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Article

Root morphology, growth, and physiology in Begonia (*Malus* × *micromalus*) grown in copper hydroxide containers

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Abstract

A major concern with container seedlings is root circling and deformation that will affect post-planting performance and stability. To improve root quality, 3-year-old Begonia (*Malus* × *micromalus*) plants were grown in the containers treated on interior surfaces with different concentrations of copper hydroxide ($Cu(OH)_2$) (0, 40, 80, 120, 160, and 200 g L^{-1}) for 1 year. Compared with the standard container control (SC) and carrier asphalt container control (AC), the number of terminal lateral roots and lateral root volume were increased by 21% and 13% at 80 and 120 g L^{-1} $Cu(OH)_2$ but decreased by 8% and 10% at 200 g L^{-1} $Cu(OH)_2$. Only 80 g L^{-1} $Cu(OH)_2$ increased the plant height and root weight, while other concentrations of $Cu(OH)_2$ resulted in the declines. Phosphorus and potassium were improved with lower concentrations of $Cu(OH)_2$ but decreased with 160 and 200 g L^{-1} $Cu(OH)_2$. No significant difference in the concentrations of soluble protein and sugars in leaves was observed between $Cu(OH)_2$ treatments and the controls. AC decreased nitrogen concentration in leaves by 12% over the SC across the whole growing season and increased taproot diameter by 17%. Our results indicate that 80 g L^{-1} $Cu(OH)_2$ was the optimum concentration for root pruning and the maintenance of physiological function. Disadvantages in growth and physiology gradually showed up with increased concentrations.

Key words: carrier, container, physiological function, root morphology, root pruning

Introduction

Container seedling quality is closely related to root form that subsequently affects nutrient uptake, stability, and toppling after transplanting. Seedlings can produce serious circling, matted, and kinked roots when grown in containers over time. Mechanical root pruning before transplanting might damage up to 80% root system (Codling et al. 2019). Copper (Cu)-treated containers have been used to reduce root deformation and spiraling to form a well-developed root system and improve seedling quality in many species (Arnold 1992; Ruter 1994; Dunn et al. 1997; Aldrete et al. 2002; Tsakaldimi and Ganatsas 2006; Sword-Sayer et al. 2009; Marchioretto et al. 2020). The theory of root pruning is that when root tips encounter high concentrations of Cu ions inside the container walls, cell division at the root apex and root elongation are inhibited. More lateral roots and root branching are created, which is beneficial to resume normal root growth and nutrient uptake after outplanting (Barnett and McGilvray 2002). Copper hydroxide [$Cu(OH)_2$], as a chemical root pruning reagent, has been widely used to improve the root quality of container-grown seedlings (Arnold et al. 1993; Aldrich et al. 1996; Pomper et al. 2002; Chang and Lin 2006; Marchioretto et al. 2020). A 100 g L^{-1} $Cu(OH)_2$ concentration successfully reduced root circling and spiraling in *Betula nigra*, *Taxodium distichum*, and *Platanus occidentalis* (Ruter 1994). The same concentration of $Cu(OH)_2$ increased root surface, plant height, and dry weight of fine roots (<2 mm in diameter) of *Alstonia scholaris* (Chang and Lin 2006). *Quercus shumardii* seedlings treated with 100 g L^{-1} $Cu(OH)_2$ regenerated more roots less than 1.0 mm in diameter and gained increases in root mass, height, and stem diameter after transplanting (Arnold 1996). *Pinus halepensis* seedlings grown in quickpots coated with 8.3 and 33 g L^{-1} $CuCO_3 \cdot Cu(OH)_2$ had significantly greater root quality, height, diameter, shoot, and root biomass (Tsakaldimi and Ganatsas 2006).

Seedlings with improved root quality have the potential to acquire nutrients and water more efficiently, resulting in increased plant growth (Aldrete et al. 2002). *Physalis peruviana* seedlings treated with 16–24 g L^{-1} $Cu(OH)_2$ had a better nutritional status with increased nitrogen (N), phosphorus (P), potassium (K), and Cu contents (Marchioretto et al. 2020). Arnold and Struve (1993) reported that seedlings grown in

$Cu(OH)_2$-treated containers had more Cu, N, calcium, magnesium, and zinc concentrations than nontreated seedlings. However, Cu treatment does not always produce beneficial effects on growth, biomass, and other physiological parameters although root spiraling was successfully controlled and root morphology was improved. For instance, 100 g L^{-1} $Cu(OH)_2$ did not affect the height of *B. nigra*, and the final height of *T. distichum* and *P. occidentalis* was reduced (Ruter 1994). The root and shoot dry weight of *Eucalyptus globulus* did not respond to 25 g L^{-1} $CuCO_3{\cdot}Cu(OH)_2$. The difference in root morphology caused by Cu treatment did not increase plant survival and growth 4 years after planting (Fernández et al. 2007). The seedling height, stem collar diameter, and shoot dry weight of *P. peruviana* reduced linearly with the increase in $Cu(OH)_2$ concentrations (Marchioretto et al. 2020). *Asimina triloba* seedlings did not benefit from the application of 100 g L^{-1} $Cu(OH)_2$ because the root dry weight and chlorophyll levels were decreased (Pomper et al. 2002). Therefore, when the root circling is controlled by a chemical root pruning reagent, the root function may not be improved, or even destroyed to some extent. Theoretically, the structure and function should be unified if the Cu concentration is appropriate and reasonable.

