

# Herbicide/Copper Combinations for Improved Control of *Hydrilla verticillata*

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## ABSTRACT

Greenhouse aquariums were used to evaluate the efficacy of the dipotassium salt of endothall in combination with other products against hydrilla (*Hydrilla verticillata* (L.f.) Royle). Copper, diquat, or the mono(N,N-dimethylalkalamine) salt of endothall were combined with the dipotassium salt of endothall to determine if equal or improved hydrilla control could be obtained by combining products and using them in low concentrations. Treatments included 1, 2, and 3 mg dipotassium salt of endothall L<sup>-1</sup> used alone and in combination with 0.5 mg Cu L<sup>-1</sup>, 0.5 mg diquat L<sup>-1</sup>, or 0.2 mg endothall (mono(N,N-dimethylalkalamine salt)) L<sup>-1</sup>. A 24-hour dissipation half-life was simulated and shoot biomass was harvested 3 and 6 weeks after treatment (WAT). Endothall (dipotassium salt) applied singularly at 2 and 3 mg L<sup>-1</sup> resulted in significant reductions in biomass 6 WAT compared to the untreated reference, or >95% control. All combinations significantly decreased the above ground biomass 6 WAT compared to the untreated reference. The dipotassium salt of endothall combined with copper or diquat provided >99% control even at the lowest rate of endothall (1 mg L<sup>-1</sup>). Possible benefits of such applications include reduced herbicide quantity, improved selectivity and control of target plants, and reduced contact times.

**Key words:** Endothall, diquat, copper, Aquathol K, Hydrothol 191, Cutrine-Plus, Reward, herbicide combination.

## INTRODUCTION

Combinations of herbicides have frequently been used to control weeds in agricultural settings. Use of herbicide combinations is increasing in the management of invasive aquatic plants. However, only anecdotal evidence on the effectiveness of herbicide combinations has been reported and laboratory experimentation has been limited. Previous research

has shown that control of target plants may be increased by using herbicides in combination (Mackenzie and Hall 1967, Sutton et al. 1970, Sutton et al. 1972). Mackenzie and Hall (1967) demonstrated a "quicken knockdown" of hydrilla (*Hydrilla verticillata* (L.f.) Royle) when copper sulfate was applied in combination with diquat; however, the authors were reluctant to fully attribute the decline to the combination.

In two related trials, the effect of diquat on the uptake of copper (Sutton et al. 1970) and the effect of copper on the uptake of diquat (Sutton et al. 1972) were evaluated. When combinations of copper and diquat were used to treat hydrilla and southern naiad (*Najas guadalupensis* (Sprengel) Magnus), 77 percent and 38 percent more copper, respectively, was found in the plant tissue compared to using copper alone. Additionally, reduced amounts of copper were found in the water column of pools treated with the combination of diquat plus copper, suggesting increased uptake of copper (Sutton et al. 1970). By using <sup>14</sup>C labeled diquat, higher levels of radioactivity and decreased dry plant weight were found when diquat was combined with copper sulfate versus diquat applied alone (Sutton et al. 1972). Improved diquat uptake may have been due to increased permeability caused by the copper (Sutton et al. 1972), which increases free-radical production (Gupta et al. 1996).

Identifying and understanding interactions between herbicides may permit more effective aquatic weed control by extending control periods, reducing overall herbicide rates, improving selectivity of target species, and enhancing control in flowing systems that have short herbicide contact times.

Concentration exposure time (CET) relationships indicate that if the dipotassium salt of endothall (7-oxabicyclo [2,2,1] heptane-2,3-dicarboxylic acid) is exposed to the target species for an extended period, less herbicide is required to achieve the desired level of control (Netherland et al. 1991). CET relationships using endothall indicate 2.0 mg ae equivalent (ae) L<sup>-1</sup> is required to adequately control hydrilla when the exposure time is at least 48 hours. Reduced rates of product may be required if CET and herbicide combination relationships are established.

