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Integrated Weed Management Strategies for Control of Hydrilla

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PURPOSE: This technical note describes the results of a laboratory investigation conducted to evaluate the effectiveness of the herbicide diquat (6,7-dihydrodipyrido[1,2-*a*:2',1'-*c*]pyrazinediium), and the fungal pathogen *Mycoleptodiscus terrestris* (Gerd.) Ostazeski, applied alone and in combination with one another, as an integrated weed management strategy against the nuisance aquatic plant, hydrilla (*Hydrilla verticillata* (L.f.) Royle).

BACKGROUND: Integrated weed management (IWM) can be defined as the application of multiple technologies (chemical, biological, and cultural) for the purpose of reducing pest plant populations. It involves the deliberate selection, integration, and implementation of effective weed control strategies, while considering economic, ecological, and sociological consequences (Thill et al. 1991). Adopting an IWM program can reduce costs and chemical loading to the environment, provide better and longer-term weed control, reduce the development of herbicide-resistant weed populations, and expand product selectivity.

Integrated weed management practices have been largely overlooked as an approach for controlling submersed aquatic weeds. However, recent studies have shown that combining the indigenous fungal pathogen, *M. terrestris*, with low doses of herbicides has excellent potential as an integrated strategy for long-term management of hydrilla (Shearer and Nelson 2002; Nelson et al. 1998; Netherland and Shearer 1996). Netherland and Shearer (1996) showed that applying a sub-lethal dose of fluridone (2 μg L^{-1}) with *M. terrestris* at rates of 100 and 200 colony-forming units (cfu) ml^{-1} reduced hydrilla biomass by more than 90 percent and was more efficacious than applying either control agent alone. The integrated treatment provided the benefits of rapid biomass reduction exhibited by *M. terrestris* and the long-term prevention of hydrilla regrowth by fluridone. In addition, these researchers estimated that integrated treatments reduced fluridone exposure requirements by approximately 50 days, which could potentially broaden the use of this product in many aquatic systems. Further studies conducted under the Aquatic Plant Control Research Program have shown that fluridone contact time can be reduced by as much as 69 days when combined with *M. terrestris* (unpublished data). A 21-day contact to fluridone at 5 μg L^{-1} combined with 0.028 g L^{-1} *M. terrestris* (as a dry inoculum) controlled 97 percent hydrilla with no regrowth during the 90-day study. Another advantage for integrating herbicides with *M. terrestris* is increased selectivity. Nelson et al. (1998) showed that integrated treatments using lower fluridone rates minimized injury to native plant species. Similar results have also been documented when integrating *M. terrestris* with the herbicide endothall (Shearer and Nelson 2002). Overall, the results of these early investigations demonstrated that *M. terrestris*, combined with herbicides, can reduce herbicide use rates, minimize contact time requirements, and improve selectivity.

The contact herbicide diquat is effective for controlling hydrilla when high use rates are applied under static conditions (Frank et al.1979; Skogerboe et al. 2006). However, diquat performance can be variable under some field conditions (Frank et al. 1979; Van et al. 1987). Inconsistent performance can often be attributed to a failure to maintain sufficient herbicide contact time requirements. Diquat dissipates rapidly in waters of natural aquatic systems largely due to adsorption by sediments, suspended particulate matter, and aquatic vegetation (Ritter et al. 2000; Simsiman and Chesters 1975). Efficacy is greatly improved when diquat is combined with copper, but copper toxicity to non-target organisms has prevented widespread use of this application method (Richardson 2008; Chiconela et al. 2007; Sutton et al. 1972; Frank et al. 1979). Combining diquat with *M. terrestris* may provide an alternate method for using diquat to successfully control hydrilla. Currently, there is no information on the compatibility of diquat applied as an integrated treatment with *M. terrestris*. The objectives of this study were to: compare the efficacy of diquat, *M. terrestris*, and integrated (diquat + *M. terrestis*) treatments against hydrilla; and determine the effect of herbicide contact time on treatment efficacy.