Previous studies rarely simultaneously compared the effects of a series of concentrations of Cu on the growth and physiology of container-grown seedlings, so it is possible to miss the optimal treatment concentration. The optimal concentration refers to not only improving root structure but also maintaining or enhancing physiological functions. Begonia (*Malus* × *micromalus*) is an important ornamental plant that is widely used in urban beautification and greening. In this study, we used six concentrations of $Cu(OH)_2$ to control the roots of *M.* × *micromalus*. The objectives of this study were (*i*) to screen and evaluate the appropriate root pruning $Cu(OH)_2$ concentration(s) through root structure, function, and leaf physiology, not just the root system appearance; and (*ii*) to elucidate the possible reasons of poor root pruning effects caused by higher or lower Cu concentrations.

Materials and methods

Plant materials

In April 2018, 175 (25 replications for each treatment) three-year-old *M.* × *micromalus* (Siping Nursery, Jilin, China) were planted into 3-gallon black straight-walled round plastic containers (55 cm in height, 1.5 cm × 1.5 cm drainage holes) containing two peat moss:one vermiculite:one perlite (*v*/*v*) media. Media was amended with a slow-release fertilizer (Aolv: total N 14%, NO_3^- 8.2%, NH_4^+ 5.8%, P 14%, and K 14%), 2 g per container. The initial height and root collar diameter were 122.0 cm and 26.1 mm, respectively. The bulk density, electrical conductivity, and pH values of the substrates were 0.31 g cm^{-3}, 0.39 mS cm^{-1}, and 6.1. All plants were hand-watered twice or thrice a week during the whole experimental period.

The experiment was carried out in the botanical garden of Shanghai Institute of Technology (30°92′N, 121°47′E), Shanghai, China. The annual mean air temperature (2010–2019) was 17.8 °C with the highest temperature in July and August of 29.4 °C and the lowest temperature in January of 5.7 °C (He et al. 2021). The average annual precipitation was 1132.9 mm (1971–2010) and the average increase rate was 50.9 mm $10a^{-1}$ (Fang et al. 2012).

Experimental design and measurements

The interior surfaces of containers were coated with six concentrations of $Cu(OH)_2$ (0, 40, 80, 120, 160, and 200 g L^{-1}) with asphalt paint as the carrier that was evenly sprayed to the inner walls of the containers (20 mg cm^{-2}, 0.05 mm in thickness). We set up two controls: standard container control without asphalt and $Cu(OH)_2$ (SC) and asphalt container control without $Cu(OH)_2$ (AC).

After 1 year treatment, the plant height (from the media surface to the highest bud on the plant) was measured monthly and the root collar diameter (above the media surface) was measured every 2 months from March to September. Mature and healthy leaves on the top of the plant canopy in different treatments were randomly selected for the measurements of physiological parameters. The nutrients such as N, P, and K, chlorophyll, soluble protein, and soluble sugars were measured in the middle of May, July, and September. The leaves and roots were dried at 80 °C to constant weight and then were ground into powder. The samples were heated in the presence of sulphuric acid at 380 °C until the solution was clear. Nitrogen was determined by the Kjeldahl method. The clear solution was distilled with the addition of concentrated sodium hydroxide (PEIOU SKD-800, China) and then was determined using titrimetric analysis. Phosphorus was analyzed using the molybdenum blue method. After adding dinitrophenol and molybdenum antimony into the solution, the mixture was measured at 700 nm wavelength. Potassium was determined by flame photometer (FP640) (Bao 2000). Dry leaf powder was extracted by 80% ethanol for 30 min, three times in total. The extract was measured using the anthrone method (Seifter et al. 1950). Crude protein was gained by grounding fresh leaves in distilled water. After centrifuging at 5000*g* for 10 min, supernatant was mixed with G-250 Coomassie Brillian Blue and then measured at 595 nm wavelength (Gao 2006). The branches, twigs, and leaves were collected to measure shoot weight at the end of the experiment, excluding the stem. Fresh leaves were used to measure chlorophyll and protein contents. Fresh leaves were cut into pieces and ground in 80% ethanol. After filtering, the absorbance of the extract was measured at 645 and 663 nm wavelength to calculate total chlorophyll concentrations (C_T) with the following formula (Arnon 1949).

$$C_T = 17.32A_{645} + 7.18A_{663}$$

Destructive sampling of roots was done at the end of the growing season after all above-ground measurements were finished. The entire roots were removed carefully from the containers and the soil on the roots was rinsed away carefully. The length of taproots and lateral roots were measured with a ruler. Taproot diameter was measured at the midpoint of the root length by a digital caliper (Arnold 1996). The volume of all roots and lateral roots was determined by water discharged by the roots. The number of terminal lateral roots was counted. The dry weight of shoots and roots was separately measured to calculate the ratios of shoots to roots after they were oven-dried at 70 °C to constant weight.

Statistical analysis

All statistical analyses reported in this paper were performed with SPSS 16.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA was used to analyze the significance of different concentrations of $Cu(OH)_2$ on nutrient concentrations, plant height, root collar diameter, leaf physiology, and root parameters. Duncan's multiple comparisons were used to examine the differences between groups.