The objectives of this study were to evaluate the efficacy of the dipotassium salt of endothall (Aquathol K, Cerexagri, Inc., Philadelphia, PA, hereafter referred to as AK) used alone and in combination with either diquat (6,7-dihydrodipyrido (1,2-a:2',1'-c) pyrazinediium dibromide, Reward, Syngenta, Wilmington, DE), chelated copper (Cutrine-Plus, Applied Biochemists, Milwaukee, WI), or the mono(N,N-dimethylalkalamine) salt of endothall (Hydrothol 191, Cerexagri, Inc., Philadelphia, PA, hereafter referred to as H191) on hydrilla.

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## METHODS AND MATERIALS

This greenhouse study was conducted at the Lewisville Aquatic Ecosystem Research Facility (LAERF), Lewisville, TX, USA (33°04'45"N, 96°57'33"W) during the summer of 1999. Sediment was obtained from a dry LAERF pond, amended with 2 g ammonium sulfate, 1.5 g Osmocote (18-6-12), mixed, placed in 250 ml plastic containers, and covered with sand to prevent loss of sediment and nutrients into the overlying water. Each container was planted with two apical shoots of hydrilla collected from the San Marcos River, San Marcos, Texas (29°56'14"N, 97°55'28"W).

At the time of planting, shoots were approximately 15 to 20 cm long and generally had at least one axillary branch. Eight planted containers were placed in each of eight 50-L aquariums. Eight aquariums were placed in six larger tanks (1.5 m × 0.8 m × 1 m), filled with water, and used as water baths to maintain a constant temperature at 30 ± 2C using chillers (Remcor Products Company, Glendale Heights, IL). Day length was maintained at 14 hours by using two Aquasun (Ultraviolet Resources International, Cleveland, OH) wide spectrum bulbs (110 watt) over each large tank. Water for the aquariums was obtained from Lewisville Lake, a nearby Corps of Engineers reservoir. Prior to use, water was treated with aluminum sulfate and filtered through sand to reduce algae growth. Plants were grown for approximately three weeks and averaged about 60 cm in height prior to treatment.

Treatment combinations were replicated three times and included 16 herbicide/copper combinations and an untreated reference (Table 1). Herbicide treatments were applied using a simulated 24-hour dissipation half-life. At 24 hours after treatment, half the water was drained from each aquarium, replaced with untreated water and the process was repeated once a day for two additional days. Biomass samples were obtained by removing four containers from each aquarium and harvesting above ground biomass at 3 and 6 weeks after treatment (WAT). Biomass samples were dried to a constant weight at 80C.

Effects of endothall (at three levels) in combination with other compounds on biomass were tested with two-way ANOVA at three and six weeks ( $p < 0.05$ ) (Statgraphics Plus for Windows 3.1 1997). ANOVAs were applied using log (x + 1)-transformed data. Multiple comparison of means were made using least significant difference (LSD) test ( $p < 0.05$ ).

## RESULTS AND DISCUSSION

There was a significant interaction effect between AK and the other compounds on biomass at three and six weeks. The effect of AK on biomass was dependent upon the other product present. At 3 WAT, the biomass of all treated plants, except those treated with H191 alone, was significantly reduced compared to the untreated reference (Figure 1). By using AK alone at progressively increasing concentrations (1, 2, and 3 mg L<sup>-1</sup>), significant reductions in biomass were measured, though viable plant tissue was still observed even at the recommended label rate of 3 mg L<sup>-1</sup> AK (Figure 1A). When 2 or 3 mg L<sup>-1</sup> AK was used in combination with H191 biomass was significantly reduced compared to the untreated reference and H191 used alone. Viable shoot material was still present at 3 WAT (Figure 1B). All application rates of diquat provided improved control compared to the untreated reference and a significant reduction in biomass was observed in all combination levels of AK + diquat (Figure 1C). Additionally, all application rates of copper provided significant biomass reduction compared to the untreated reference and additional decreases were observed when copper was combined with all rates of AK (Figure 1D).

