

Integrated Use of Fluridone and A Fungal Pathogen for Control of Hydrilla

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ABSTRACT

Combinations of the herbicide fluridone {1-methyl-3-phenyl-5-[3-(trifluoromethyl) phenyl]-4(1H)-pyridinone} and the microbial pathogen *Mycocleptodiscus terrestris* (Gerde-mann) Ostazeski (*Mt*) were tested for efficacy under controlled-environment conditions against dioecious hydrilla (*Hydrilla verticillata* (L.f.) Royle. Fluridone rates of 2, 5, and 12 µg/L, *Mt* rates of 25, 50, 100 and 200 colony forming units (CFU) per ml, and integrated treatments of 2, 5, and 12 µg/L + 100 and 200 CFU/ml, and 12 µg/L + 25 and 50 CFU/ml were tested. Although a dose response was noted among fluridone rates, all treatments resulted in linear decreases in biomass, photosynthesis (PTS) and chlorophyll from 14 through 94 days posttreatment. In contrast, *Mt* applications of 25 and 50 CFU/ml were ineffective throughout the study. *Mt* at 25 and 50 CFU/ml + 12 µg/L fluridone showed no differences from the 12 µg/L fluridone treatment alone. As *Mt* rates were increased to 100 and 200 CFU/ml, severe initial injury was noted within 5 d; however, PTS and chlorophyll showed strong signs of recovery by 14 d and biomass was fully recovered by 28 d posttreatment. Fluridone at all rates + *Mt* at 100 and 200 CFU/ml produced rapid injury and biomass reductions of > 90% by 28 d posttreatment. The lack of intact viable tissue prevented sampling of physiological variables past 28 d. No differences were noted between the integrated treatments, indicating a lack of dose response. *Mt* at rates of 100 and 200 CFU/ml resulted in rapid plant injury; however, only short-term control was achieved. Continuous exposure to fluridone resulted in a steady reduction in biomass over time. Integrating fluridone with *Mt* at rates of 100 and 200 CFU/ml greatly enhanced control, reduced exposure requirements, and increased susceptibility of hydrilla to fluridone at a rate (2 µg/L) that was not otherwise lethal.

Key words: *Hydrilla verticillata*, mycoherbicide, integrated control, *Mycocleptodiscus terrestris*.

INTRODUCTION

The systemic herbicide fluridone has been shown to inhibit growth of the submersed exotic plant hydrilla at concentrations ranging from 1 to 10 µg/L; however, at these rates an extended exposure period (> 10 weeks) is required to achieve plant control ((MacDonald et al. 1993, Doong et al. 1993, Netherland et al. 1993, Fox et al. 1994, Netherland

and Getsinger 1995a and b). These exposure time requirements continue to limit fluridone use in many aquatic systems. Fluridone application rates are approaching their lower threshold, and therefore new strategies for reducing exposure time requirements may provide the best alternatives for improving control. One approach that deserves consideration is the possible integration of fluridone applications with microbial pathogens to enhance efficacy.

Although many microbes associated with aquatic macrophytes may act as weak pathogens, healthy plants are able to ward off infection and disease epidemics are rare. Several investigators have proposed using sub-lethal herbicide rates to stress submersed vegetation or inhibit growth and increase susceptibility to pathogens (Charadattan 1986, Sorsa et al. 1988, Kerfoot 1989). Integrating the contact herbicide endothall and the endemic fungal pathogen *Colletotrichum* sp. for control of *Myriophyllum spicatum* (Sorsa et al. 1988) or applying the systemic herbicide fluridone with isolates of various endemic fungal species on *Ceratophyllum demersum* (Smit et al. 1990) has been reported to increase efficacy in comparison with either treatment alone.

