J. Aquat. Plant Manage. 53: 138–140

NOTE

Comparative efficacy of chelated copper formulations alone and in combination with diquat against hydrilla and subsequent sensitivity of American lotus

GRAY TURNAGE, J. D. MADSEN, AND R. M. WERSAL*

INTRODUCTION

Hydrilla [*Hydrilla verticillata* (L. f.) Royle], a nonnative plant from Asia, has become established in lakes and rivers across North America (Sutton and Blackburn 1971, Langeland 1996). Hydrilla has been nicknamed "the perfect aquatic weed" due to its reproductive capabilities and ability to grow in almost any type of aquatic habitat while outcompeting neighboring plants for resources (Langeland 1996). Hydrilla can reproduce through production of turions, tubers, rhizomes, and stem fragments, as well as by sexual reproduction (Langeland and Sutton 1980, Van and Steward 1990, Sutton et al. 1992, Madsen and Smith 1999). There are two biotypes of hydrilla, a monoecious biotype and dioecious biotype. The monoecious biotype has typically been problematic in the northern-tier states, and the dioecoius biotype is a problem in the southeastern United States. However, additional information is needed on basic biological and ecological aspects of monoecious hydrilla as it relates to its dioecious counterpart. Certainly, the plasticity with which hydrilla responds to environmental factors, competition with other species, and management techniques makes this plant extremely difficult to control.

In the 1960s, the first studies evaluating efficacy of various herbicides in controlling hydrilla found copper sulfate mixed with diquat to be one of the best control options (Blackburn and Weldon 1970). Additional studies were conducted with copper and other herbicides as new chelated copper formulations were developed (Sutton and Blackburn 1971, Sutton et al. 1971, Gangstad 1978, Pennington et al. 2001). Currently, there are a number of different copper formulations labeled for use in aquatic habitats, some of which have not been tested in combination with diquat. Each copper formulation has different chelators, pH, and overall chemical composition that might cause them to perform differently, depending upon environmental factors present during application. A popular tank mix for hydrilla control today includes diquat with the ethylenediamine formulation of copper.

Herbicide mixes that are used for hydrilla tend to be nonselective and can negatively affect native and nontarget plant populations (Sutton and Blackburn 1971, Haller et al. 1990), although little data exists on quantifying tank-mix effects on multiple species. One native species that often co-occurs with hydrilla and is beneficial in many southeastern United States reservoirs is American lotus (*Nelumbo lutea* Willd.). American lotus is the dominant native plant species in the Ross Barnett Reservoir (the largest reservoir in Mississippi) (Cox et al. 2014), and is considered to be desirable for wildlife habitat. Anecdotal reports during operational management of hydrilla on the Ross Barnett Reservoir have indicated that damage to American lotus beds adjacent to hydrilla sites was occurring when hydrilla was treated using tank mixes of diquat and the ethylenediamine formulation of copper.

Therefore, the purpose of this study was to: 1) evaluate the efficacy of different formulations of chelated coppers alone and in combination with diquat on hydrilla, 2) establish if differences exist between chelated copper formulations with respect to American lotus sensitivity, and 3) develop recommendations on treatment regimens for hydrilla using copper and/or diquat while minimizing damage of American lotus.

MATERIALS AND METHODS

The study was conducted at the R. R. Foil Plant Science Research Center, Mississippi State University, Starkville, MS in 132, 378-L (135 cm long by 79 cm wide by 64 cm deep) tanks from June 2012 through September 2012. Hydrilla was propagated from stock cultures at Mississippi State Univer-

*First author: Research Associate, Geosystems Research Institute, Mississippi State University, Box 9627, Mississippi State, MS 39762. Second author: Research Biologist, USDA, ARS, EIWRU, University of California–Davis, Department of Plant Sciences, Mail Stop 4, Robbins Hall 274, One Shields Ave., Davis, CA 95616. Third author: Aquatic Plant Scientist, Applied Biochemists, Arch Chemicals, Inc., now part of Lonza, Alpharetta Innovation and Technology Center, 1200 Bluegrass Parkway, Alpharetta, GA 30004. Corresponding author's E-mail: ryan.wersal@lonza.com. Received for publication March 6, 2014 and in revised form June 9, 2014.

