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# Impacts of Copper Leaching From Copper Hydroxide-treated Containers on Water Recycling, Nursery Runoff, and Growth of Baldcypress and Corn

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ABSTRACT. **Studies were conducted using *Zea mays* L. and *Taxodium distichum* L. seedlings as model systems to study Cu leaching from $Cu(OH)_2$-treated containers. Initial experiments developed Cu toxicity curves (as $CuSO_4$) in an inorganic (sand) or organic (bark–sand) medium with single (acute) or multiple (chronic) applications. A second pair of experiments investigated short-term (35 days) Cu accumulation and plant responses to irrigation with water (125 mL/plant per day) recycled through a fixed reservoir volume (9.5 L) from 0.7-L $Cu(OH)_2$-treated containers filled with an inorganic or organic medium. Finally, plant responses and Cu leaching were monitored during growth in 2.3-L $Cu(OH)_2$-treated containers filled with two organic media fertigated with high (8.0) or low (6.5) pH solutions. Different $Cu(OH)_2$ concentrations and application methods were tested. Leachate data from the latter studies were used to calculate potential Cu concentrations in nursery runoff using various water application methods and pot spacings. Expression of Cu toxicity symptoms depended on exposure, concentration, and medium for each species. Plants subjected to chronic exposure and grown in an inorganic medium developed toxicity symptoms at lower doses than plants subjected to acute exposure and grown in an organic medium. Several measures of plant growth were greater for both species when grown in 0.7-L $Cu(OH)_2$-treated containers, but not in 2.3-L containers. Plants in $Cu(OH)_2$-treated containers seldom exhibited Cu toxicity symptoms in shoot tissues, even with an inorganic medium. Soluble Cu content of the recycled solution from Spin Out-treated containers increased slightly (<1.2 $mg \cdot L^{-1}$) during the 35-day experiment. Longer-term studies with nonrecycled leachate from 2.3-L containers indicated that Cu leaching increased after 60 to 90 days. Copper leaching was greater with the combination of applied solution of pH 6.5 and bark–sand–peat medium than with the combination of applied solution of pH 8.0 and bark–sand medium, and increased with greater concentrations of $Cu(OH)_2$ in container wall treatments or when containers were filled before latex carrier was dried. Calculations of potential nursery runoff indicated that the levels of soluble Cu in effluent for most concentrations and spacings projected were below EPA action levels for potable water (1.3 $mg \cdot L^{-1}$) when overhead irrigation was used.**

Copper-treated containers are used to reduce the development of kinked, matted, and spiraled roots at the container wall–medium interface (Appleton, 1993; Struve et al., 1994). A labeled product, Spin Out (Griffin Corp., Valdosta, Ga.), containing $Cu(OH)_2$ at 100 $g \cdot L^{-1}$ latex carrier is commercially available. While much is known concerning the efficacy of Cu-treated containers in altering root morphology and plant growth during woody plant production (Arnold and Struve, 1989a, 1989b, 1993; Beeson and Newton, 1992; Struve et al., 1994), no published information is available on the potential for leaching of Cu from treated containers or the potential for Cu accumulation in irrigation water that is recycled from treated containers.

The potential for Cu to leach from $Cu(OH)_2$-treated containers is of interest for several reasons. Retention and reuse of irrigation water to reduce freshwater consumption or nursery runoff has increased [U.S. Environmental Protection Agency (EPA), 1992; Skimina, 1996; Wilkerson, 1996]. Capture and reuse of nursery runoff is one strategy to reduce the risk of surface-water contamination from applied nutrients and pesticides (Skimina, 1996; Wilkerson, 1996). With recycling, the potential for Cu to accumulate to phytotoxic levels could be a concern. Copper uptake and concentrations that induce toxicity symptoms in plants vary greatly with species (Cataldo and Wildung, 1978; Heale and Ormrod, 1982), medium or soil type (Mengel and Kirkby, 1982), pH (Allan and Jarrell, 1989), and copper formulation (Arnold et al., 1994). Copper leaching from treated containers would also be of interest if runoff water from nurseries is discharged from the site. Concentrations of nutrients in discharge water must meet state and federal water-quality standards. These standards are based on EPA guidelines, but state standards may be more stringent than EPA guidelines. The maximum Cu contaminant level in potable water (action level), or concentration at which remediation efforts are required, in Texas is 1.3 $mg \cdot L^{-1}$ (EPA, 1995). The allowable Cu concentration in discharge (runoff) water depends on the volume, flow rate, and calcium carbonate concentration of the receiving body (Texas Water Commission, 1991), thus varying with location.

The objectives of these experiments were to A) determine phytotoxic concentrations of Cu in solution in root zones of corn and baldcypress (Expt. 1), B) determine the concentration and potential phytotoxicity of Cu that might be eluted from $Cu(OH)_2$-treated container walls and accumulated in a recycling reservoir

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(Expt. 2), C) determine the pattern of copper leaching in container effluent over time (Expt. 3), D) determine differences in leaching of Cu from wet coatings compared with dried coatings (Expt. 4), E) determine effects of various copper applications on root deflection (Expts. 2, 3, and 4), and F) estimate Cu concentration in surface runoff from nurseries with various copper application methods and different container spacings (modeling using data from Expts. 3 and 4).

## Materials and Methods

All experiments were conducted in a greenhouse with 33/17 °C max/min temperatures, as recorded with a mini-drum hygrothermograph (Oakton model 08369-70; Cole-Parmer Instrument Co., Vernon Hills, Ill.), and natural photoperiods interrupted from 00:00 to 04:00 HR with incandescent light (100-W bulbs on 1-m centers suspended 0.7 m above the containers). Corn (*Zea mays*) and baldcypress (*Taxodium distichum*) were chosen as contrasting model species; *Z. mays* is a rapidly growing annual monocot with a vigorous adventitious root system, whereas *T. distichum* is deciduous conifer with a coarse taproot system, maintains a vigorous central leader, and is an important landscape species over much of North America. *Zea mays* seeds (Pioneer hybrid 3192, courtesy S. Voss, Pioneer Hi-Bred Intl. Inc., Johnston, Iowa) used in the following studies were planted 2 cm deep near the center of the containers. The first corn seedling to emerge from the media was retained, the rest were removed at emergence. *Taxodium distichum* seeds (K&S Jeane Seed, Inc., Quitman, La.) were moist stratified (4 °C, 4 months), germinated in flats containing coarse builders sand or 3 milled pine bark : 1 sand (v/v), and transplanted to individual containers (as specified below) when one to two true leaves were present.