Results

Effects of $Cu(OH)_2$ treatments on root morphology

Over 120 g L^{-1} $Cu(OH)_2$ decreased taproot length by 19% compared with the controls (Fig. 1*a*). The taproot diameter in the AC was 17% higher than that in the SC and all Cu treatments. There was no difference in taproot diameter among $Cu(OH)_2$ treatments (Fig. 1*b*). Compared with the controls, 80 and 120 g L^{-1} $Cu(OH)_2$ treatments led to a 21% increase in the number of terminal lateral roots and an average 61% enhancement in the sum of the lateral root length. The number of terminal lateral roots in 160 and 200 g L^{-1} $Cu(OH)_2$ treatments was decreased by 8% (Figs. 1*c* and 1*d*). The lateral root volume was increased by 13% in 80 and 120 g L^{-1} $Cu(OH)_2$ treatments but was decreased by 10% in 200 g L^{-1} $Cu(OH)_2$ treatment (Fig. 1*e*). The volume of all roots in 80 g L^{-1} $Cu(OH)_2$ treatment was significantly higher than other treatments (Fig. 1*f*).

Effects of $Cu(OH)_2$ treatments on growth and biomass

The growth of all plants increased rapidly from March to May, 6 cm per month (Fig. 2*a*). There was a significant difference in plant height between SC and AC ($P < 0.05$) and between $Cu(OH)_2$ treatments and AC ($P < 0.05$). The final plant height in September in the AC was 6% taller than that in the SC. The height in 160 and 200 g L^{-1} $Cu(OH)_2$ and SC was lower than others. $Cu(OH)_2$ treatment resulted in a decrease in height compared with the AC except for 80 g L^{-1} $Cu(OH)_2$. Compared with the SC, 80 g L^{-1} $Cu(OH)_2$ treatment increased the height by 4% and other treatments did not significantly affect the height.

The root collar diameters increased 1 mm every 2 months (Fig. 2*b*). No significant difference in root collar diameter was observed between the controls and the treatments ($P > 0.05$), but plants treated with 200 g L^{-1} $Cu(OH)_2$ had lower collar values than others ($P < 0.05$).

The dry weight of shoot (twigs and leaves) in 160 and 200 g L^{-1} $Cu(OH)_2$ treatments was 6% lower than that in other $Cu(OH)_2$ concentrations and the controls ($P < 0.05$) (Fig. 2*c*). $Cu(OH)_2$ treatment led to a significant decrease in root dry weight except 80 g L^{-1} concentration. Consequently, controls and 80 g L^{-1} $Cu(OH)_2$ treatment had the highest ratios of roots to shoots (Fig. 2*c*).

Effects of $Cu(OH)_2$ treatments on nutrients

No significant differences in the concentrations of N, P, and K in roots were observed between the controls and the $Cu(OH)_2$ treatments (Fig. 3). The average N, P, and K concentrations in roots were 3, 0.2, and 4 mg g^{-1} at the end of the growing season, respectively.

The significant seasonal fluctuation in the concentrations of N, P, and K in leaves was observed with the highest values in May for both controls and treatments (Fig. 4). The N in plants treated with 80 and 120 g L^{-1} $Cu(OH)_2$ was 17% higher than that in the AC in May ($P < 0.05$), but no difference was found in July and September except for those treated with 200 g L^{-1} $Cu(OH)_2$ (Fig. 4*a*). AC resulted in 12% decrease in N over the SC across the whole growing season (Fig. 4*a*). $Cu(OH)_2$ treatments significantly increased P concentrations in leaves in May except 200 g L^{-1} compared with the AC. P concentration in the AC was 18% lower than in the SC in May (Fig. 4*b*). Treatment of plants with 40–120 g L^{-1} $Cu(OH)_2$ increased K concentration by 14% compared with the AC across the whole growing season (Fig. 4*c*). The K concentrations in plants treated with 160 and 200 g L^{-1} $Cu(OH)_2$ were significantly lower than the controls and plants subjected to other $Cu(OH)_2$ treatments.

