Greenhouse Cucumber Growth and Yield Response to Copper Application

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Abstract. To determine the nutrient solution copper (Cu^{2+}) level above which *Cucumis sativus* L. (cucumber, cv. LOGICA F1) plant growth and fruit yield will be negatively affected, plants were grown on rockwool and irrigated with nutrient solutions containing Cu^{2+} at 0.05, 0.55, 1.05, 1.55, and 2.05 $\text{mg}\cdot\text{L}^{-1}$. Copper treatment began when plants were 4 weeks old and lasted for 10 weeks. During this 10-week period, plants were harvested at 3 weeks (short-term) and 10 weeks (long-term) after the start of Cu^{2+} treatment. Neither visible leaf injury nor negative Cu^{2+} effect was observed on plant growth (leaf number, leaf area, leaf dry weight, and stem dry weight) after 3 weeks of continuous Cu^{2+} treatment. However, after 10 weeks of continuous Cu^{2+} application, cucumber leaf dry weight was significantly reduced by Cu^{2+} levels 1.05 $\text{mg}\cdot\text{L}^{-1}$ or greater; leaf number, leaf area, and stem dry weight were significantly reduced by Cu^{2+} levels 1.55 $\text{mg}\cdot\text{L}^{-1}$ or greater. Copper (Cu^{2+} levels 1.05 $\text{mg}\cdot\text{L}^{-1}$ or greater) also caused root browning. Some plants under the 2.05 $\text{mg}\cdot\text{L}^{-1}$ Cu^{2+} treatment started to wilt after 6 weeks of continuous Cu^{2+} treatment. Copper treatment did not result in any change in leaf greenness until after Week 9 from the start of the treatments. There was no sign of a negative Cu^{2+} effect on cucumber fruit numbers after the first 2 weeks of production, but plants under the highest Cu^{2+} concentration treatment (2.05 $\text{mg}\cdot\text{L}^{-1}$) gradually produced fewer cucumber fruit than the control (0.05 $\text{mg}\cdot\text{L}^{-1}$) and eventually resulted in lower cucumber yield. Nutrient solution can be treated with 1.05 $\text{mg}\cdot\text{L}^{-1}$ of Cu^{2+} in cucumber production greenhouses; however, it is not recommended to use Cu^{2+} concentrations 1.05 $\text{mg}\cdot\text{L}^{-1}$ or greater continuously long-term (more than 3 weeks). When applying Cu^{2+} , it is suggested that cucumber roots be examined regularly because roots are a better indicator for Cu^{2+} toxicity than leaf injury.

Greenhouse operations create favorable environmental conditions for plants; these conditions also favor growth of many plant pathogen and algae species. To control the spread of plant pathogens and algae, commercial greenhouse operations use conventional control methods (i.e., pesticides and biological control agents), but there has been much exploration on new control methods such as ozone and ultraviolet treatment of nutrient solutions (Graham et al., 2009; Zheng et al., 2005). Copper (Cu^{2+}) is one of the essential micronutrient elements for plants (Salisbury and Ross, 1992), but excessive Cu^{2+} is toxic to living organisms, including plants. Because copper is a toxic heavy metal, some growers are using 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; Scheck and Pscheidt, 1998) to control diseases or pathogens both in the greenhouse and in field crop production. However, more research is still needed to determine copper levels that can be used to control greenhouse diseases and algae without negatively affecting major greenhouse crop species. Much of the research on Cu^{2+} toxicity has been on the responses of plants to copper-polluted soil (Borkert et al., 1998; Fernandes and Henriques, 1991; Panou-Filotheou et al., 2001). Recently, research has been done on Cu^{2+} toxicity on greenhouse ornamental crops and sweet pepper (Zheng et al., 2004, 2005), but there are no data on rockwool-grown greenhouse cucumber plants. Different plant species, the same species at different development stages, and plants grown in different growing substrates have different sensitivities to copper toxicity (Zheng et al., 2004, 2005).

Cucumber is one of the three major greenhouse vegetable crops in both Europe and North America, and rockwool is the primary growing substrate used in greenhouse hydroponic production.

The objectives of this study were to: 1) determine the nutrient solution Cu^{2+} levels above which cucumber plant growth and fruit

yield will be negatively affected; and 2) study the responses of cucumber plants to short-term and long-term copper applications in the nutrient solution.

