoot ratio were significantly ($P < 0.05$) reduced by solutions with Cu ≥ 1.0 $mg \cdot L^{-1}$ (Table 1). No significant ($P > 0.05$) treatment effect was observed for specific leaf area (data not show).

Discussion

Pepper plants grown on rockwool were more sensitive to Cu toxicity at the early growth stage (Experiment II, Cu treatments initialized at 4.5 weeks) and became more resistant when plants were older (Experiment I, Cu treatments initialized at 11 weeks). The calculated Cu toxicity threshold, based on 10% shoot biomass reduction, was 0.19 $mg \cdot L^{-1}$ at the seedling stage and it increased to somewhere between 0.55 to 1.05 $mg \cdot L^{-1}$ when plants were older. Different plant species have different Cu sensitivities. Previous research found the threshold ($mg \cdot L^{-1}$) for taro = 0.08 (Hill et al. 2004), for miniature rose is from 0.15 to 0.3, chrysanthemum = 0.32, and geranium = 0.5 (Zheng et al., 2004). All the aforementioned thresholds were resulted from solution culture experiments. Since rockwool is a chemically inert material, one would expect that the results in terms of Cu toxicity threshold from both rockwool and solution culture experiments should be similar. When plants are grown in organic matter containing substrates, the absorption of Cu by roots from the solution is reduced when Cu is complexed with soluble organic compounds (Rey and Tsujita, 1987). Therefore, the Cu threshold must be higher for plants grown on organic matter containing substrates, such as peat moss and coir. Further research is needed to investigate Cu thresholds for different species and different growing media combination.

Copper did not have any effect on pepper fruit yield when treatments were started with older plants (11 weeks old), even when the Cu concentration in the nutrient solution reached 2.05 $mg \cdot L^{-1}$. When applying Cu in greenhouse vegetable production, one of the concerns is food safety. Based on the dry fruit copper content and fruit water content measurements, it was calculated that the fresh fruit copper concentration was below 0.95 $mg \cdot kg^{-1}$. This is below the drinking water standard (1.3 $mg \cdot kg^{-1}$) set by the U.S. Environmental Protection Agency (EPA, 2002). Lower fruit copper may due to the fact that copper tends to accumulate in roots rather than move up to the aerial parts (Alva et al., 1999; Hill et al., 2000; Zheng et al., 2004), and the mobility of copper in plants is restricted (Fernandes and Henriques,1991). Zheng et al. (2004) reported that copper concentration in the roots can be 20 to 30 times higher than that in the aerial parts. Even so, it is suggested that nutrient solution Cu concentration used in hydroponic pepper production should not exceed 0.19 $mg \cdot L^{-1}$ at the early growing stage (<4 to 5 weeks), and not exceed 1.0 $mg \cdot L^{-1}$ at older stages (e.g., older than 11 weeks). At higher Cu concentrations, plant roots can be injured long before any aerial damage is evident as shown in Experiment III and Zheng et al. (2004), and the injured roots may be more susceptible to root pathogens (Javis. 1992).

Currently, 1 $mg \cdot L^{-1}$ Cu is used in some commercial greenhouse and nurseries with the intent to control diseases and green algae. Our study has found that 1.15 $mg \cdot L^{-1}$ Cu can prevent or delay the appearance of green algae in nutrient solutions (not published). Based on the Cu toxicity thresholds generated from Experiments I and II, the current industry practice certainly can not be applied in greenhouse hydroponic pepper production, especially when inert materials such as rockwool are used as growing media or in solution culture. As an alternative strategy, we investigated the intermittent application of Cu to the nutrient solution. However, pepper seedlings exposed to solutions containing Cu ≥ 1.0 $mg \cdot L^{-1}$ for only 2 $h \cdot d^{-1}$ resulted in significant root browning, leaf chlorosis and reduction in growth. Our previous research also showed that miniature rose, chrysanthemum, and geranium started to show root injury symptoms after only 24 h of exposure to Cu of 0.5 $mg \cdot L^{-1}$ or even lower (Zheng et al., 2004). The leaf chlorosis most likely was due to excess Cu-induced Fe deficiency (Taylor and Foy, 1985). This is supported by the fact that high Cu concentration can reduce root Fe uptake and leaf tissue Fe content (Zheng et al., 2004). To avoid plant injury, but effectively control diseases and green algae, it is suggested that roots, especially at early growth stage, should not be directly exposed to high level Cu, even for a short time, in solution culture or hydroponic systems with inert growing substrates. However, it may be feasible to design a system which can remove Cu or convert Cu to forms which are not available to plants after the disinfection process and before the Cu treated nutrient solution or water be delivered to hydroponic production system.

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