**PHYTOTOXICITY OF CU IN APPLIED SOLUTIONS (EXPT. 1).** Effects of acute and chronic exposures to increased concentrations of Cu in irrigation water during growth of corn and baldcypress in two contrasting media were studied to characterize growth and toxicity responses for comparison with plant responses observed in $Cu(OH)_2$-treated container experiments. A completely random factorial design with 2 media × 5 Cu concentrations × 2 exposure levels × 5 replications was used for each species. On 31 May 1993, baldcypress seedlings were transplanted to 0.7-L black plastic containers (TLC Polyform Inc., Moorow, Ga.) filled with a coarse builders sand or a 3 milled pine bark : 1 sand (v/v) medium. On an adjacent bench, *Zea mays* seeds were planted in identical containers and media. Seedlings of each species were irrigated daily with 125 mL of fertilizer solution [distilled water plus N at 100 $mg \cdot L^{-1}$ from a 20N–8.7P–16.6K water soluble fertilizer (Peters, Scotts Co., Marysville, Ohio)], at a pH adjusted to 6.5 with NaOH and HCl, spiked with sufficient $CuSO_4 \cdot 5H_2O$ to produce Cu solutions of 0.018 (Cu from base fertilizer only, no additional Cu added), 5, 10, 100, or 1000 $mg \cdot L^{-1}$. Half of the seedlings of each species and medium type were irrigated with the various Cu treatments only at planting (acute exposure) and thereafter with the base fertilizer solution. The remaining seedlings received Cu-treated solutions at each irrigation (chronic exposure). Height, stem diameter (2 cm from media surface), leaf number, presence of chlorosis or necrosis, and survival were recorded weekly. Leaf, stem, and root dry mass and the presence of Cu toxicity symptoms on the roots were determined after 28 d for corn and 35 d for baldcypress.

Despite the high water solubility (pH 7.0) of $CuSO_4 \cdot 5H_2O$ at 316,000 $mg \cdot L^{-1}$ (Weast, 1975), a white precipitate formed after a few days in the 100 and 1000 $mg \cdot L^{-1}$ pH 8.0 Cu solutions and the 1000 $mg \cdot L^{-1}$ pH 6.5 Cu solution. Most of this material would disperse with agitation. To minimize this problem during the rest of the study, new solutions were mixed frequently to avoid precipitate formation.

**RECYCLED IRRIGATION WATER FROM CU-TREATED CONTAINERS (EXPT. 2).** These experiments were conducted to determine the short-term effects of recycling container leachate from $Cu(OH)_2$-treated containers with a fixed volume of bulk irrigation water. This is analogous to irrigation from a fixed-volume holding tank in a nursery. A completely random factorial design with 2 media × 3 $Cu(OH)_2$ concentrations × 5 replications was used for each species. Containers (0.7-L, TLC Polyform Inc., Moorow, Ga.) were painted on interior surfaces with $Cu(OH)_2$ at 0, 100, or 200 $g \cdot L^{-1}$ of latex carrier formulated as Spin Out. Five seedlings of each container treatment and species (*Z. mays* and *T. distichum*) combination were grown in coarse builders sand or in a 3 milled pine bark : 1 sand medium. Six 9.5-L reservoirs were filled with the base fertilizer solution. Five plants of each species, medium, and container treatment combination were watered daily with 125 mL of water from the reservoirs. Leachate from plants grown in each medium and container treatment (10 plants) were pooled across species and returned to their respective reservoirs. The reservoirs were then brought to capacity with the base fertilizer solution at pH 6.5. With daily irrigation, this represented more than four passes of the bulk solution volume through the container medium. A 60-mL water sample was drawn from each reservoir after 0, 7, 14, 21, 28, and 35 d and analyzed for soluble Cu concentration. At 7, 14, 21, 28, and 35 d, a pooled 60-mL sample of leachate from each medium and container treatment combination was also analyzed for soluble Cu concentration. Similar growth and toxicity measures were recorded as described in Expt. 1.

**MEDIA AND FERTIGATION PH AND CONTAINER WALL TREATMENT EFFECTS ON CU LEACHING (EXPT. 3).** These studies were conducted to estimate the potential for Cu to leach from $Cu(OH)_2$-treated containers coated on interior surfaces with various levels of $Cu(OH)_2$ containing polymers and varying in medium composition and fertigation pH. Experiments 3, 4, and 5 were conducted concurrently using common control treatments. *Zea mays* and *T. distichum* seedlings were grown for 90 and 120 d, respectively, in 2.3-L black plastic nursery containers (Lerio Corp., Mobile, Ala.) on 0.5-m spacings in a greenhouse as previously described. Before planting, containers were painted on interior surfaces with a latex polymer containing $Cu(OH)_2$ at 100, 200, or 300 $g \cdot L^{-1}$ (SpinOut) or not painted. An average of 5.49 g of dried polymer was applied per container. Medium was added after the latex dried. Two medium–fertigation regimes were tested with each species and container wall treatment. One treatment was a 3 milled pine bark : 1 sand (v/v) medium amended with 2.4 $kg \cdot m^{-3}$ lime (Vulcan Materials Co., Tarrant, Ala.), 1.2 $kg \cdot m^{-3}$ gypsum (Standard Gypsum Corp., Fredericksburg, Texas), 4.7 $kg \cdot m^{-3}$ 18N–3.1P–8.3K controlled-release fertilizer (Sierablen, Scotts Co., Marysville, Ohio), and 0.88 $kg \cdot m^{-3}$ trace elements (Micromax, Scotts Co.), and plants subsequently irrigated with 300 mL of fertilizer solution [distilled water plus N at 100 $mg \cdot L^{-1}$ from a 20N–8.7P–16.6K water soluble fertilizer (Peters, Scotts Co.)], pH adjusted to 8.0 with NaOH and HCl. The second medium–irrigation treatment was a 3 milled pine bark : 1 sand : 1 sphagnum peat medium amended with 1.2 $kg \cdot m^{-3}$ dolomite, 2.4 $kg \cdot m^{-3}$ gypsum, 4.7 $kg \cdot m^{-3}$ 18N–3.1P–8.3K controlled-release fertilizer, and 0.88 $kg \cdot m^{-3}$ trace elements and plants subsequently irrigated with 300 mL of fertilizer solution (distilled water plus N at 100 $mg \cdot L^{-1}$ from 20N–8.7P–16.6K), pH adjusted to 6.5 with NaOH and HCl.

Each plant was irrigated with 300 mL of the appropriate solution each day between 08:00 and 10:00 HR through the first month of growth. Thereafter, if needed, a second daily irrigation was applied between 02:00 and 04:00 HR. Media cores were extracted from each dry $Cu(OH)_2$-treated container four times during the experiment and pooled by medium type. Three subsamples were used to determine medium pH via a saturated paste method. A final medium pH was determined for each container at harvest. Plant growth and toxicity responses were measured as described for toxicity studies. Efficacy of $Cu(OH)_2$-treated containers to prevent root deflection at container wall–medium surfaces was evaluated on a 1 to 5 rating scale: 1 = no apparent reduction in root elongation following contact with container walls, numerous deflected roots on side walls and matted roots at bottom of rootball; 2 = several roots on side walls elongating > 1 cm after contacting treated side walls and circling or matted roots present at container bottom; 3 = several roots at side walls and bottom, elongating after contact; 4 = escaped roots confined to bottom of the rootball surface; and 5 = few to no roots elongating more than 1 cm after contacting container surfaces.