Table 1. Herbicide treatments (% ai) and biomass reductions, as compared to untreated reference plants, of hydrilla and America lotus 4 wk after treatment with diquat and chelated copper applied alone and in combination. Negative biomass numbers indicate an increase in biomass or plant growth. Values sharing the same letter within a column are not different according to a Fisher's Protected LSD at a $P \leq 0.05$ significance level. Analyses were conducted within species.

Herbicide	Concentration (mg L^{-1})	Hydrilla biomass reduction (%)	American Lotus biomass reduction (%)
Untreated reference	0.0	0 a	0 ab
Diquat[2]	0.37	83 e–h	94 de
Copper ethylenediamine granular 3.4%[3]	0.5	71 c–f	3 ab
Copper ethylenediamine granular 3.4%	1.0	92 e–h	62 b–e
Diquat + copper ethylenediamine granular 3.4%	0.37 + 0.5	100 h	100 e
Diquat + copper ethylenediamine granular 3.4%	0.37 + 1.0	99 h	100 e
Emulsified copper ethanolamine 9%[4]	0.5	56 b–e	29 a–d
Emulsified copper ethanolamine 9%	1.0	51 abc	16 abc
Diquat + emulsified copper ethanolamine 9%	0.37 + 0.5	100 h	100 e
Diquat + emulsified copper ethanolamine 9%	0.37 + 1.0	100 h	100 e
Emulsified copper ethanolamine 3.8%[5]	0.5	39 ab	9 ab
Emulsified copper ethanolamine 3.8%	1.0	38 a	−21 a
Diquat + emulsified copper ethanolamine 3.8%	0.37 + 0.5	97 fgh	100 e
Diquat + emulsified copper ethanolamine 3.8%	0.37 + 1.0	99 h	100 e
Copper ethanolamine 9%[6]	0.5	48 a–d	78 cde
Copper ethanolamine 9%	1.0	63 cde	−2 ab
Diquat + copper ethanolamine 9%	0.37 + 0.5	68 d–g	100 e
Diquat + copper ethanolamine 9%	0.37 + 1.0	100 h	100 e
Copper ethylenediamine 8%[7]	0.5	79 e–h	−9 a
Copper ethylenediamine 8%	1.0	80 e–h	−13 a
Diquat + copper ethylenediamine 8%	0.37 + 0.5	99 h	100 e
Diquat + copper ethylenediamine 8%	0.37 + 1.0	98 gh	100 e

sity and American lotus seeds were obtained from the U.S. Army Engineer Research and Development Center, Lewisville Aquatic Ecosystem Research Facility in Lewisville, TX. Planting occurred at the end of June for both species.

For hydrilla, planting consisted of placing two 20-cm apical shoots into 3.78-L containers filled with sediment that was purchased commercially. American lotus seeds were scarified with a file and allowed to sit in a shallow container of water until the seeds sprouted. Once the seeds sprouted, two plants were placed into 3.78-L containers filled with sediment. The sediment was amended with Osmocote®[1] (19–6–12) fertilizer at rate of 2 g L^{-1} of soil to maintain plant growth. Five potted containers of hydrilla were placed into 66 of the 132, 378-L mesocosms, and three potted containers of American lotus were placed in each of the remaining 66 tanks. Tanks were filled with pond water to a depth of 48 cm. Plants were allowed to grow until they reached the water's surface (48 cm, approximately 4 wk). Pretreatment biomass was harvested using 1 pot $tank^{-1}$. All harvesting consisted of clipping living plant shoots at the sediment surface. Plants were washed to remove dirt and debris, placed into labeled paper bags, and dried at 70 C for 5 d.

After the pretreatment harvest, herbicides were applied using the herbicides and concentrations outlined in Table 1. Liquid herbicides were applied to the water as a concentrated aqueous solution to achieve the target concentrations. Granular herbicides were weighed to the appropriate mass, placed in a permeable container, and suspended approximately 20 cm below the water surface within the appropriate mesocosm. All treatments were made using a 12-hr exposure time and were replicated in three tanks. To achieve the exposure time, granular herbicide containers were removed and all tanks were drained and refilled with fresh water to remove herbicide residues. At 4 wk after treatment (WAT), all living aboveground plant tissues were harvested, washed, and dried in a similar manner as pretreatment samples.