MATERIALS AND METHODS: This research was conducted in 55-L aquarium systems housed in a large, controlled-environment growth chamber located at the U.S. Army Engineer Research and Development Center (ERDC),Vicksburg, MS. Lighting, photoperiod, and temperature were maintained for optimal hydrilla growth (580 $\pm$ 50 μmol photons m^{-2} sec^{-1}, 14:10-hr light:dark photoperiod; 22 °C).

Hydrilla was collected from a research pond located at the Center for Aquatic and Invasive Plants, Gainesville, FL, and shipped to ERDC for use in this study. Hydrilla was propagated from 15-cm apical stem cuttings and planted in sediment-filled containers (946 ml). Sediment was collected from Brown's Lake, Vicksburg, MS, and amended with ammonium chloride at a rate of 0.2 g L^{-1} sediment. Six containers of hydrilla were placed in each aquarium filled with 52 L of an aquatic plant culture solution (Smart and Barko 1984). Plants were allowed to grow for 4 weeks prior to treatment application.

Treatments included 0.370 mg L^{-1} diquat at contact times of 2, 4, and 8 hr; 0.185 mg L^{-1} diquat for 8 hr; 0.014, 0.028, and 0.042 g L^{-1} *M. terrestris*; all combinations of both agents at each rate and contact time; and an untreated control. Diquat was applied as the commercial formulation Reward (Syngenta Crop Protection, Greensboro, NC). After each designated diquat contact time, aquariums were emptied and refilled with fresh water three times to remove herbicide residues. The fungal pathogen was applied as a dry, granular inoculum currently under development as a mycoherbicide at the U.S. Department of Agriculture, Agriculture Research Service, National Center for Agriculture Utilization Research (USDA-ARS-NCAUR) in Peoria, IL, in cooperation with ERDC (Shearer and Jackson 2003, 2006; Shearer 2002). Dry inoculum was spread evenly onto the water surface by hand and allowed to naturally dissipate onto plant surfaces. Herbicide and pathogen treatments were applied sequentially, with the fungal inoculum applied immediately following each herbicide contact time drain-fill procedure. Plant biomass was harvested 6 weeks after treatment (WAT) and dried to a constant weight at 70 °C.

Treatments were randomly assigned and replicated three times. Data were subjected to analysis of variance procedures using Statistical Analysis System (SAS) software. When significant treatment effects were found, means were separated using the Waller-Duncan k-ratio t test at the 0.05 level of significance.

RESULTS AND DISCUSSION: Treatment effects on hydrilla shoot biomass are presented in Table 1. Compared to untreated plants, *M. terrestris* applied alone at 0.028 and 0.042 g L^{-1} reduced hydrilla by 38 percent. Lower rates of *M. terrestris* were ineffective.

All rates and contact times of diquat applied alone significantly reduced hydrilla growth. The most effective treatment was an 8-hr exposure to 0.370 mg diquat L^{-1}, which reduced plant biomass by 87 percent. Diquat was less effective when applied alone at shorter contact times (< 8 hr). Studies by Van et al. (1987) also showed that diquat efficacy was reduced as contact time decreased.

Combining diquat with *M. terrestris* improved hydrilla control under these experimental conditions. Compared to untreated plants, a 2-hr exposure to 0.370 mg diquat L^{-1} combined with 0.042 g L^{-1} *M. terrestris* reduced hydrilla by 99.6 percent. Diquat and pathogen applied alone at these rates and contact times only controlled plants by 70 and 41 percent, respectively. Similar synergistic interactions were noted with treatment combinations of 0.370 mg diquat L^{-1} with either 0.028 or 0.042 g *M. terrestris* L^{-1} for a 4-hr contact time. An 8-hr exposure to diquat at 0.185 mg L^{-1} combined with *M. terrestris* (all rates) was also effective, reducing hydrilla by an average of 91 percent. Similar exposure to diquat alone at this rate only controlled 52 percent of hydrilla.

