J. Aquat. Plant Manage. 55: 113–115

Note

Evaluation of copper on Texas wild rice, creeping primrose-willow and waterstargrass

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INTRODUCTION

Texas wild rice (*Zizania texana* Hitchc.; TWR) is a federally endangered plant endemic to the upper reach of the San Marcos River, Hays County, Texas (Poole et al. 2007). The U.S. Fish and Wildlife Service's San Marcos Aquatic Resources Center (SMARC; 29°50′23.9″N, 97°58′33.8″W) maintains refugia populations of TWR for reestablishment in the San Marcos River in the event of catastrophic floods or droughts. In addition, the SMARC propagates TWR and other native aquatic plants for restoration efforts in the San Marcos River that include floating waterprimrose (*Ludwigia repens* Frost) and waterstargrass [*Heteranthera dubia* (Jacq.) MacM.]. Control and maintenance of algae infestations in tanks, raceways, and outdoor ponds containing aquatic plants is time consuming and labor intensive. Improving strategies for managing threatened and endangered species with herbicides has been listed as a research priority (Getsinger et al. 2008).

The most common types of algae in tanks and ponds at the SMARC are green (e.g., *Spirogyra*, *Anabaena*, *Cladophora*, *Draparnaldia*, *Hydrodictyon*, *Chlorococcum*, and *Ankyra*), yellow-green (e.g., *Tribonema* and *Vaucheria*), red (*Batrachospermum*), diatoms (e.g., *Asterionella*, *Fragilaria*, *Amphipleura*) and muskgrass (*Chara* spp.). Filamentous green algae grow throughout the water column but often form dense floating mats on the surface and block sunlight. Muskgrass competes with TWR and native aquatic plants by rooting in the pots, competing for nutrients, and blocking sunlight. Decaying algae fall to the bottom, forming a nutrient pool that further perpetuates algal blooms and increases turbidity. A recent genetic analysis of TWR found the SMARC refugia population will require an additional 300 plants to match the genetic diversity of the wild population (Wilson et al. 2015). With the increasing production of TWR and other aquatic vegetation at SMARC required to meet the goals of the Edwards Aquifer Habitat Conservation Plan (EARIP 2011) and *ex situ* genetic management, manual control of algae will require increased time from a limited staff. SMARC staff manages approximately 300 m^2 of greenhouse tanks, 54 m^2 of outdoor raceways, and 720 m^2 of outdoor ponds by manually removing algae on a daily basis. Algae management at SMARC staff consumes a minimum of 12 to 14 h wk^{-1}.

The most common method of algae control in ponds and natural water bodies is the use of copper-based products (Lembi 2009). Copper is a contact algaecide and herbicide that at low concentrations ($\leq$ 1.0 ppm) controls algae with no harm to most vascular plants. Cutrine-Plus©[1] is a liquid chelated copper algaecide that controls a broad range of species. The product contains ethanolamine, a chelating agent that slows the precipitation of copper with carbonates and bicarbonates in water and is labeled for use in fish ponds and raceways (Applied Biochemists 2012). For increased production and maintenance of TWR and other native aquatic plants at the SMARC, alternative methods to manual control of algae need to be evaluated. It is hypothesized that copper ethanolamine used one or two times over a 4-wk period will result in $\leq$ 5% mortality to TWR and native aquatic plants, reduce the time and labor devoted to algae control, and result in more efficient aquatic plant production. An integrated management approach to algae control in tanks and raceways could be best achieved using a variety of techniques, of which copper is one component. The objective of this project is to determine the effects of copper concentrations on TWR, floating waterprimrose, and waterstargrass.