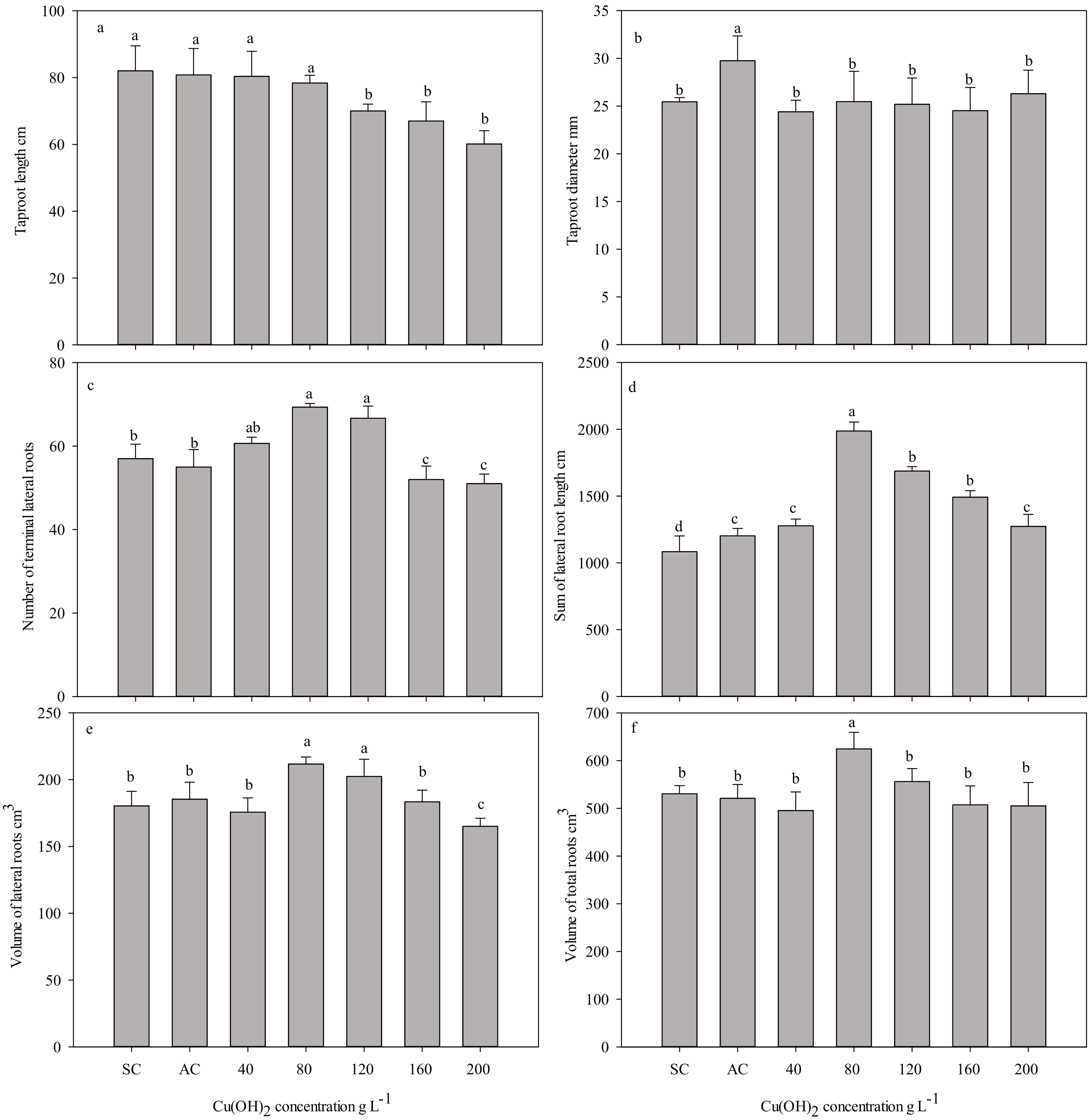
Effects of $Cu(OH)_2$ treatments on physiological characteristics

No significant difference in soluble protein concentration in leaves was observed between $Cu(OH)_2$ treatments and the controls (Fig. 5*a*). The average soluble protein concentrations were 29, 20, and 26 mg g^{-1} in May, July, and September, respectively. In July, $Cu(OH)_2$ treatments decreased chlorophyll concentration by 34%, but no significant differences found in May and September (Fig. 5*b*). There was no significant difference in the soluble sugars between the treatments and the controls (Fig. 5*c*). The soluble sugar concentrations ranged from 17 to 24 mg g^{-1} during the growing season.

Discussion

Cu is an essential microelement for plant growth and development. Plants fail to grow healthily without adequate Cu, but excess available Cu is toxic to plant growth and other physiological processes (Yruela 2005). Root circling was reduced by higher concentrations of Cu ions. However, if excess Cu was cumulated in roots and transferred to the aboveground part, the negative effects on physiological activities and growth would show. Li et al. (2019) reported that 300–500 μmol L^{-1} (excess) Cu directly damaged root function, thus inhibiting shoot growth of citrus. Thus, Cu treatment often yields positive and negative effects, which might relate to different combinations of containers, species, substrates, Cu forms and concentrations, etc. (Dumroese et al. 2013). If there was a negative effect of Cu, it was not the optimal concentration for the specific plants. The optimal concentration refers

Fig. 1. The taproot length (*a*), taproot diameter (*b*), number of terminal lateral roots (*c*), sum of lateral root length (*d*), volume of lateral roots (*e*), and total root volume (*f*) of container *Malus* × *micromalus* after 1 year $Cu(OH)_2$ treatments (mean ± SE, *n* = 5). Different letters indicate significant difference among treatments at the level of 0.05.



to not only controlling root deformation but also maintaining normal physiological functions.

Plant growth and root morphology

In our study, a series of concentrations of $Cu(OH)_2$, ranging from 40 to 200 g L^{-1}, were used. The lowest concentration of 40 g L^{-1} was too low to efficiently improve root morphology and growth of *M.* × *micromalus*. In contrast, 16–24 g L^{-1} $Cu(OH)_2$ decreased the root area, volume, and dry weight of *P. peruviana* (Marchioretto et al. 2020), suggesting that *P. peruviana* might be more sensitive to Cu than *M.* × *micromalus*. According to the morphology of roots, 80 and 120 g L^{-1} $Cu(OH)_2$ were the optimal concentrations for *M.* × *micromalus*, but only 80 g L^{-1} $Cu(OH)_2$ increased plant height and root dry weight. $Cu(OH)_2$ over 120 g L^{-1} decreased plant height compared with the controls, but root circling was still decreased and root appearance was good (pictures not shown). At 120 g L^{-1} $Cu(OH)_2$, *Pinus ponderosa* had finer and fibrous roots than the seedlings without Cu, but no significant differences were observed in height, root collar diameters, and total biomass values (Dumroese and Wenny 1997). The almost same results of no change in shoot height, dry weight, and stem diame-

Fig. 2. The plant height (*a*) and root collar diameter (*b*) of container *Malus* × *micromalus* treated with different concentrations of $Cu(OH)_2$ were measured during the growing season (mean ± SE, $n = 25$). The shoot dry weight (excluding stem), total root dry weight, and the ratio of root to shoot (*c*) were measured at the end of the growing season (mean ± SE, $n = 5$).