Percent biomass reduction was calculated as one minus the quotient of average treatment biomass and reference biomass. An analysis of variance (ANOVA) was used to analyze treatment effects using Statistix 9 (Analytical software 2009). If a significant difference was detected, then means were separated using the Fisher's Protected LSD procedure. All analyses were conducted at $P \leq 0.05$ significance level.

RESULTS AND DISCUSSION

Hydrilla was actively growing throughout the study as biomass increased 67% (3.6 to 10.8 g dry weight [DW] pot^{-1}) when comparing pretreatment biomass to untreated reference plants 4 WAT. Likewise, American lotus was actively growing as biomass increased 66% (7.3 to 21.4 g DW pot^{-1}) when comparing pretreatment biomass to untreated reference plants 4 WAT. Overall, all treatments reduced hydrilla aboveground biomass with respect to untreated reference plants 4 WAT, except the emulsified copper ethanolamine 9% at 1.0 mg L^{-1}, emulsified copper ethanolamine 3.8% applied at 0.5 and 1.0 mg L^{-1}, and copper ethanolamine 9% at 0.5 mg L^{-1}. In particular, the combination treatments resulted in 97 to 100% biomass reductions for hydrilla regardless of the copper formulation, with exception of copper ethanolamine 9% applied in combination with diquat at 0.5 mg L^{-1} where only 68% hydrilla biomass reduction was achieved (Table 1).

Diquat applied alone at 0.37 mg L^{-1} resulted in 83% reduction in hydrilla biomass. The liquid formulation of copper ethylenediamine applied alone resulted in 79 and 80% reductions in hydrilla biomass when applied at 0.5 and 1.0 mg L^{-1}, respectively (Table 1). Similarly, the granular formulation resulted in 71 and 92% reductions when applied at the same concentrations. Copper ethylenediamine, both the liquid and granular formulations, were the only copper formulations applied alone to provide similar hydrilla control as the combination treatments. The other copper formulations tested needed to be combined with diquat to achieve greater than 70% control of hydrilla.

Although diquat and the combination of diquat and copper is very effective at controlling hydrilla (Blackburn and Weldon 1970, Sutton et al. 1970, 1971, Pennington et al. 2001), it also has the potential to cause nontarget injury, depending upon concentrations used and exposure. When diquat was applied alone or combined with copper, regardless of the formulation, it caused significant reductions in American lotus biomass. Biomass reduction of American lotus was 94% when diquat was applied alone at 0.37 mg L^{-1}, and 100% when combined with copper, regardless of the formulation (Table 1). The use of copper alone did not result in significant biomass reductions of American lotus using a 12-h exposure time, with the exception of granular copper ethylenediamine at 1.0 mg L^{-1} and copper ethanolamine at 0.5 mg L^{-1}. In fact, when liquid copper ethylenediamine was applied at 0.5 and 1.0 mg L^{-1} American lotus biomass increased 9 and 13%, respectively, indicating no herbicidal effects at 4 WAT.

Given the results of this study, there exists a tradeoff between selectivity (which plants are affected) and sensitivity (how dramatic the effect is on a given plant). The tradeoff between selectivity and sensitivity depends upon the species present at a treatment site, site-specific water characteristics, and the uses of the water body. In dense hydrilla infestations, it might be beneficial to use the diquat + copper combination to rapidly remove biomass from an area. The use of copper ethylenediamine could be considered when treating hydrilla alone or in mixed stands of floating or emergent vegetation when selectivity is desired. With the development of fluridone resistance in hydrilla (Arias et al. 2005, Puri et al. 2007), a greater emphasis needs to be placed on product stewardship through rotating multiple modes of action and revisiting older use patterns of current chemistries such as copper and diquat. Future studies should verify efficacy and copper sensitivity of additional native plants to copper ethylenediamine, and determine effective ratios of diquat and copper in combination to maximize hydrilla control and minimize nontarget injury, especially because only the maximum label rate of diquat was tested in this study.

SOURCES OF MATERIALS

[1]Osmocote 19–6–12 fertilizer, Scotts-Sierra Horticultural Products Company, 14111 Scottslawn Rd., Marysville, OH 43041.

[2]Reward® Landscape and Aquatic Herbicide (Diquat dibromide), Syngenta Crop Protection, Inc., P.O. Box 18300, Greensboro, NC 24719.