METHODS AND MATERIALS

TWR seedlings were propagated from seeds collected from plants held at the SMARC and cultured until shoot length was 12 to 14 cm, and one TWR seedling was planted per pot. Floating waterprimrose and waterstargrass were propagated by potting three 12- to 14-cm apical tips collected from the San Marcos River. The apical tips were inserted 6 to 7 cm into soil and the plants were grown to 15 cm above soil level. Plants were potted with a mixture of commercially purchased top soil (80%), sand (15%), and pea gravel (5%) in 10.5-cm^2 plastic pots 12 cm in height and placed in 1.3 by 0.4 by 0.4–m (208-L) fiberglass tanks filled with Edwards Aquifer water in a greenhouse. The pH and water temperature were maintained at 6.95 (SE = 0.13) and 23.1 C (SE = 0.18), respectively. Following potting of TWR seedling and apical tips of floating waterprimrose and waterstargrass, the plants were maintained in the flow-

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through tanks with aquifer water inflow and outflow of 3.8 L min^{-1} for 2 wk until treatment. Water retention time in the tanks was 70 min.

Plants were treated with 0.06, 0.12, 0.25, 0.50, 1.00, and 2.00 ppm copper ion as Cutrine-Plus[1] and nontreated plants served as controls. Copper ion concentrations were poured into the tanks and mixed for 3 min for complete distribution of the copper in each of the 208-L tanks. Inflow and outflow of water into the tanks was stopped for 24 h to allow adequate contact time between the copper and plants. After 24 h, inflow and outflow were restored to the tanks.

Ten replicates of each plant species were used per treatment, and the experiment was repeated for a total of 20 plants. All vegetation within each pot was visually evaluated for chlorosis and necrosis at 2 and 4 wk after treatment (WAT) based on a scale of 0 (no plant damage) to 100 (complete plant destruction) (Frans et al. 1986) and summary statistics were calculated for survival. At 2 WAT, five plants were harvested for dry weight, separating the roots and leaves. The remaining five plants were treated a second time with copper at 2 WAT. At 4 wk after initial treatment, the remaining five plants receiving two copper treatments were harvested for dry weight. Total leaf (aboveground biomass [AGB]) and root lengths (belowground biomass [BGB]) were measured (cm) for TWR. All fibrous roots were measured for TWR plants, but fine roots coming off fibrous roots were not measured. All plants were clipped and divided as AGB or BGB. Plants parts were placed in a paper bag and oven dried for 7 d at 60 C to determine dry weight.

ANOVA was used to determine differences ($P < 0.05$) between dry weight for all species and total leaf length for TWR for copper concentrations. If differences were detected with the ANOVA, means were separated using Tukey's multiple comparison test ($P < 0.05$). The effective concentration that reduces biomass by 50% compared to untreated plants (EC_{50}) and 95% confidence intervals (Cis) were calculated using nonlinear regression for dry weight of all species using the Emax model and SAS 9.3.[2]

RESULTS AND DISCUSSION

The survival rate combining plants from both experiments was 95% for TWR and 100% for floating waterprimrose and waterstargrass, with only one TWR plant treated with copper dying at 3 WAT. No chlorosis or necrosis was observed on TWR or floating waterprimrose. Foliar symptoms including chlorosis and necrosis were observed on waterstargrass at 1 WAT. At 2 WAT, 25 and 50% of the waterstargrass leaves were chlorotic for plants treated with 1 and 2 ppm copper ion, respectively.

No significant differences in dry weight were detected on TWR treated with copper for 2 WAT AGB ($P = 0.75$) and BGB ($P = 0.94$) following one treatment with copper ions, and 4 WAT AGB ($P = 0.83$) and BGB ($P = 0.44$) and two applications of copper ions. Aboveground dry weight biomass increased 390 to 838% at 2 WAT compared to seedlings biomass at pretreatment ($P < 0.001$) after a single copper treatment and 919 to 1,674% at 4 WAT after two copper treatments compared to seedling biomass at pretreatment. At 2 WAT there were no differences detected for lengths of AGB ($P = 0.81$) and BGB ($P = 0.96$), and 4 WAT AGB ($P = 0.42$) and BGB ($P = 0.48$) following treatment with copper. The EC_{50} values for TWR roots and leaves at 2 WAT with one copper ion application were calculated to be 6.8 (CI = 5.6 to 8.2) and 7.3 (CI = 7.0 to 5.0) ppm, respectively, indicating that that TWR is highly tolerant to copper ethanolamine (Table 1). At 4 WAT following two treatments with copper ions, EC_{50} values for TWR roots and leaves were calculated to be 7.2 (CI = 5.9 to 9.1) and 2.0 (CI = 1.7 to 2.3) ppm, respectively, indicating that Texas wild rice is not affected by two treatments of copper ethanolamine within 4 wk.