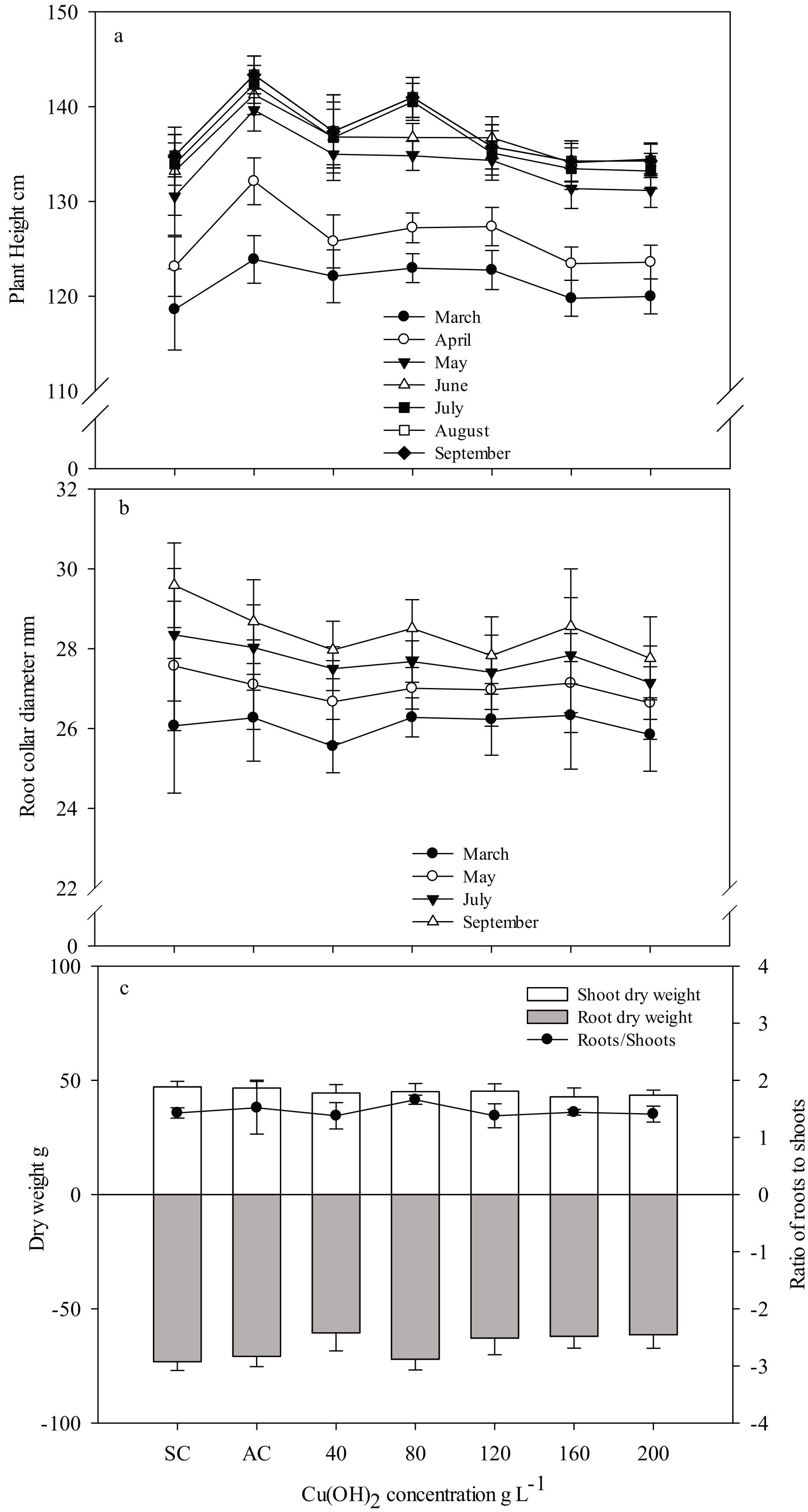
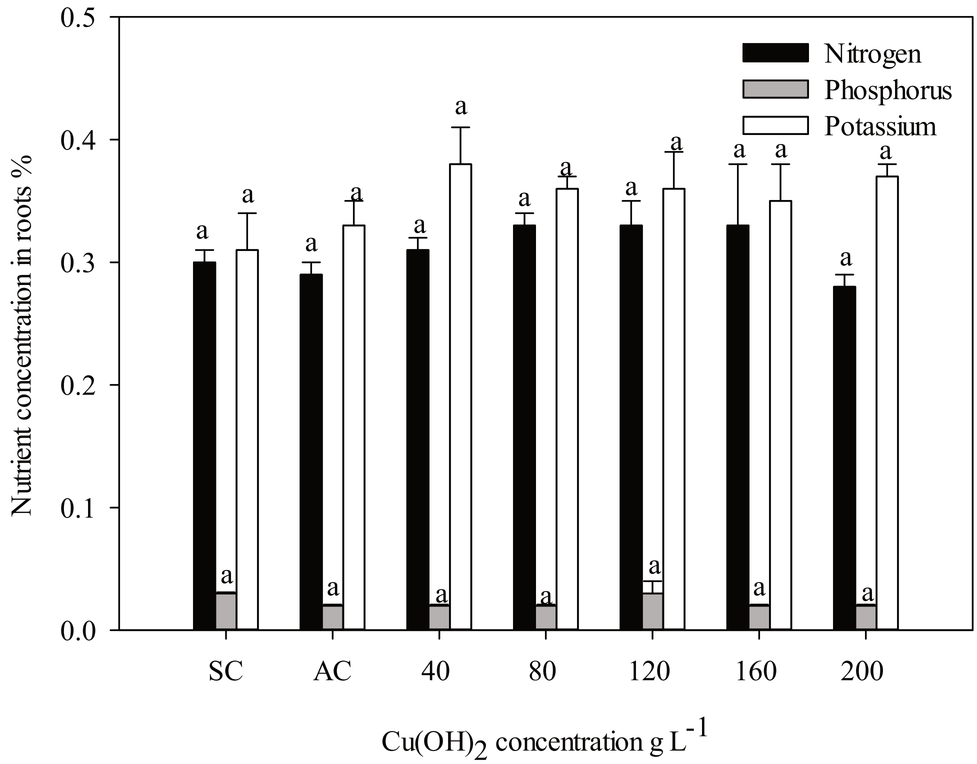