Materials and Methods

Plant materials and culture. *Cucumis sativus* L. (cucumber, cv. LOGICA F1) seeds were sown in rockwool cubes on 20 Feb. 2003 in one of the research greenhouses at the University of Guelph (Guelph, Ontario, Canada) (lat. 43°34' N). Greenhouse temperature was kept at 23/21 °C at day/night; the relative humidity was ≈60%. Seedlings were transplanted onto rockwool blocks (10 × 10 × 8 cm) on 28 Feb. 2003. After 2 weeks, plants on blocks were transplanted onto rockwool slabs (15 × 91.5 × 7.5 cm) in the vegetable fruit production system in the greenhouse. Plants were then irrigated when needed with the nutrient solution recommended by the Ontario Ministry of Agriculture and Food (Ontario Ministry of Agriculture, Food and Rural Affairs, 2001) for commercial greenhouse cucumber production in Ontario without additional Cu^{2+} in the nutrient solution for an additional week. The nutrient solution contained macronutrients (in mM): 11.8 $\text{NO}_3\text{-N}$, 4.0 $\text{NH}_4\text{-N}$, 1.5 phosphorus, 3.6 calcium, 5.0 potassium, 2.0 sulfur, and 1.0 magnesium; and micronutrients (in μM): 5.0 manganese, 3.5 zinc, 20 boron, 0.5 molybdenum, 0.05 Cu^{2+} , and 25 iron as FeCl_3 . The Cu^{2+} treatments were initiated after the cucumber seedlings were 4 weeks old.

Copper treatment. The experiment was a randomized complete block design with three blocks. There were five treatments [0.05 (control), 0.55, 1.05, 1.55, and 2.05 $\text{mg}\cdot\text{L}^{-1}$ Cu^{2+} concentrations] with eight plants per experimental unit. The Cu^{2+} concentration in each treatment was achieved by adding reagent-grade $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$ to the nutrient solution. The nutrient solution was changed twice a week to ensure sufficient nutrients for plant growth and to keep solution Cu^{2+} levels close to the targeted levels.

Short-term study. Three weeks after the start of the treatment (10 Apr. 2003), four of the eight plants in each experimental unit were harvested to assess the effects of short-term copper application on the growth of cucumber plants. The remaining four plants in each experimental unit were left for cucumber production and a long-term growth study (another 7 weeks for a total of 10 weeks). At harvest, plant leaf number was determined before the plant was separated into leaves and stems. Leaf area of each plant was measured using a leaf area meter (LI-300; LI-COR, Inc., Lincoln, NE). All plant tissues were dried separately in a forced-air oven at 65 °C until dry weights remained constant.

Long-term study. On the first day of treatment (21 Mar. 2003), leaf greenness was measured on the last fully expanded leaf of four plants with a SPAD meter (Minolta SPAD-501, Osaka, Japan) and once a week on the same day until the end of the study. SPAD meters are commonly used for

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measuring leaf greenness, which is an indicator of chlorophyll content, and thus is used as a diagnosis tool for some environmental stress or nutrient deficiency (Blackmer and Schepers, 1995). Fruit was harvested when mature. At harvest, fruit length, diameter, and fresh weight were determined. The first fruit harvest was on 29 Apr. 2003 and the final fruit harvest was at the final harvest of the whole experiment on 29 May 2003. At the final harvest, plant leaf number, leaf area, and leaf and stem dry weights were measured as described in the short-term study.

During the entire experiment, all crop maintenance practices were consistent with those of Ontario greenhouse cucumber producers.

Statistics. 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 honestly significant difference or a linear regression analysis was performed after the log transformation of the dependent variable. Statistical analysis was conducted using SAS (Version 9.1; SAS Institute Inc., Cary, NC).

Results and Discussion

Visible injury. No visible symptoms of Cu^{2+} toxicity or nutrient deficiency were observed on leaves of any of the cucumber plants before 20 May 2003 (≈ 6 weeks after the start of Cu^{2+} treatments). Several plants under the $2.05 \text{ mg}\cdot\text{L}^{-1}$ Cu^{2+} treatment in two of the three blocks started to wilt on 20 May 2003. SPAD measurements also showed that Cu^{2+} treatments did not result in any change in leaf greenness until after Week 9 from the start of the treatments (Fig. 1). After 10 weeks of continuous Cu^{2+} application, leaf greenness was significantly reduced by the nutrient solution with a Cu^{2+} concentration of $2.05 \text{ mg}\cdot\text{L}^{-1}$ and was 86% lower than that of the control ($0.05 \text{ mg}\cdot\text{L}^{-1}$; Fig. 1). Our previous study showed that within 2 weeks after the start of Cu^{2+} treatment ($1.05 \text{ mg}\cdot\text{L}^{-1}$ or greater), young leaves of rockwool-grown young sweet pepper plants started to show chlorosis (Zheng et al., 2005). It suggests that rockwool-grown young cucumber plants are more resistant to Cu^{2+} toxicity than young sweet pepper plants. The plant wilting might have been the result of the root injury caused by the higher Cu^{2+} level in the fertigation solution. At the final harvest, brown roots were observed for plants fertigated with solutions containing 1.05 , 1.55 , and $2.05 \text{ mg}\cdot\text{L}^{-1}$ of Cu^{2+} . Our results confirmed that visible leaf symptom or greenness are not good indicators for Cu^{2+} phytotoxicity; visible root injury is more sensitive in this regard; and roots may be injured long before any aerial damage is evident at higher Cu^{2+} concentrations (Zheng et al., 2004, 2005). Other studies (Taylor and Foy, 1985; Zheng et al., 2004, 2005) also demonstrated that high Cu^{2+} caused root injury that increased with increasing Cu^{2+} levels. Chrysanthemum and geranium roots exposed to Cu^{2+} concentrations as low as $0.32 \text{ mg}\cdot\text{L}^{-1}$