Table 1. Plant species, EC_{50} values indicating relative susceptibility of species to copper, and 95% confidence intervals on three aquatic plants at 2 and 4 wk after treatment.

Species	Plant Part	2 wk			4 wk		
		EC_{50}[1]	95% CI	r^2	EC_{50}[1]	95% CI	r^2
Texas wild rice	Roots	6.8	5.6–8.2	0.69	7.2	5.9–9.1	0.61
	Leaves	7.3	7.0–7.5	0.72	2.0	1.7–2.3	0.79
Floating waterprimrose	Roots	0.9	0.7–1.1	0.82	1.0	0.7–1.3	0.79
	Leaves	1.0	0.9–1.1	0.92	1.0	0.8–1.2	0.90
Waterstargrass	Roots	0.8	0.6–1.0	0.83	1.0	0.5–1.5	0.84
	Leaves	1.0	0.8–1.2	0.96	1.0	0.6–1.4	0.95

[1]EC_{50} = Effective copper concentration (ppm) that reduces biomass by 50% compared to untreated plants; CI = confidence interval.

Significant differences were present in floating waterprimrose dry weight at 4 WAT AGB ($P = 0.01$) and BGB ($P = 0.03$) but no difference was found for floating waterprimrose at 2 WAT BGB ($P = 0.33$). No pattern of decreasing dry weight with increasing copper concentration was observed with floating waterprimrose. Plants treated with 0.12 and 2.00 ppm copper had root : shoot ratios of 1 : 4 and 1 : 3.7, respectively. Plants treated with 0.06 and 1.00 ppm had root : shoot ratios of 1 : 0.8 and 1 : 0.9, respectively, while the root : shoot ratio of controls was 1 : 1.2. The EC_{50} values for floating waterprimrose roots and leaves at 2 WAT with one copper ion application were calculated to be 0.9 (CI = 0.7 to 1.1) and 1.0 (CI = 0.9 to 1.1) ppm, respectively, indicating that use of the maximum label rate of copper ethanolamine results in 50% reduction in biomass (Table 1). At 4 WAT following two treatments with copper ions, EC_{50} values for floating waterprimrose roots and leaves were calculated to be 1.0 (CI = 0.7 to 1.3) and 1.0 (CI = 0.8 to 1.2) ppm, respectively.

Waterstargrass dry weight was impacted by copper ion treatment at 2 WAT AGB ($P = 0.02$), 4 WAT AGB ($P = 0.0001$), and 4 WAT BGB ($P = 0.0003$), but no difference was detected at 2 WAT BGB ($P = 0.40$) dry weight. There was a decreasing pattern in dry weight for waterstargrass from 0.00 to 2.00 ppm copper, and all copper treatments ≥ 0.25 ppm at 2 and 4 WAT resulted in AGB dry weight lower than at 0 WAT. Although waterstargrass biomass was reduced at 4 WAT, survival was 100%, and the plants appeared healthy and exhibiting recovery following two treatments. The EC_{50} values for waterstargrass roots and leaves at 2 WAT with one copper ion application were calculated to be 0.8 (CI = 0.6 to 1.0) and 1.0 (CI = 0.8 to 1.2) ppm, respectively, indicating that use of the maximum label rate of copper ethanolamine results in 50% reduction in biomass (Table 1). At 4 WAT following two treatments with copper ions, EC_{50} values for waterstargrass roots and leaves were calculated to be 1.0 (CI = 0.5 to 1.5) and 1.0 (CI = 0.6 to 1.4) ppm, respectively.