Nonrecycled container leachate was collected using modified pour-through extraction (Yeager et al., 1983) immediately after planting, and at 7, 30, 60, 90, and 120 d as follows: all plants were irrigated with their respective solutions on the afternoon before sampling; the following morning, 300 mL of distilled water was applied to the media surface of each container and allowed to drain for 20 min into plastic saucers; additional aliquots of 50 mL distilled water were added until collected leachate equaled or exceed 50 mL. Leachate samples were placed in 60-mL opaque Nalgene bottles (Nagle Co., Rochester, N.Y.), coded to conceal treatments during analysis and sent by priority air freight to the Griffin Corp. Biological Laboratory (Valdosta, Ga.) for Cu determinations. Each sample was analyzed for soluble Cu. One sample from each treatment combination and samples from the bulk fertilizer solutions were analyzed for total Cu. The difference between applied and collected volumes of solution for five containers of each medium type were used to calculate leaching fraction, 37.3%. Data were analyzed separately for each species as a completely randomized factorial design; 2 medium–fertigation pH combinations × 4 dry $Cu(OH)_2$ container wall treatments × 5 replications × 6 sample dates for *T. distichum* or 5 sample dates for *Z. mays*. Destructive harvest measures were conducted only on the final sample date.

**ELIMINATION OF DRYING STEP AND CU LEACHING (EXPT. 4).** Five containers for each species and medium treatment were painted with $Cu(OH)_2$ at 100 $g \cdot L^{-1}$ polymer carrier immediately before filling with medium and planting seedlings to determine the effects of eliminating the drying phase of $Cu(OH)_2$ applications. Wet paint remained a liquid to moist paste-like consistency throughout the experimental period (120 d), drying fully only where exposed to ambient atmosphere, predominantly above the container media. Plants in these containers were compared to those treated with $Cu(OH)_2$ at 100 $g \cdot L^{-1}$ and allowed to dry before potting. Data collection was as described in Expt. 3. Data were analyzed separately for each species as a completely randomized factorial design; 2 medium–fertigation pH combinations × 2 wet/dry container wall treatments × 5 replications × 6 sample dates for *T. distichum* or 5 sample dates for *Z. mays*.

**SPIKED CU FERTIGATION (EXPT. 5).** Ten seedlings of each species were grown in nontreated containers, five in each medium–fertigation pH combination as described in Expt. 3, but with the addition of Cu (from $CuSO_4$) solution at 5 $mg \cdot L^{-1}$ (spiked samples). Results of these treatments were compared with data from seedlings grown similarly, but with Cu only at the base level contained in the fertigation treatments (0.018 $mg \cdot L^{-1}$). Data collection was as described in Expt. 3. Data were analyzed separately for each species as a completely randomized factorial design; 2 medium–fertigation pH combinations × 2 Cu spike treatments × 5 replications × 6 sample dates for *T. distichum* or 5 sample dates for *Z. mays*.

**CALCULATION OF POTENTIAL CU CONCENTRATIONS IN NURSERY RUNOFF.** Measured Cu concentrations in leachate may represent levels obtained using drip or mini-spray stake irrigation where all water is applied directly to the container medium surface with no loss due to water falling outside the container, as typically occurs with overhead applications. Leachate data from Expts. 3 and 4 were used to calculate theoretical concentrations of Cu in nursery runoff water based on the use of overhead irrigation and 0.16- (pot-to-pot, container diameter = 16 cm), 0.3-, and 0.6-m centers on a square pattern. Calculations assumed are as follows: 1) 300 mL of water reached each container surface (1.49 $ml \cdot cm^{-2}$) per irrigation event; 2) irrigation water contained Cu at 0.018 $mg \cdot L^{-1}$; 3) there was no influence of plant canopy on the distribution of water reaching the container surfaces; 4) there was uniform distribution of water over the containers; 5) there was no binding of Cu on soil, gravel, or other underlying substrates below the containers or in water diversion pathways; and 6) there was uniform leaching volumes and Cu concentrations among containers within the same treatment combination. These six conditions represent an extreme situation in which the greatest runoff of Cu-contaminated water would occur. Contact of effluent with plants, soil, or organic matter before leaving the site might remove Cu from the runoff resulting in lower Cu concentrations; thus, calculated values should be interpreted only as relative comparisons among container treatments. Theoretical runoff concentrations of Cu = [(Cu concentration in leachate × volume of leachate) + (0.018 $mg \cdot L^{-1}$ of Cu × the volume of water falling outside the containers (calculated as the proportion of the irrigated surface area not covered by containers for a given spacing × the total volume of water applied/unit surface area irrigated))]/(leachate volume + volume of water falling outside the containers). Mean leachate volume collected per container was 112 mL.

Twelve replicate 60-mL samples were collected in Spring 1996 from the concrete retention tank for runoff from a 878-$m^2$ container nursery to provide a comparison for calculated runoff values. The nursery contained about 3000 plants growing in 2.3- to 11.8-L $Cu(OH)_2$-treated containers and an additional number of plants in nontreated containers, all irrigated (pH 6.5 to 6.7) with mini-spray stakes. Underlying nursery soil was covered with 6-mm plastic sheeting covered with about 10 cm of river gravel with diameter $\leq 5$ cm. Six of the samples were drawn from runoff collected following irrigation applications, while the remaining six samples were collected following a 4-cm rain event. Samples were analyzed for soluble and total Cu as previously described for leachate samples.

Analyses were determined separately for *Z. mays* and *T. distichum* in all experiments. An analysis of variance was performed using the general linear models procedures in SAS (SAS Institute Inc., Cary, N.C.) to determine the significance of interactions and main effects in each experiment. Significant ($P \leq 0.05$) higher-order interactions and significant lower-order interactions and main effects, not involved in significant higher-order interactions, were further analyzed. Growth characteristics (height, stem diameter, leaf number, and dry tissue mass) were analyzed using expected least squares means procedures, if qualitative in nature, or using polynomial regression procedures, if quantitative in nature. Frequency data (development of foliar chlorosis, tissue necrosis, and survival) were analyzed using a modified chi-square test, for smaller sample sizes.

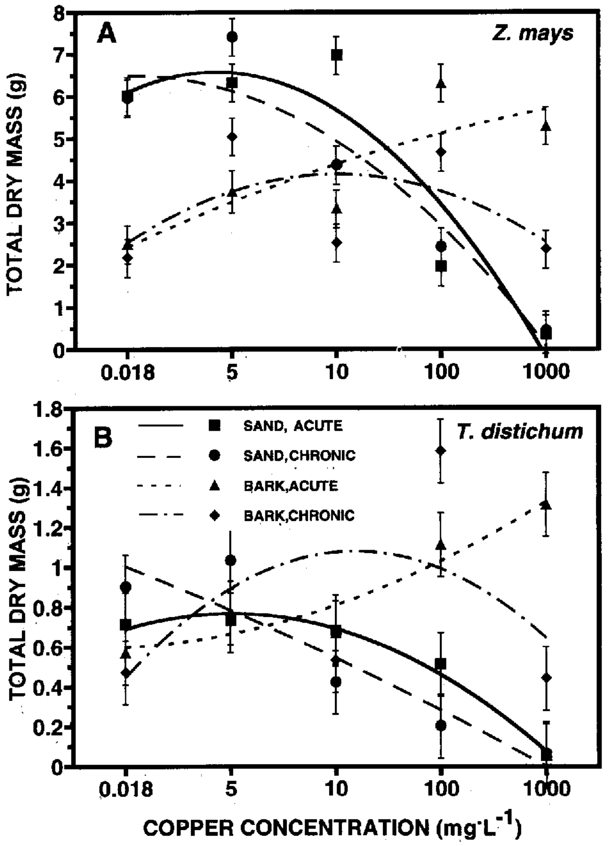


Fig. 1. Total plant dry matter accumulation of *Zea mays* (A) during 28 d and *Taxodium distichum* (B) during 35 d in response to acute (■, ▲) or chronic (●, ◆) applications of various Cu concentrations when grown in 0.7-L containers filled with a sand (■, ●) or pine bark–sand (▲, ◆) medium. Symbols represent means ± standards errors, n = 5. Lines are second-order polynomial regression equations for medium and exposure combinations over Cu concentrations; $R^2$ for *Z. mays* (A) is 0.30 to 0.91 and for *T. distichum* (B) is 0.28 to 0.98, $P \leq 0.05$.