At 6 WAT, biomass of all treatments except for H191 alone was again significantly lower than the untreated reference (Figure 1). Significant decreases in biomass were observed between 1 mg L<sup>-1</sup> AK and the 2 and 3 mg L<sup>-1</sup> AK treatments, and viable stem tissue was absent in those aquariums treated with 3 mg L<sup>-1</sup> AK (Figure 1A). Similar to 3 WAT, combination of H191 with AK reduced biomass significantly compared to the untreated reference and using the H191 alone; however, viable shoot material was still present at all treatment levels at 6 WAT (Figure 1B). All combinations of AK + diquat (Figure 1C) and AK + copper (Figure 1D) continued to provide substantial control through 6 WAT and no appreciable viable plant material was observed in any copper/AK combinations.

According to CET relationships of dipotassium salt of endothall, a 24-hour exposure time should reduce hydrilla biomass by about 70% when treated with 2 mg ae L<sup>-1</sup> and 85-100% when treated with 3 mg ae L<sup>-1</sup> (Netherland et al. 1991). Results of this small-scale evaluation demonstrated that a 24-hour exposure time resulted in >95% control using a reduced rate of the dipotassium salt of endothall (1 mg ai L<sup>-1</sup>) in combination with copper (0.5 mg L<sup>-1</sup>) or diquat (0.5 mg L<sup>-1</sup>),

TABLE 1. HERBICIDE/COPPER COMBINATIONS USED AGAINST *HYDRILLA VERTICILLATA* (L.F.) ROYLE. APPLICATION RATES ARE GIVEN AS MG ACTIVE INGREDIENT (AI) L<sup>-1</sup>. THE DIPOTASSIUM SALT OF ENDOTHALL AS AQUATHOL K, IS REFERRED TO AS AK AND THE MONO(N,N-DIMETHYLALKYLAMINE) SALT OF ENDOTHALL AS HYDROTHOL 191, IS REFERRED TO AS H191.

Product	Concentration (mg ai L <sup>-1</sup> )	Endothall (AK)			
		0.0	1.0	2.0	3.0
Untreated Reference		X			
Endothall (AK)			X	X	X
Endothall (H191)	0.2	X	X	X	X
Diquat	0.5	X	X	X	X
Copper	0.5	X	X	X	X