Fig. 3. The concentrations of nitrogen, phosphorus, and potassium in the roots of container *Malus* × *micromalus* treated with different concentrations of $Cu(OH)_2$ were measured at the end of the growing season (mean ± SE, $n = 5$). The same letters indicate no significant difference among treatments at the level of 0.05.



ter by 100 g L^{-1} $Cu(OH)_2$ treatment for rhododendron, river birch, sycamore, bald cypress, and weeping willow were observed (Ruter 1994; Svenson 2002). For *M.* × *micromalus*, 80 g L^{-1} was better than 120 g L^{-1} if growth and biomass were simultaneously considered. Similar results were also reported in *Pinus* sp. whose shoot dry weight and root collar diameter were increased (Aldrete et al. 2002; Tsakaldimi and Ganatsas 2006).

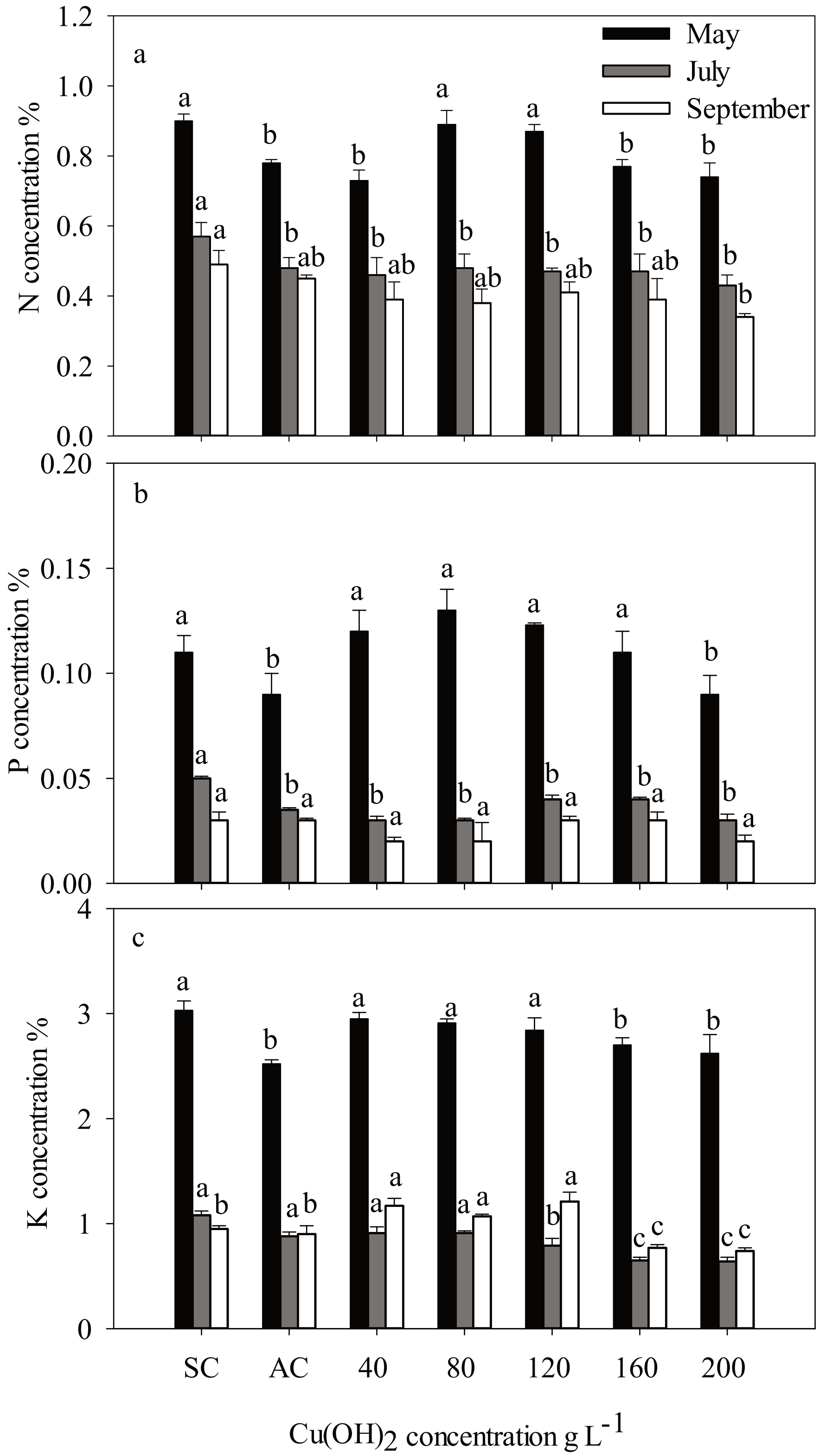
In our study, $Cu(OH)_2$ treatment did not significantly affect the volume of all roots except 80 g L^{-1} $Cu(OH)_2$. The responses of root volume to Cu treatment are complicated. Cu-treated container seedlings generally produced more finer and fibrous roots, but their volume or weight might be small. The spiraled roots accounted for 33% greater root volume for *Ponderosa* pine and 13% for *Pinus palustris* (Dumroese and Wenny 1997; Dumroese et al. 2013). The increased root volume at 80 g L^{-1} $Cu(OH)_2$ was mainly attributed to the enhancement in the lateral root volume, which is beneficial for water and nutrient uptake.