[3]Harpoon® Granular Aquatic Herbicide (Copper Ethylenediamine Granular 3.4%), Applied Biochemists, A Lonza Business, W175N11163 Stonewood Dr. Ste. 234, Germantown, WI 53022.

[4]Cutrine Ultra® Algaecide/Herbicide/Cyanobactericide (Emulsified Copper Ethanolamine Complex, Mixed 9%), Applied Biochemists, A Lonza Business, W175N11163 Stonewood Dr. Ste. 234, Germantown, WI 53022.

[5]Clearigate® (Emulsified Copper Ethanolamine 3.8%), Applied Biochemists, A Lonza Business, W175N11163 Stonewood Dr. Ste. 234, Germantown, WI 53022.

[6]Cutrine®-Plus Algaecide and Herbicide (Copper Ethanolamine Complex, Mixed 9%), Applied Biochemists, A Lonza Business, W175N11163 Stonewood Dr. Ste. 234, Germantown, WI 53022.

[7]Harpoon® Aquatic Herbicide (Copper Ethylenediamine 8%), Applied Biochemists, A Lonza Business, W175N11163 Stonewood Dr. Ste. 234, Germantown, WI 53022.

ACKNOWLEDGEMENTS

We would like to thank Bradley Sartain, Marvin (Trey) Higginbottham, John Perren, John Mark Curtis, Stephen Wray, Kris Madsen, Lee Bryant, Daisy Polk, and Sandor Dibble for assistance with this project. Approved for publication as Journal Article No. J-12480 of the Mississippi Agricultural and Forestry Experiment Station, Mississippi State University.

LITERATURE CITED

Analytical Software. 2009. Statistix 9. User's manual. Analytical software. Florida State University Tallahassee, FL.

Arias RS, Netherland MD, Scheffer BE, Puri A, Dayan FE. 2005. Molecular evolution of herbicide resistance to phytoene desaturase inhibitors in *Hydrilla verticillata* and its potential use to generate herbicide-resistant crops. Pest Manage. Sci. 61:258–268.

Blackburn RD, Weldon LW. 1970. Control of *Hydrilla verticilatta*. Hyacinth Control J. 8B:4–9.

Cox MC, Wersal RM, Madsen JD, Gerard PD, Tagert ML. 2014. Assessing the aquatic plant community within the Ross Barnett Reservoir, MS. Invasive Plant Sci. Manage. 7:375–383.

Gangstad EO. 1978. Chemical control of hydrilla. J. Aquat. Plant Manage. 16:38–40.

Haller WT, Fox AM, Shilling DG. 1990. Hydrilla control program in the upper St. Johns River, Florida, USA. Proceedings European Weed Research Society, 8th Symposium on Aquatic Weeds. 8:111–116.

Langeland KA. 1996. *Hydrilla verticillata* (L. f.) Royle (Hydrocharitaceae), "The Perfect Aquatic Weed." Castanea 61:293–304.

Langeland KA, Sutton DL. 1980. Regrowth of hydrilla from axillary buds. J. Aquat. Plant Manage. 18:27–29.

Madsen JD, Smith DH. 1999. Vegetative spread of dioecious hydrilla colonies in experimental ponds. J. Aquat. Plant Manage. 37:25–29.

Pennington TG, Skogerboe JG, Getsinger KD. 2001. Herbicide/copper combinations for improved control of *Hydrilla verticillata*. J. Aquat. Plant Manage. 39:56–58.

Puri A, MacDonald GE, Haller WT. 2007. Stability of fluridone-resistant hydrilla (*Hydrilla verticillata*) biotypes over time. Weed Sci. 55:12–15.

Sutton DL, Blackburn RD. 1971. Uptake of copper in hydrilla. Weed Sci. 11:47–53.

Sutton DL, Blackburn RD, Steward KK. 1971. Influence of herbicides on the uptake of copper in hydrilla. Weed Sci. 11:99–105.

Sutton DL, Van TK, Portier KM. 1992. Growth of dioecious and monoecious hydrilla from single tubers. J. Aquat. Plant Manage. 30:15–20.

Van TK, Steward KK. 1990. Longevity of monoecious hydrilla propagules. J. Aquat. Plant Manage. 28:74–76.