Fig. 1. Leaf greenness (SPAD readings) of the most recent fully expanded leaves of cucumber plants (cv. LOGICA F1) measured at 1-week intervals after the start of the experiment. The 0.05 , 0.55 , 1.05 , 1.55 , and $2.05 \text{ mg}\cdot\text{L}^{-1}$ in the figure are the different copper (Cu^{2+}) concentrations. Each point represents the mean of three replications (12 subsamples) $\pm$ the SE of the mean. Points for the same day, bearing the same letter (beside the symbol) indicate that the means are not significantly different at the $P \leq 0.05$ level by Tukey's Studentized range (honestly significant difference) test.

had brown tips within 24 h of treatment, and miniature roses had brown tips after 1 week of treatment with $0.32 \text{ mg}\cdot\text{L}^{-1}$ (Zheng et al., 2004). Injured roots may then be more susceptible to root pathogens (Jarvis, 1992). Studies have also shown that Cu^{2+} can inhibit root elongation in taro (Hill et al., 2000), rice (Lidon and Henriques, 1992), corn (Ouzounidou et al., 1995), and other plant species (Ouzounidou et al., 1994).

Growth. The growth analysis showed that after 3 weeks of continuous Cu^{2+} application, plant leaf number (12 ± 0.1 per plant), leaf area ($4728 \pm 95 \text{ cm}^2/\text{plant}$), leaf dry weight ($11.9 \pm 0.28 \text{ g/plant}$), stem dry weight ($7.7 \pm 0.16 \text{ g/plant}$), and plant height ($102 \pm 1.8 \text{ cm}$) were not significantly ($P > 0.05$) affected by any of the Cu^{2+} treatments initiated when plants were 4 weeks old. However, after 10 weeks of continuous Cu^{2+} application, plant growth was significantly reduced by nutrient solutions with Cu^{2+} concentration $1.05 \text{ mg}\cdot\text{L}^{-1}$ or greater (Fig. 2). These results are consistent with visual leaf injury and SPAD results in which no Cu^{2+} treatment effects were observed during the first 6 weeks. These results indicate that Cu^{2+} toxicity is accumulative, and short-term (3 weeks) Cu^{2+} application may not have any effect on cucumber plant growth, but long-term (10-week) Cu^{2+} application can result in a decrease in plant growth.

Our previous study showed that plant growth attributes such as root length, root dry weight, leaf area, and total plant dry weight were reduced in chrysanthemum ($0.32 \text{ mg}\cdot\text{L}^{-1}$ or greater of Cu^{2+}), geranium ($0.25 \text{ mg}\cdot\text{L}^{-1}$ or greater of Cu^{2+}), and miniature

roses ($0.15 \text{ mg}\cdot\text{L}^{-1}$ or greater of Cu^{2+}) after only 4 weeks of Cu^{2+} application in solution culture (Zheng et al., 2004). Rockwool-grown young pepper seedling leaf number, leaf area, leaf biomass, specific leaf area, stem length, and shoot biomass were reduced by high Cu^{2+} concentrations ($0.55 \text{ mg}\cdot\text{L}^{-1}$ or greater) after 3 weeks of treatment, but 11-week-old pepper plants only showed reduction in leaf and shoot dry weights when the irrigation solution Cu^{2+} concentration reached $1.05 \text{ mg}\cdot\text{L}^{-1}$ when growth was measured after 18 weeks of treatment (Zheng et al., 2005). Differences in the results indicate that different plant species have different sensitivity to Cu^{2+} and plant sensitivity is also based on the age of the plant (Zheng et al., 2004). Other literature also indicates 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).