Overall, the results of this study showed that diquat is compatible with *M. terrestris* as an integrated application strategy for controlling hydrilla. Combining herbicide and pathogen reduced hydrilla biomass better than either agent applied alone. Enhanced weed control when herbicides are applied in combination with fungal pathogens has been reported on other plant species (both terrestrial and aquatic) (Nelson and Shearer 2005; Christy et al. 1993; Hoagland 1996; Sorsa et al. 1988; Wymore et al. 1987). Diquat contact time and rate were also reduced when herbicide and pathogen were applied in tandem. The results demonstrate a viable and consistent alternative for controlling hydrilla using an IWM approach.

Efforts are ongoing to formulate *M. terrestris* as a marketable bioherbicide for use in aquatic plant management. Knowledge of potential use patterns for this product will expedite successful operational use in the future. Furthermore, herbicide resistance and enhanced degradation issues that have been reported in Florida (Richardson 2008; Michel et al. 2004) will dictate the development and use and/or rotation of alternate management strategies in the near future. While diquat resistance has not been reported in hydrilla, it has been documented in another aquatic plant, *Landoltia punctata* (G. Mey.) D.H. Les & D.J. Crawford (Koschnick et al. 2006). Identifying new application tactics that may lower use rates and contact times, provide long-term efficacy, prevent the development of herbicide resistance and minimize injury on non-target species, would allow greater flexibility for using herbicides in aquatic systems.

Table 1. Mean dry weight biomass of hydrilla 6 weeks following treatment with Diquat, *M. terrestris*, and combined treatments of Diquat and *M. terrestris*. Contact time varied among treatments as noted.

Treatment	Dry Weight Biomass (g)
Untreated Control	16.06 a
***M. terrestris* alone:**	
0.014 g L^{-1}	16.09 a
0.028 g L^{-1}	10.62 b
0.042 g L^{-1}	9.40 b
Diquat alone:	
0.370 mg L^{-1} for 2 hr	4.78 cde
0.370 mg L^{-1} for 4 hr	8.05 bc
0.370 mg L^{-1} for 8 hr	2.10 ef
0.185 mg L^{-1} for 8 hr	7.70 bcd
Simultaneous Applications (Diquat + *M. terrestris*):	
0.370 mg L^{-1} + 0.014 g L^{-1} for 2 hr	7.48 bcd
0.370 mg L^{-1} + 0.028 g L^{-1} for 2 hr	4.12 c–f
0.370 mg L^{-1} + 0.042 g L^{-1} for 2 hr	0.06 f
0.370 mg L^{-1} + 0.014 g L^{-1} for 4 hr	3.69 c–f
0.370 mg L^{-1} + 0.028 g L^{-1} for 4 hr	3.41 def
0.370 mg L^{-1} + 0.042 g L^{-1} for 4 hr	0.51 ef
0.370 mg L^{-1} + 0.014 g L^{-1} for 8 hr	2.15 ef
0.370 mg L^{-1} + 0.028 g L^{-1} for 8 hr	0.01 f
0.370 mg L^{-1} + 0.042 g L^{-1} for 8 hr	0.01 f
0.185 mg L^{-1} + 0.014 g L^{-1} for 8 hr	3.34 def
0.185 mg L^{-1} + 0.028 g L^{-1} for 8 hr	0.75 ef
0.185 mg L^{-1} + 0.042 g L^{-1} for 8 hr	0.11 f

Means followed by a different letter are significantly different according to Waller-Duncan k-ratio t test at $p \geq 0.05$; n = 3.

FUTURE WORK: Future research will explore the integration of *M. terrestris* with new herbicides currently under investigation for use in aquatic habitats as well as determine the mechanism of synergy between herbicide and pathogen. In addition, collaborative research between ERDC, USDA-ARS-NCAUR, and SePRO Corporation will continue under a Cooperative Research and Development Agreement (CRADA) and the Aquatic Plant Control Research Program to develop *M. terrestris* as a bioherbicide formulation for submersed aquatic weed control.

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