One strategy that has received limited study in aquatic systems but has proved successful in some terrestrial systems (Templeton et al. 1979, Harris 1993), is inundation of a susceptible plant population with a known pathogen. Implicit in the inundative biocontrol approach is the derivation of the control organism from the plant ecosystem, to which it is returned as an invasive and ultimately lethal agent (Gunner et al. 1990). Charadattan (1990) reviewed inundative biocontrol in aquatic systems and expressed concern regarding the practicality, effectiveness, and environmental consequences of inoculating large water bodies to produce epidemics on the scale necessary to control aquatic plants. Nonetheless, he encouraged research to identify more efficacious pathogens and integration of these pathogens with herbicides.

The fungal pathogen *Mycocleptodiscus terrestris* (*Mt*), was recently isolated from hydrilla tissue and has been identified as a candidate for inundative control of hydrilla (Joye 1990, Shearer 1993). Electron microscopy studies confirmed that *Mt* mycelia can penetrate through the cell wall, spread throughout plant tissues, and cause the rapid collapse of hydrilla (Joye and Paul 1991). Although laboratory and field studies have shown that *Mt* provides a rapid knockdown of hydrilla, vigorous regrowth has been noted within 2 to 4 weeks posttreatment (Shearer personal communication).

Integrating a slow-acting systemic herbicide such as fluridone with a rapid-acting microbial herbicide such as *Mt* may improve hydrilla control by providing: 1) initial biomass control and loss of carbohydrate reserves following *Mt* treatment; 2) increased susceptibility of injured hydrilla to

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fluridone; and 3) reduced fluridone exposure time requirements. The objectives of this study were to compare the efficacy of fluridone, *Mt*, and integrated fluridone + *Mt* treatments against dioecious hydrilla.

MATERIALS AND METHODS

Studies were conducted in an experimental system that has been previously described for fluridone concentration and exposure time studies conducted at the US Army Engineer Waterways Experiment Station (WES), Vicksburg, MS (Netherlands et al. 1993, Netherlands and Getsinger 1995b). The system consists of 52, 55 L aquaria located in a controlled-environment room with a temperature of 26 ± 2 C, light intensity of 570 ± 60 $\mu\text{mol}/\text{m}^2/\text{sec}$, and photoperiod of 14L:10D. Aquaria were filled with a water culture solution recommended for aquatic macrophyte growth by Smart and Barko (1984).

Dioecious hydrilla was obtained from the Suwannee River, FL, and sediment (enriched with 200 mg $\text{NH}_4\text{Cl}/\text{L}$) was collected from Brown's Lake at the WES. Eleven glass beakers (300 ml) filled with 250 ml of sediment and containing four 10 to 15 cm hydrilla apical cuttings were placed in each aquarium. One volume of water was exchanged every 48 h via peristaltic pumps and air was gently bubbled into each aquarium to provide water circulation.

Hydrilla formed a surface canopy by 28 days. At this time, one beaker was removed from each aquarium to provide an estimate of pretreatment biomass. Following collection, plants were dried at 70C for 48 h. Pretreatment dry weight (DW) of shoot and root biomass in each aquarium was 11.7 ± 1.3 g DW (128 g DW m^2) and 1.8 ± 0.22 g DW respectively. Shoot biomass approximates spring to early summer values reported for hydrilla (Harlan et al. 1985).

At the time of treatment, the flow-through water system was deactivated. Fluridone stock solutions were prepared from the commercial formulation Sonar[®] AS (479 grams active ingredient per liter) and treatment concentrations are reported as $\mu\text{g}/\text{L}$. *Mt* was applied as the live fungal mycelium with the final fungal slurry having a thick consistency and a colony forming unit (CFU) count of 1×10^6 CFUs/ml. *Mt* treatment concentrations are reported as CFU/ml. Fluridone and *Mt* were dispensed to the water surface in individual pipettors and allowed to disperse through the water column. Treatment concentrations are reported in Table 1. Each treatment was replicated three times using a completely randomized design.