## Results and Discussion

PHYTOTOXICITY OF CU IN APPLIED SOLUTIONS (EXPT. 1). Significant ($P \leq 0.05$) interactions among medium type and concentration of Cu application were observed for nearly all growth and toxicity symptom characteristics. Total plant dry mass and the presence of chlorotic leaf tissue were selected as being representative of growth responses (Fig. 1) and toxicity symptoms (Fig. 2), respectively. There were significant interactions of copper concentration in applied solution and medium type on plant growth and foliar chlorosis (Figs. 1 and 2). With sand medium, chronic or acute applications above 10 mg·L$^{-1}$ of Cu significantly reduced plant growth, severely stunting or killing plants with Cu at 100 to 1000 mg·L$^{-1}$. In a similar study, growth reductions or toxicity symptoms were reported on four woody plant species following 110 d of exposure in solution culture to Cu at 4 to 20 mg·L$^{-1}$ (Heale and Ormrod, 1982). Growth of *T. distichum* was similar for all media and exposure treatments at Cu concentrations below 10 mg·L$^{-1}$ (Fig. 1B), while growth of *Z. mays* was greater in sand than bark medium below 10 mg·L$^{-1}$ (Fig. 1A). Acute exposures to high Cu concentrations appeared to be mildly growth promoting in bark medium, particularly for *T. distichum*, suggesting that the ambient level of Cu in the base fertilizer solution (0.018 mg·L$^{-1}$) was below optimal. Alternatively, when growth was not limited by high Cu concentrations, there may have been some growth inhibitory compound present in the bark compared to the sand medium that had a greater influence on *Z. mays* than on *T. distichum*. Data from the recycled irrigation water experiment tends to support the latter explanation (Fig. 3). The high organic content in bark medium may have buffered the effects of chronic exposures to greater Cu concentrations (Fig. 1). For example, even a single application of Cu at 1000 mg·L$^{-1}$ severely stunted or killed both species in the sand medium but had no negative effects on biomass accumulation in the bark medium.

There were more chlorotic *Zea mays* plants at lower Cu concentrations than *T. distichum* plants (Fig. 2 A vs. B). Corn seedlings exhibited typical interveinal chlorosis with chronic Cu exposure as low as 5 mg·L$^{-1}$ in sand, and chlorosis was common in nearly all chronic Cu application seedlings at 10 mg·L$^{-1}$ (Fig. 2A). In contrast, occasional chlorosis developed in baldcypress with Cu at 5 and 10 mg·L$^{-1}$ in sand medium, but large numbers of chlorotic seedlings did not occur until 100-mg·L$^{-1}$ chronic Cu applications and 1000-mg·L$^{-1}$ acute Cu exposure (Fig. 2B). Acute or chronic exposure to Cu at 1000 mg·L$^{-1}$ in sand medium often resulted in the death of corn seedlings (data not presented). Only two *T. distichum* seedlings exhibited foliar chlorosis in bark medium, and then only in response to chronic Cu exposure at 1000 mg·L$^{-1}$ (Fig. 2B). This was consistent with reports that *T. distichum* exhibits only partial prevention of deflected roots at Cu-treated container wall surfaces (Arnold, 1992; Beeson and Newton, 1992; Ruter, 1994; Struve et al., 1994), suggesting a high tolerance of *T. distichum* roots to Cu

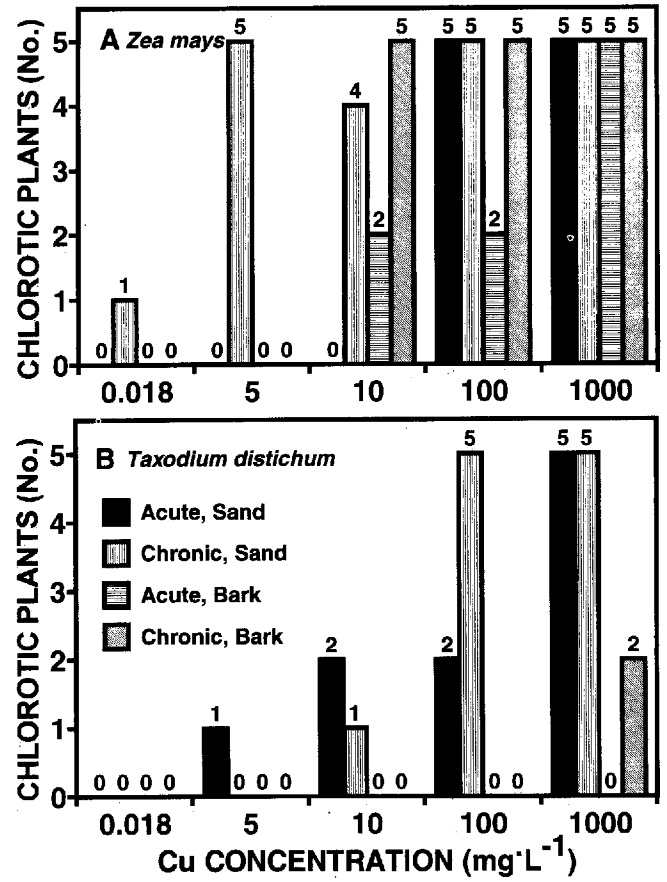


Fig. 2. Development of chlorotic foliage of *Zea mays* (A) during 28 d and *Taxodium distichum* (B) during 35 d in response to acute (solid and horizontal fill columns) or chronic (vertical and diagonal fill columns) applications of various Cu concentrations when grown in 0.7-L containers filled with a sand (solid and vertical fill columns) or pine bark–sand (horizontal and diagonal fill columns) medium. Columns represent the number of plants exhibiting chlorosis out of five possible, n = 5. Differential frequencies ($P \leq 0.05$) were determined using a modified chi-squares test for small sample sizes.