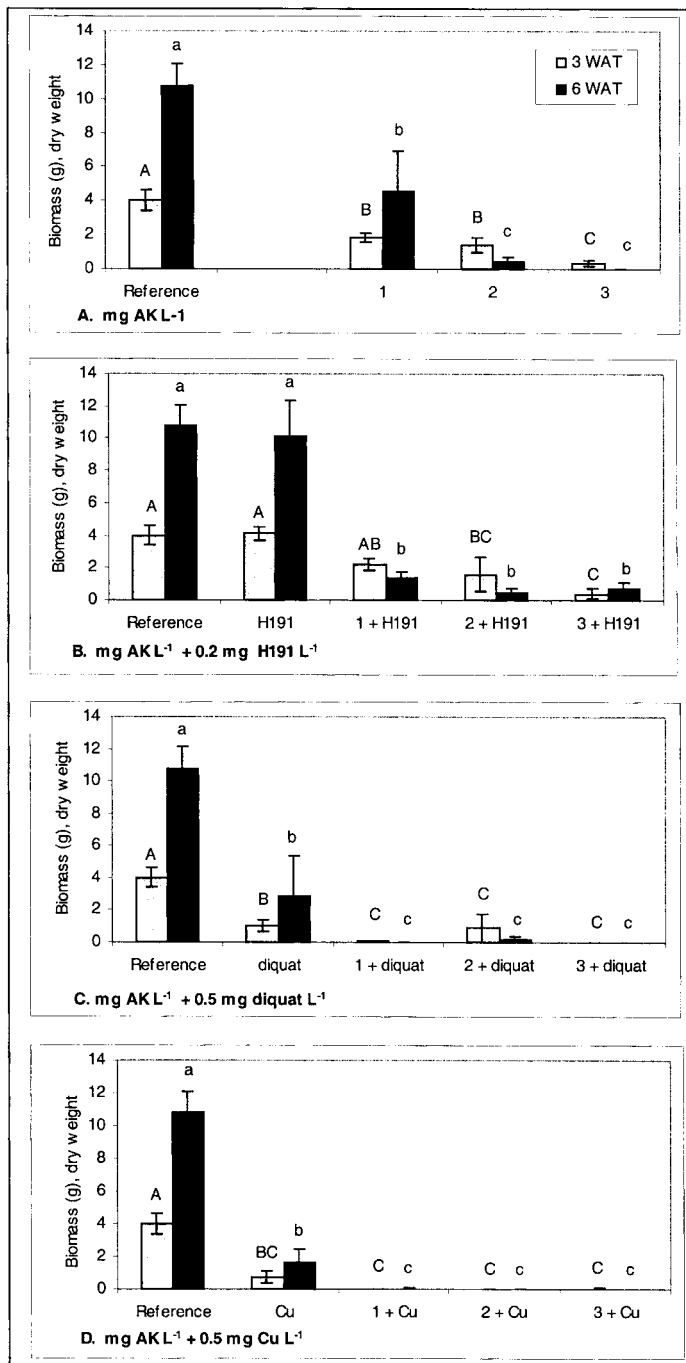


Figure 1. Shoot biomass (g dry weight), of *Hydrilla verticillata* (L.f.) Royle after treatment with A) the dipotassium salt of endothall as Aquathol K (AK) at 1, 2, and 3 mg active ingredient (ai) L<sup>-1</sup>, B) AK in combination with the mono(N,N-dimethylalkalamine) salt of endothall as Hydrothol 191 (H191) at 0.2 mg ai L<sup>-1</sup>, C) AK in combination with diquat as Reward at 0.5 mg/a.i. L<sup>-1</sup>, and D) AK in combination with chelated copper as Cutrine-Plus at 0.5 mg/a.i. L<sup>-1</sup>. Gray bars indicate biomass after 3 weeks after treatment (WAT) and black bars indicate biomass after 6 WAT. Upper case letters indicate least significant differences (LSD) ( $\alpha < 0.05$ ) between treatments 3 WAT while lower case letters indicate LSD between treatments 6 WAT. Error bars denote  $\pm$ SE where n = 3.

resulting in the same level of control as endothall applied alone at 3 mg ai L<sup>-1</sup>.

The Cutrine-Plus label for copper recommends using 0.4 to 1.0 mg Cu L<sup>-1</sup> for the control of hydrilla. In this study, 0.5 mg Cu L<sup>-1</sup>, reduced biomass at 3 WAT, but plants were recovering by 6 WAT. Application of Cu in combination with AK resulted in greater and longer control of hydrilla than Cu applied at the low rate alone.

The Reward label for diquat recommends using 18.76 L ha<sup>-1</sup> (2 gallons acre<sup>-1</sup>) when the average depth is 1.83 m (6 feet), the approximate equivalent of 0.46 mg L<sup>-1</sup>. In this study, when 0.5 mg L<sup>-1</sup> was applied alone substantial re-growth was observed by the 6 WAT evaluation; however, when combined with even 1 mg ai L<sup>-1</sup> AK, more than 95% control was observed.

Because the lowest rates of AK combined with Cu and AK combined with diquat were as effective in controlling hydrilla as the higher combination rates, the lower limits of herbicide and copper application rates were not determined. Further reductions in product rates, particularly copper and diquat, may still be possible without sacrificing hydrilla control.

A 24-hour half-life dissipation was used in this study to mimic conditions with reduced flow and calm conditions. Under higher water exchange conditions, such as unprotected windy areas, exposure times could be significantly less than were simulated in this study. Under such circumstances, higher rates of herbicides may be required to achieve adequate plant control. Depending on the actual exposure time, even the maximum label rate of a herbicide may not provide adequate control, and repeated applications may be necessary. Combining the dipotassium salt of endothall with copper or diquat could aid in mitigating short exposure time conditions and improve target plant control.

Results of these evaluations indicate that herbicides applied in combination, specifically Aquathol K/Cutrine-Plus and Aquathol K/Reward, can provide excellent control of hydrilla using lower application rates. Combinations may provide improved control of target plants growing in areas impacted by high water exchange rates. Field demonstrations will be conducted on Toledo Bend Reservoir, Louisiana using rates from these small-scale studies.

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