Our results suggest that 200 g L^{-1} $Cu(OH)_2$ was too high to produce beneficial effects. Reducing or eliminating root spiraling with Cu exceeding the appropriate concentration will damage part of root functions. For *M.* × *micromalus*, 80 g L^{-1} was the ideal concentration of $Cu(OH)_2$. Pardos et al. (2001) also found that 80 g L^{-1} copper carbonate-treated container effectively prevented root deformation in *Quercus suber* and increased lateral root branching frequency. Because of more lateral roots at 120 g L^{-1} $Cu(OH)_2$ concentration, we predict that these plants have the potential to grow fast after outplanting although we saw no significantly increased growth in containers. Haywood et al. (2012) reported that increased height and volume of seedlings grown in Cu-treated containers were observed 5 years after outplanting, without any short-term benefit (South et al. 2005; Sung et al. 2010).

Physiological responses

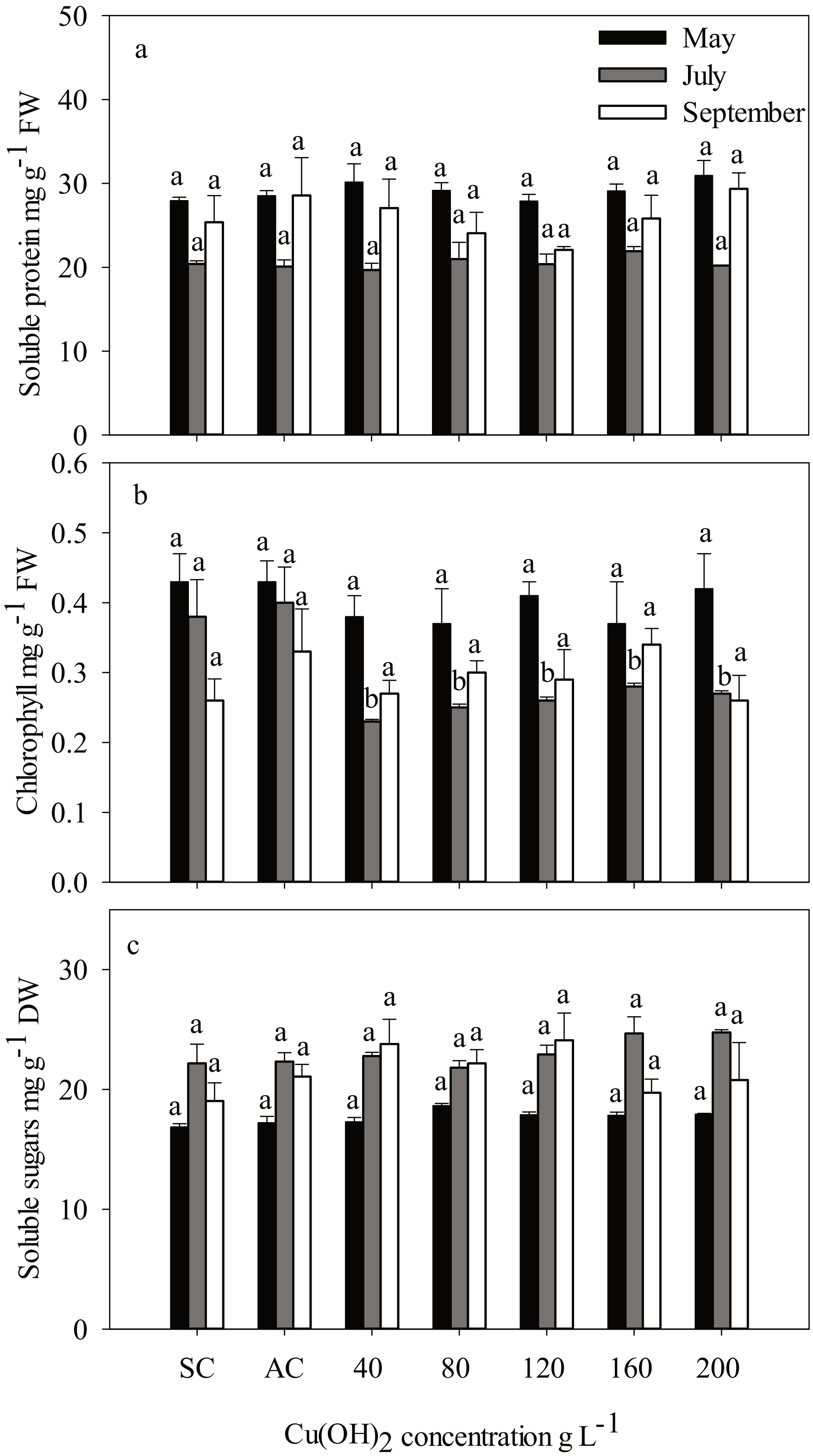
The main function of roots is to uptake water and mineral nutrients. Few studies reported the nutrient status and other physiological parameters of chemical root pruning of container seedlings. The growth advantage after transplanting is not only related to root morphology but also related to root function. The nutrient uptake status of roots was often inferred through fine root morphology and plant growth instead of directly measuring N, P, and K contents in leaves and roots in previous studies. High levels of Cu in roots can hamper nutrient uptake, resulting in nutrient deficiency (Festa and Thiele 2011). In the present study, $Cu(OH)_2$ treatment did not affect N contents; K was decreased in plants treated with 160 and 200 g L^{-1} $Cu(OH)_2$; P was declined in plants treated with 200 g L^{-1} when compared with the AC across the whole growing season. Mineral nutrient uptake and accumulation were affected when $Cu(OH)_2$ concentration was over 160 g L^{-1} for *M.* × *micromalus*. A recent study also proved that Cu stress led to a decrease in calcium, P, and K contents in roots and stem (Es-sbihi et al. 2020). The N, P, and K at 80 g L^{-1} $Cu(OH)_2$