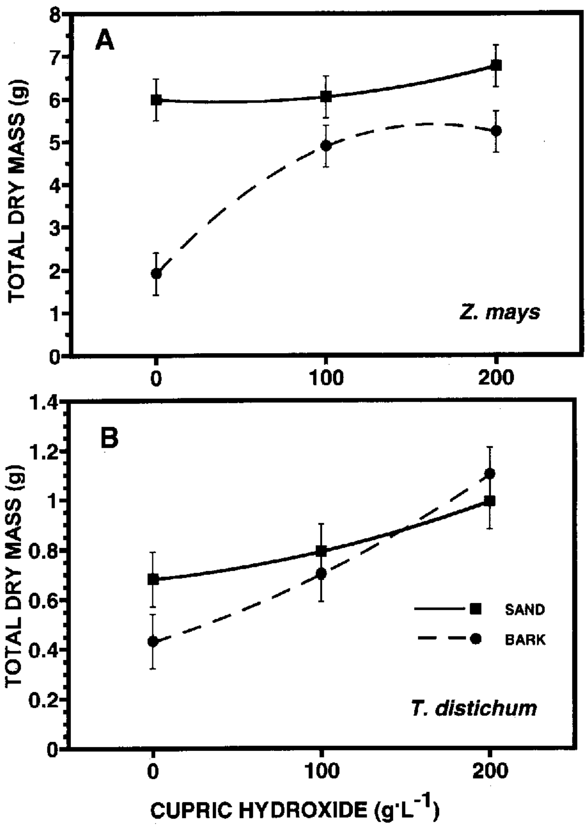


Fig. 3. Total plant dry matter accumulation of *Zea mays* (**A**) during 25 d and *Taxodium distichum* (**B**) during 35 d when grown in 0.7-L containers painted on interior surfaces with various concentrations of Cu(OH)$_2$ filled with sand (■) or pine bark–sand (●) media. Symbols represent means ± standards errors, n = 5. Lines are second-order polynomial regression equations for media types over Cu(OH)$_2$ concentrations, $R^2$s for equations are 0.98 to 0.99, $P \le 0.05$.

exposure. Ninety percent of corn plants had some bulbous roots, a typical symptom of mild copper toxicity, with as little as a single exposure to Cu at 5 mg·L$^{-1}$, while only 13% of baldcypress seedlings developed symptoms of Cu toxicity in the roots at Cu concentrations ≤10 mg·L$^{-1}$ for either acute or chronic exposures. This is consistent with reports that toxicity symptoms from Cu uptake are typically manifested first in the root system (Mengel and Kirkby, 1982). Expression of toxicity symptoms was concentration and formulation dependent with chronic exposure during in vitro rooting of *Betula pubsecens* J.F. Ehrh. X *Betula papyrifera* Marsh. cuttings, ranging from 2.5 mg·L$^{-1}$ of Cu as Cu(CH$_3$COO)$_2$ to 10 mg·L$^{-1}$ for Cu as CuCl$_2$ (Arnold et al., 1994).

**Recycled irrigation water from Cu-treated containers (Expt. 2).** Growth of both species in bark medium increased in 0.7-L containers with increasing Cu(OH)$_2$ concentration on inner walls (Fig. 3). A similar but less pronounced trend was apparent in sand medium. Without Cu(OH)$_2$ treatments, dry matter accumulation was greater in both species in sand than in bark, but Cu(OH)$_2$ treatments tended to negate differential responses to medium (Fig. 3). Increased growth in response to Cu-treated containers has been previously reported for *T. distichum* (Arnold, 1992) and other species (Arnold and Struve, 1989a, 1989b, 1993; Struve et al., 1994).

While soluble Cu concentrations were elevated in the leachate from Cu(OH)$_2$-treated containers (Fig. 4), all but a few samples fell below the maximum contaminant level of 1.3 mg·L$^{-1}$ allowed in drinking water (EPA, 1995). All samples from the fixed-volume reservoirs contained Cu at <1.3 mg·L$^{-1}$ (Fig. 4). No adverse effects on plant growth (Fig. 3) or development of foliar toxicity symptoms (data not presented) occurred in seedlings grown with irrigation water recycled from Cu(OH)$_2$-treated containers. The concentrations of soluble Cu, determined by analysis of reservoir solution, were lower than those determined to be phytotoxic in Expt. 1. Caution should be exercised in extrapolating these short-term results to longer-term field practices, fixed-volume reservoirs with differential turnover rates of recycled water, or reservoirs where the soluble Cu might bind with organic matter or soil particles, be taken up by plant tissues, or rendered insoluble by chemical reactions with anionic complexes.

**Media and fertigation pH and container wall treatment effects on Cu leaching (Expt. 3).** Total plant dry mass, height, stem diameter, and leaf number did not differ statistically ($P \le 0.05$) among treatments within a species at the end of the experiment (data not presented). This may have been due to the pot-bound status of the plants by the end of the study. Plants were large in relation to the container volume; *Z. mays* seedlings after 90 d averaged 142 cm tall with a stem diameter of 15.7 mm and *T. distichum* after 120 d averaged 113 cm tall with a stem diameter of 14.8 mm. Root deflection at container surfaces did differ ($P \le 0.05$) among container treatments for each species (Table 1), but not among media types (data not presented). Most root deflection at container walls was prevented by all Cu(OH)$_2$ treatments for *Z. mays*, with a few escapes mainly at the bottom of the container occurring with the 100-g·L$^{-1}$ treatment (Table 1). Consistent with previous reports (Arnold, 1992), *T. distichum* required Cu(OH)$_2$ at 200 g·L$^{-1}$ or more to effectively inhibit root elongation.

Concentrations of Cu in nonrecycled container leachates were

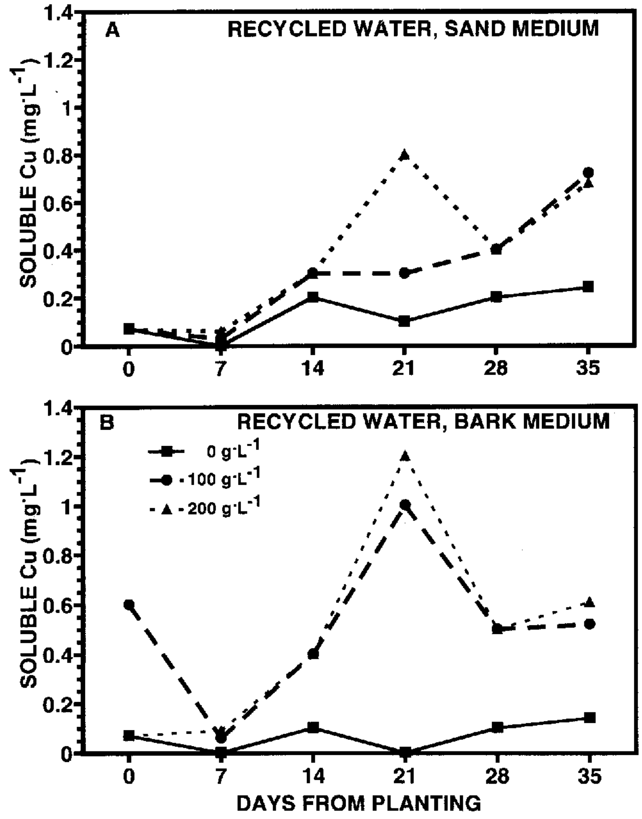


Fig. 4. Soluble Cu concentrations over time in 9.5-L fixed-volume reservoirs of nutrient solution recycled from containers treated on interior surfaces with Cu(OH)$_2$ at 0 (■), 100 (●), or 200 (▲) g·L$^{-1}$ latex polymer carrier containing a sand (**A**) or 3 pine bark : 1 sand (**B**) medium. Each value represents a single pooled water sample.