Cucumber fruit. After the first 2 weeks of fruit harvest, there was no difference in fruit number among Cu^{2+} treatments, but plants in the higher Cu^{2+} concentration treatments gradually started to produce fewer cucumbers than plants in the control ($0.05 \text{ mg}\cdot\text{L}^{-1}$; Fig. 3). The fruit number in the $2.05 \text{ mg}\cdot\text{L}^{-1}$ Cu^{2+} -treated plants began to have a significant decrease compared with the control 16 d after the initial harvest. At the end of the experiment, there was a 65% difference in the number of fruits produced by the control and the $2.05 \text{ mg}\cdot\text{L}^{-1}$ Cu^{2+} -treated plants. There were no differences in the four lower-level treatments.

The average fresh weight per cucumber and the fruit diameter of the $2.05 \text{ mg}\cdot\text{L}^{-1}$

Fig. 2. The responses of cucumber (cv. LOGICA F1) plants to long-term (10 weeks) application of copper (Cu^{2+}) at five different concentrations (0.05, 0.55, 1.05, 1.55, and 2.05 $\text{mg}\cdot\text{L}^{-1}$). Data represent the total harvested tissues including leaves removed during the experiment and at the end of the 10-week copper treatment. Data are the mean $\pm$ the SE of three replications (four subsamples in each replication). Bars bearing the same letter are not significantly different at the $P \leq 0.05$ level by Tukey's Studentized range (honestly significant difference) test.

Fig. 3. Accumulated number of cucumber (cv. LOGICA F1) fruits per plant on different days from the first harvest (29 Apr. 2003). Data are the mean $\pm$ the SE of three replications (four subsamples in each replication). Points for the same day, bearing the same letter (beside the symbol), indicate that the means are not significantly different at the $P \leq 0.05$ level by Tukey's Studentized range (honestly significant difference) test. Days with data points bearing no letter mean there is no treatment effect.

Cu^{2+} -treated plants were significantly ($P < 0.05$) lower than those of the other four treatments among which there were no significant differences; however, in terms of fruit dry weight (16.7 ± 0.41 g/cucumber) and length (34.1 ± 0.78 cm), there was no difference between the control and 2.05 $\text{mg}\cdot\text{L}^{-1}$ Cu^{2+} treatment (Fig. 4). In our previous study, Cu^{2+} treatments did not have any significant effect on numbers of ripe and green pepper fruit, fresh and dry weight of ripe and green fruit, and total fresh and dry

weights of fruit (Zheng et al., 2005). The lack of treatment effect in pepper fruit may be because copper treatments did not start until the plants were more than 11 weeks old and older plants are more resistant to copper toxicity (Zheng et al., 2005).

In conclusion, the rockwool-grown cucumber (LOGICA F1) plants were not as sensitive to nutrient solution Cu^{2+} toxicity as pepper plants (Zheng et al., 2005). There was no negative effect on cucumber plant growth under 3 weeks of continuous Cu^{2+} treatment,

Fig. 4. Fruit fresh weight and diameter of cucumber (cv. LOGICA F1) irrigated with five different copper (Cu^{2+}) concentrations (0.05, 0.55, 1.05, 1.55, and 2.05 $\text{mg}\cdot\text{L}^{-1}$). Data are the mean $\pm$ the SE of three replications (four subsamples in each replication). Bars bearing the same letter are not significantly different at the $P \leq 0.05$ level by Tukey's Studentized range (honestly significant difference) test.

even with Cu^{2+} concentrations as high as 2.05 $\text{mg}\cdot\text{L}^{-1}$, and at the early plant development stage. However, long-term (more than 9 weeks) continuous Cu^{2+} application caused cucumber leaf wilting, root browning, and slower growth when Cu^{2+} concentrations were 1.05 $\text{mg}\cdot\text{L}^{-1}$ or greater. There was no sign of negative Cu^{2+} effect on cucumber fruit numbers after the first 2 weeks of production, but plants under higher Cu^{2+} concentration treatment (2.05 $\text{mg}\cdot\text{L}^{-1}$) gradually produced fewer cucumber fruits than the control

(0.05 mg·L⁻¹) and eventually resulted in lower cucumber yield. Nutrient solution can be treated with 1.05 mg·L⁻¹ of Cu²⁺ in cucumber production greenhouses, but it is not recommended to use Cu²⁺ concentrations 1.05 mg·L⁻¹ or greater continuously for long-term (more than 3 weeks). When applying Cu²⁺, it is suggested that cucumber roots be examined regularly because roots are a better indicator for Cu²⁺ toxicity than leaf injury. If color change occurs in the root, the Cu²⁺ concentration is too high or a high Cu²⁺ concentration has been in the system too long.

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