The research conducted in this trial indicates that TWR and floating waterprimrose were minimally impacted by copper at concentrations $\leq$ 1 ppm. Conversely, waterstargrass biomass was reduced significantly with copper at concentrations $\geq$ 0.06 ppm; however, no mortality was observed at any concentration and the plants appeared to be healthy at 4 WAT. Nevertheless, our study suggests that copper can be used as part of an integrative management strategy combined with siphoning, hand removal, netting, tank flushing, and increased current velocity for algae control in tanks and raceways containing aquatic plants. Since some aquatic macrophytes such as common elodea (*Elodea canadensis* Michx.) can be negatively affected by copper (Mai et al. 2002), additional trials are suggested for delta arrowhead [*Sagittaria platyphylla* (Engelm.) J.G. Sm.], Illinois pondweed (*Potamogeton illinoensis* Morong), pennywort (*Hydrocotyle* spp.), fanwort (*Cabomba caroliniana* Gray), and variable watermilfoil (*Myriophyllum heterophyllum* Michx.), which are native to the San Marcos River and propagated at the SMARC for restoration efforts. These data would also add value to other restoration projects throughout the nation.

TWR, like many other rooted macrophytes, acquires macro- and micronutrients through its roots (Smart and Barko 1984). It is presumed that the release of these nutrients from submerged soils is the primary driver for algae growth. TWR is also an obligate CO_2 plant unable to consume HCO_3–, which is positively and directly related to its biomass productivity via water current velocity (Power and Doyle 2004). As a result, alternative methods for seed germination and plant propagation that can be more easily managed for algae growth such as hydroponics, aeroponics, aquaponics, and axenic culture systems appear to be unreliable options given TWR's unique life history and metabolic requirements. Although our results suggest that copper can be used for algae control in refugia containing TWR with no damage to the plants, other aquatic plants are known to accumulate copper, which may eventually interfere with physiological functions such as photosynthesis and membrane formation (Hu et al. 2007, Monferran et al. 2009). To ensure that no effects from copper bioaccumulation are occurring in TWR, monitoring should occur for 30 d posttreatment following each exposure, particularly when multiple or sequential treatments are required to control algae during a season or the lifecycle of the plant.

Copper is also known to kill aquatic nuisance species when used as an algaecide (Boyd 1990; Francis-Floyd et al. 1997; Mitchell 2002). In the current study, a single treatment at 1 ppm resulted in 100% mortality of *Physa virgata* (Gould), a native freshwater snail (data not shown). An additional use of copper could be to treat TWR plants while held in quarantine for control of not only algae but also nonnative snails such as *Melanoides tuberculata* (Muller, 1974) or other species prior to moving plants into refugia tanks. Not only does this directly benefit the plants being maintained in captivity but it may also improve the longevity of aquatic system infrastructure such as pumps, chiller/heaters, and pipes. Collectively our results suggest that the use of copper to control algae for captive propagation and refugia efforts affords a number of logistical and monetary benefits while imposing little to no negative consequence on TWR plants.

SOURCES OF MATERIALS

[1]Cutrine-Plus, Applied Biochemists, W175N11163 Stonewood Dr., Suite 234, Germantown, WI 53022.

[2]SAS software, version 9.3, SAS Institute Inc., 100 SAS Campus Drive, Cary, NC 27513-2414.

ACKNOWLEDGEMENTS

Funding for the project was provided by the U.S. Fish and Wildlife Service. The views expressed in this manuscript do not necessarily represent the views of the U.S. Fish and Wildlife Service. The authors thank the associate editor and an anonymous reviewer for their suggestions that improved the manuscript.

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