Table 1. Mean root ratings of *Taxodium distichum* (120 d) and *Zea mays* (90 d) seedlings grown in 2.3-L black plastic containers treated with Cu(OH)$_2$ at 0, 100, 200, or 300 g·L$^{-1}$ and allowed to dry (Expt. 3), or at 100 g·L$^{-1}$ and not allowed to dry prior to medium filling (Expt. 4), or grown in nontreated containers and irrigate with spiked (Cu at 5.0 mg·L$^{-1}$) or nonspiked fertigation solution. Data were pooled across media–irrigation regimes, n = 10.

| Experiment | Species | Container treatment | Root rating[z] |
|---|---|---|---|
| Expt. 3 | *Zea mays* | 0 g·L$^{-1}$ Cu(OH)$_2$ dry | 1.0 c[y] |
| | | 100 g·L$^{-1}$ Cu(OH)$_2$ dry | 3.9 b |
| | | 200 g·L$^{-1}$ Cu(OH)$_2$ dry | 4.4 a |
| | | 300 g·L$^{-1}$ Cu(OH)$_2$ dry | 4.7 a |
| | *Taxodium distichum* | 0 g·L$^{-1}$ Cu(OH)$_2$ dry | 1.0 d |
| | | 100 g·L$^{-1}$ Cu(OH)$_2$ dry | 2.8 c |
| | | 200 g·L$^{-1}$ Cu(OH)$_2$ dry | 4.1 b |
| | | 300 g·L$^{-1}$ Cu(OH)$_2$ dry | 4.9 a |
| Expt. 4 | *Zea mays* | 100 g·L$^{-1}$ Cu(OH)$_2$ dry | 3.9 a |
| | | 100 g·L$^{-1}$ Cu(OH)$_2$ wet | 3.4 b |
| | *Taxodium distichum* | 100 g·L$^{-1}$ Cu(OH)$_2$ dry | 2.8 a |
| | | 100 g·L$^{-1}$ Cu(OH)$_2$ wet | 3.0 a |
| Expt. 5 | *Zea mays* | 0 g·L$^{-1}$ Cu(OH)$_2$ dry | 1.0 a |
| | | 5 mg·L$^{-1}$ Cu spike | 1.0 a |
| | *Taxodium distichum* | 0 g·L$^{-1}$ Cu(OH)$_2$ dry | 1.0 a |
| | | 5 mg·L$^{-1}$ Cu spike | 1.0 a |

[z] Root ratings range from 1 = no reduction in root elongation following contact with container walls, numerous deflected roots on side walls and matted roots at bottom of rootball to 5 = few to no roots elongating more than 1 cm after contacting container surfaces.

[y] Means within an experiment, species, and column followed by the same letter are not significantly different ($P \leq 0.05$) using least squares expected means test.

dependent ($P \leq 0.05$) for each species on the medium–irrigation regime, container wall treatment, and time from planting (Fig. 5). Differential patterns of Cu leaching attributable to medium–irrigation and container wall treatments were more consistent with *T. distichum* (Fig. 5 A and C) than *Z. mays* (Fig. 5 B and D), hence leachate data from *T. distichum* seedlings were used in subsequent nursery runoff modeling calculations. Copper hydroxide applications elevated soluble Cu leachate levels across species and media–irrigation regimes. Copper leaching increased with time from planting, particularly after 60 and 90 d with *Z. mays* and *T. distichum*, respectively. The combination of a more acidic pine bark–peat–sand medium and acidic (pH 6.5) irrigation water resulted in concentrations above the action level for all Cu(OH)$_2$ treatments (Fig. 5 A and B). The combination of a bark–sand medium and high pH (8.0) irrigation water reduced Cu leaching for *T. distichum*, with concentrations near or below the action level until 90 d after planting (Fig. 5C). Copper concentrations in leachate from Cu(OH)$_2$ treatments at 200 and 300 g·L$^{-1}$ were similar on most sample dates and tended to be greater than that of the 100-g·L$^{-1}$ application (Fig. 5).

ELIMINATION OF DRYING STEP AND CU LEACHING (EXPT. 4). Filling containers with media before drying decreased the effectiveness of the Cu(OH)$_2$ treatments in reducing root deflection at the container wall–medium interface with *Z. mays*, but not with *T. distichum* (Table 1). Filling containers with media before allowing the 100-g Cu(OH)$_2$ application to dry (100 g·L$^{-1}$ wet) elevated Cu leaching levels equal to or greater than that of the 200- and 300-g·L$^{-1}$ treatments (Fig. 5 vs. 6). Copper would be expected to be more readily susceptible to leaching from the polymer in a liquid versus solid form, accounting for the greater Cu leaching from wet versus

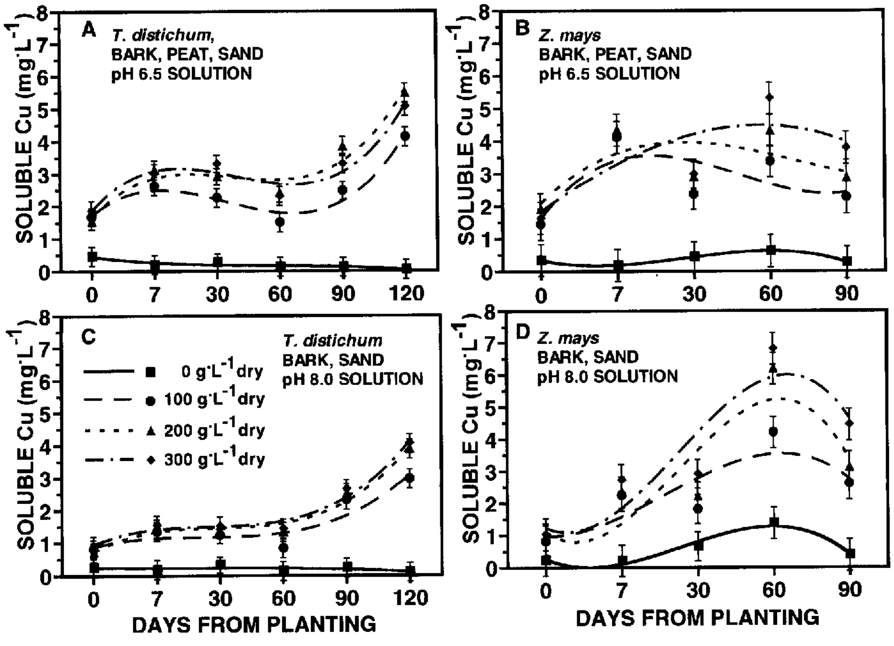


Fig. 5. Soluble Cu concentrations over time in nonrecycled leachate from *Taxodium distichum* (**A** and **C**) and *Zea mays* (**B** and **D**) seedlings grown in 2.3-L containers filled with a 3 pine bark : 1 sand : 1 peat moss (**A** and **B**) or 3 pine bark : 1 sand (**C** and **D**) medium fertigated with pH 6.5 (**A** and **B**) or pH 8.0 (**C** and **D**) nutrient solution. Before planting, containers were painted on interior surfaces with Cu(OH)$_2$ at 100, 200, or 300 g·L$^{-1}$ liquid latex polymer, or not, and allowed to dry before planting. Symbols represent means ± standards errors, n = 5. Lines are third-order polynomial regression equations for container treatments over time, $R^2$ for **A** is 0.60 to 0.80, for **B** is 0.02 (nontreated) to 0.20, for **C** is 0.35 to 0.94, and for **D** is 0.07 (nontreated) to 0.41.