Fig. 4. The concentrations of nitrogen (*a*), phosphorus (*b*), and potassium (*c*) in the leaves of container *Malus* × *micromalus* treated with different concentrations of $Cu(OH)_2$ were measured in May, July, and September (mean ± SE, $n = 5$). Different letters indicate significant differences among treatments in each month at the level of 0.05.



concentration were higher in our study. The leaf nutrient status is consistent with the root morphology to some extent. Liu et al. (2016) reported that Cu treatment decreased N and P concentrations in the stem and roots of container-grown *Quercus variabili*. The contents of N, P, and K in *P. peruviana* leaves were improved by 16–24 g L^{-1} $Cu(OH)_2$ (Marchioretto et al. 2020). Cu first accumulates in roots and then upward transports to shoots and foliage. Therefore, the Cu content

Fig. 5. The concentrations of soluble protein (*a*), total chlorophyll (*b*), and soluble sugars (*c*) of leaves of container *Malus* × *micromalus* treated with different concentrations of $Cu(OH)_2$ were measured in May, July, and September (mean ± SE, $n = 5$). Different letters indicate significant differences among treatments in each month at the level of 0.05.



in roots is higher than that in shoots and foliage (Carvalho et al. 2006; Kopittke and Menzies 2006; Bernal et al. 2007; Fernández et al. 2007; Codling et al. 2019; Yusefi-Tanha et al. 2020). If the Cu concentration in shoots and foliage is within the acceptable range, their physiological function might not be affected.

We concluded that the optimal concentration of $Cu(OH)_2$ for container *M.* × *micromalus* was 80 g L^{-1}, while 120 g L^{-1}

would be acceptable. Many previous studies used 100 g L^{-1} Cu ions at which root circling was controlled. The threshold to Cu toxicity is species specific, and also relating to plant age and container types. Low concentrations (such as 40 g L^{-1} $Cu(OH)_2$ in the present study) are not enough to exert effects, while high concentrations (such as 200 g L^{-1} $Cu(OH)_2$) cause negative impacts.

Cu ions are coated to the interior walls of the container with a carrier. Latex paint has been widely used as a carrier of Cu compounds (Arnold 1992; Armitage and Gross 1996; Chang and Lin 2006; Marchioretto et al. 2020). We tried several kinds of latex paints, whose adhesion effects were not always satisfactory. In this study, we used asphalt as the carrier that has better adhesion with the container than latex paint and will not be washed away. The effects of carrier, including latex paint, on root system were rarely studied. We found that AC increased the taproot diameter and decreased N compared with the SC. Wenny et al. (1988) mentioned in their conclusion that white latex paint had little or no effect on root and shoot growth of *Ponderosa* pine, western white pine, and Douglas fir. As chemical carriers, latex paint and asphalt may affect root tip growth. The root circling along the container walls and the effects of $Cu(OH)_2$ and carrier on root pruning are shown in Fig. S1.

Conclusions

In the present study, we compared the root structure, physiological parameters, and growth of container-grown *M.* × *micromalus* treated with different concentrations of $Cu(OH)_2$ and the controls. We found that the carrier had some effects on container-grown seedlings, which was ignored in previous studies. It is uncertain whether carriers react with Cu compounds, which deserves to be further studied in the future. The root entanglement could be efficiently alleviated when $Cu(OH)_2$ concentration was over 80 g L^{-1}, but only 80 g L^{-1} $Cu(OH)_2$ increased plant height and root dry weight. The negative effects of excess Cu ions increased with increase in $Cu(OH)_2$ concentration although apparently root circling was inhibited. In addition, the P and K absorption via roots was reduced when $Cu(OH)_2$ exceeded 160 g L^{-1}, indicating root function has been damaged. To sum up, the appropriate concentration of root pruning agent should ensure that the root function is not affected.

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Data available upon request.

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Investigation: DL, ZY, YL
Project administration: YZ
Supervision: WW
Writing – original draft: YZ
Writing – review & editing: YZ

Competing interests

The authors declare there are no competing interests.

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Supplementary material

Supplementary data are available with the article at https://doi.org/10.1139/CJPS-2022-0090.

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