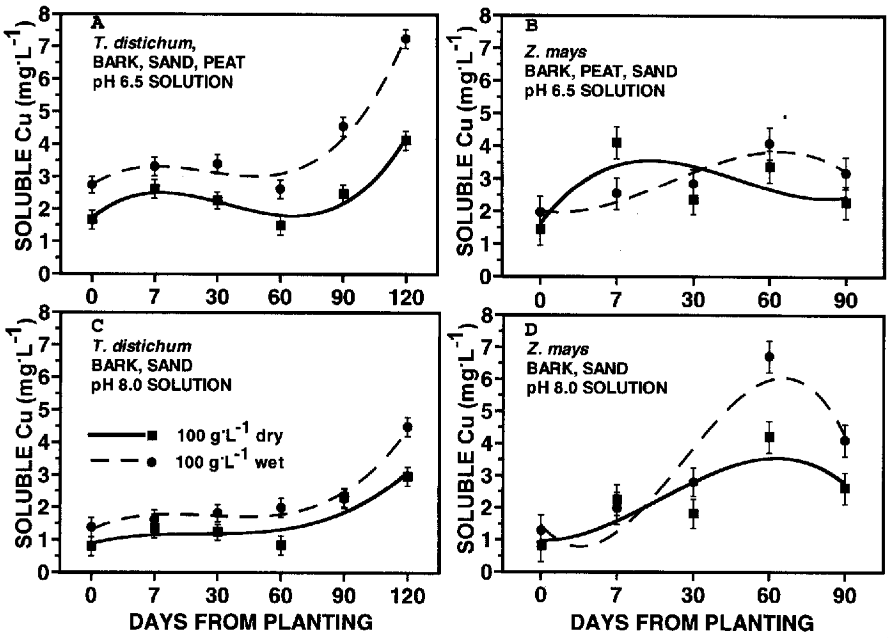


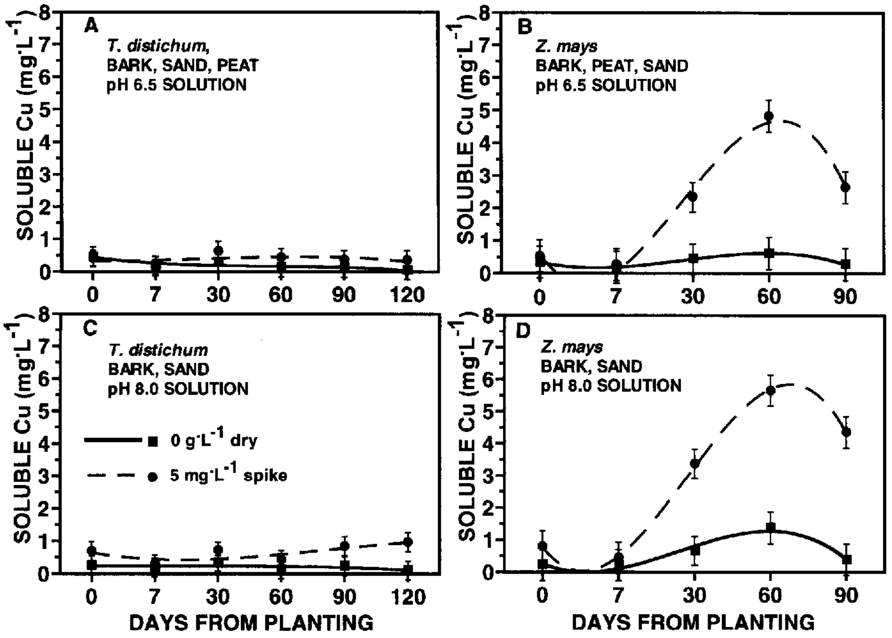
Fig. 6. Soluble Cu concentrations over time in nonrecycled leachate from *Taxodium distichum* (**A** and **C**) and *Zea mays* (**B** and **D**) seedlings grown in 2.3-L containers filled with a 3 pine bark : 1 sand : 1 peat moss (**A** and **B**) or 3 pine bark : 1 sand (**C** and **D**) medium fertigated with pH 6.5 (**A** and **B**) or pH 8.0 (**C** and **D**) nutrient solution. Before planting, containers were painted on interior surfaces with $Cu(OH)_2$ at 100 g·L$^{-1}$ liquid latex polymer and filled with medium before (wet) or after (dry) the polymer dried. Symbols represent means ± standards errors, n = 5. Lines are third-order polynomial regression equations for container treatments over time, *R* for **A** is 0.60 (dry) and 0.85 (wet), **B** is 0.02 (dry) and 0.39 (wet), **C** is 0.83 (dry) and 0.89 (wet), and **D** is 0.29 (dry) and 0.40 (wet).

dry treatments of the same $Cu(OH)_2$ concentration (Fig. 6). Copper concentrations measured here likely represent worst-case scenarios for runoff levels from $Cu(OH)_2$-treated containers irrigated with drip or mini-spray stakes; as media pH was lower (bark–sand–peat, pH 4.8; bark–sand , pH 5.1) than would be typical of many production regimes, there is no accounting for binding with soil or organic particles following leaching, uptake by plants in drainage channels, or dilution via mixing with water falling outside containers when overhead irrigation is applied. Copper readily binds on soil particles and organic matter (Cataldo and Wildung, 1978). Soils typically contain from 2 to 100 µg·g$^{-1}$ of Cu. Thus, movement of leachate through unwashed gravel or over soil surfaces could bind Cu. In addition, Cu is readily absorbed by plant roots as an essential nutrient (Mengel and Kirkby, 1982), and various species of grasses and other plants frequently line drainage channels (EPA, 1992).

SPIKED CU FERTIGATION (EXPT. 5). Growth of *Z. mays* was so rapid that substantial daily drying and rewetting of the media was occurring at the 30-, 60-, and 90-d sample dates, likely allowing some of the applied water to flow between the container wall and medium rather than percolating through the medium column. This may account for the rise in Cu concentrations from the 5-mg·L$^{-1}$ spiked sample on later sample dates for *Z. mays* (Fig. 7 B and D), while most of the Cu in the spiked sample appears to have been held by medium components throughout the study in containers with *T. distichum* (Fig. 7 A and B). Leaching of Cu from nontreated containers (0 g·L$^{-1}$) was minimal throughout the study regardless of medium–irrigation treatment or species, consistent with the low Cu levels applied as fertigation or in the controlled-release fertilizer, and confirms that the increased levels of Cu observed with other treatments were due to Cu release from $Cu(OH)_2$ containing polymers (Figs. 5 and 6) or the spiked irrigation water applications (Fig. 7). Fertigation with Cu at 5 mg·L$^{-1}$ did not affect root growth at the container wall–medium interface compared to plants of either species grown in nontreated containers (Table 1).

CALCULATIONS OF POTENTIAL CU CONCENTRATIONS IN NURSERY RUNOFF. Estimated Cu concentrations in runoff from container-grown *T. distichum* seedlings (Fig. 8) illustrate the effects of dilution when overhead irrigation is applied. Projected levels of Cu in the runoff approached problem levels only with the highest $Cu(OH)_2$ treatments or wet applications of lower $Cu(OH)_2$ concentrations, and then only after 90 (Fig. 8A, bark–peat–sand) to 120 (Fig. 8B, bark–sand) d with containers placed pot to pot. Wider spacings of containers at 0.3 (Fig. 8 C and D) and 0.6 m (Fig. 8 E and F) centers reduced projected Cu concentrations in runoff to <0.5 mg·L$^{-1}$ and 0.25 mg·L$^{-1}$, respectively. Again, these calculations represent extreme situations with no binding, uptake, or rendering of

Fig. 7. Soluble Cu concentrations over time in nonrecycled leachate from *Taxodium distichum* (**A** and **C**) and *Zea mays* (**B** and **D**) seedlings grown in 2.3 L containers filled with 3 pine bark : 1 sand : 1 peat moss (**A** and **B**) or 3 pine bark : 1 sand (**C** and **D**) media fertigated with pH 6.5 (**A** and **B**) or pH 8.0 (**C** and **D**) nutrient solution. Seedlings were irrigated with identical nutrient solutions of each pH, except one contained Cu at 0.018 mg·L$^{-1}$ while the other was spiked with Cu at 5 mg·L$^{-1}$. Symbols represent means ± standards errors, n = 5. Lines are third-order polynomial regression equations for container treatments over time, $R^2$ for **A** = 0.60 (nonspiked) and 0.39 (spiked), **B** = 0.02 (non-spiked) and 0.37 (spiked), **C** = 0.35 (nonspiked) and 0.49 (spiked), and **D** = 0.07 (nonspiked) and 0.56 (spiked).



Cu insoluble in the container leachate before leaving the nursery. Nursery runoff samples collected from actual irrigation runoff averaged 0.07 and 0.11 mg·L$^{-1}$ of soluble and total Cu, respectively. Those following a rain event were even lower—0.02 mg·L$^{-1}$ and 0.05 mg·L$^{-1}$ of soluble and total Cu, respectively. These values are substantially lower than calculated theoretical values, suggesting that the values projected in the calculations may be worst-case scenarios for the time frames and Cu formulations tested.

Copper toxicity tests showed that extreme Cu concentrations in the irrigation water can adversely affect plant growth and survival. However, Cu concentrations in short-term recycling from fixed-volume reservoirs (Fig. 4), Cu(OH)$_2$-treated container leachate (Figs. 5 and 6), and projected nursery runoff (Fig. 8) were in ranges that resulted in no short-term negative growth responses in container-grown *Z. mays* and *T. distichum* seedlings (Figs. 1 and 2). While these results cannot be projected with certainty to longer-term use of Cu(OH)$_2$-treated containers or field use, they suggest that, with overhead irrigation, risks of Cu toxicity during reuse would be minimal and runoff concerns lessened. Investigations into longer-term use of Cu(OH)$_2$-treated containers would be useful in light of increased leaching of Cu over time (Figs. 5 and 6). Also, the effects of runoff flow or water reuse through retention ponds and drainage channels where exchange and cycling of soluble Cu may occur are unknown. A number of plant species have been used in phytoremediation of toxic metals, including Cu, from water and soil (Salt et al., 1995); thus, the effects of Cu uptake by typical plant species lining water diversion channels or the nursery crops during recycling would be of interest.

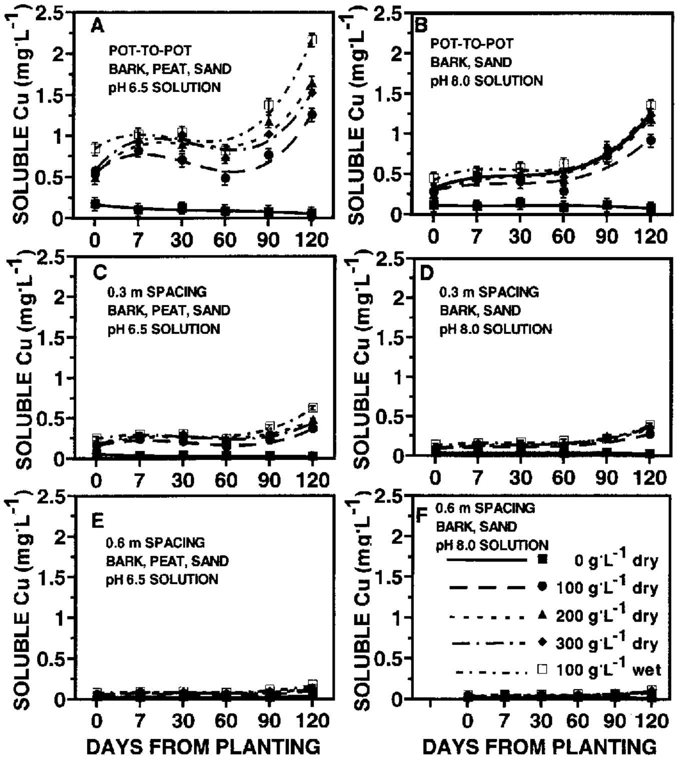


Fig. 8. Calculated soluble Cu concentrations in surface runoff for pot-to-pot (**A** and **B**), 0.3 m (**C** and **D**), or 0.6 m (**E** and **F**) spacings based on leachate collected over time from 2.3-L container-grown *Taxodium distichum* seedlings in containers filled with a 3 pine bark : 1 sand : 1 peat moss (**A**, **C**, and **E**) or 3 pine bark : 1 sand (**B**, **D**, and **F**) medium, which were fertigated with pH 6.5 (**A**, **C**, and **E**) or pH 8.0 (**B**, **D**, and **E**) nutrient solutions. Projections refer to the following treatments: before planting, containers were painted on interior surfaces with Cu(OH)$_2$ at 0, 100, 200, or 300 g·L$^{-1}$ liquid latex polymer and allowed to dry or painted with Cu(OH)$_2$ at 100 g·L$^{-1}$ polymer and filled with media and planted before drying (wet). Calculations are based on leachate data from Expts. 3 and 4. Symbols represent means ± standards errors, n = 5. Lines are third-order polynomial regression equations for container treatments over time, $R^2$s for **A**, **C**, and **E** are 0.60 to 0.85 and for **B**, **D**, and **F** are 0.34 (nontreated) to 0.94, $P \le 0.05$.

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