At 14, 28, 42, 60, and 94 days after treatment (DAT) two beakers were removed from each aquarium and DW of all shoot material was measured. Net photosynthesis (PTS) and total chlorophyll (a and b) were measured on 4-cm apical cuttings at 7, 14, 28, 42, 60, and 94 DAT. Four apices per aquarium were collected at each sample period and placed in a 300 ml BOD bottle filled with culture water. Bottles were placed in a Percival[®] growth chamber under constant temperature (25 ± 0.5 C) and light intensity (250 ± 30 $\mu\text{moles}/\text{m}^2/\text{sec}$). PTS was monitored following an incubation period of 45 to 60 minutes (Netherlands and Lembi 1992). Total chlorophyll was measured using a DMSO extraction procedure (Hiscox and Israelstam 1979).

Studies were conducted in February and June 1994 and initial data analyses indicated no significant differences existed between the studies. Therefore data from the two experiments were pooled and reanalyzed. At each sampling interval biomass, PTS, and chlorophyll were subjected to analysis of variance and treatment means compared using Fisher's LSD test at the 0.05 level. Regression analysis was used to determine the relationship between plant response over time at each treatment concentration.

RESULTS AND DISCUSSION

Results showed dramatic differences in the pattern of initial and long-term hydrilla response to fluridone, *Mt*, and the integrated fluridone + *Mt* treatments. Untreated hydrilla increased in biomass and maintained steady PTS rates and chlorophyll content throughout the study (Tables 1, 2, and 3).

Following fluridone treatment at rates of 5 and 12 $\mu\text{g}/\text{L}$, bleaching of apical tips was noted within five days. The 2 $\mu\text{g}/\text{L}$ treatment produced limited visual symptoms through 28 DAT, but was only growth inhibitory, as evidenced by static biomass levels and continued reductions in chlorophyll content and photosynthetic rates from 28 through 94 DAT (Tables 1, 2, and 3). Hydrilla response to fluridone treatments of 5 and 12 $\mu\text{g}/\text{L}$ resulted in significant linear reductions in biomass, PTS and chlorophyll concentration over time. Significant differences among treatment rates were

TABLE 1. MEAN DRY WEIGHT (DW) BIOMASS OF HYDRILLA SHOOT TISSUE OVER TIME FOLLOWING APPLICATION OF FLURIDONE, *Mt* OR A COMBINATION OF FLURIDONE AND *Mt*.

Treatment $\mu\text{g}/\text{L}$ + CFU ¹	Hydrilla biomass (g DW/harvest)					Linear response ⁵
	Days after treatment ²					
	14	28	42	60	94	
Untreated	5.5 a	9.4 a	12.7 a	16.2 a	21.5 a	$r^2=0.97$
2 + 0	5.3 a	8.6 a	9.6 b	9.5 c	7.6 c	NS
5 + 0	4.7 ab	4.5 b	3.8 c	2.5 d	1.8 d	$r^2=0.90$
12 + 0	4.1 b	3.8 bc	1.9 d	1.3 de	0.8 de	$r^2=0.81$
0 + 25	5.6 a	9.2 a	12.9 a	16.4 a	20.5 a	$r^2=0.95$
0 + 50	4.4 b	8.8 a	12.7 a	16.3 a	21.3 a	$r^2=0.96$
0 + 100	1.4 c	3.9 bc	11.8 a	15.7 a	20.8 a	$r^2=0.93$
0 + 200	1.0 cd	2.9 c	9.4 b	13.9 b	18.5 b	$r^2=0.93$
12 + 25	4.0 b	3.0 c	1.5 de	1.2 de	0.9 de	$r^2=0.77$
12 + 50	4.3 b	2.8 c	1.2 de	1.1 de	0.5 de	$r^2=0.74$
2 + 100	1.8 c	0.8 d	0.7 de	0.5 e	0.2 e	$r^2=0.76$
5 + 100	1.4 c	0.8 d	0.3 e	0.2 e	0.2 e	$r^2=0.66$
12 + 100	1.3 c	0.6 d	0.2 e	0.1 e	0.0 e	$r^2=0.70$
2 + 200	1.3 c	0.7 d	0.4 e	0.5 e	0.1 e	$r^2=0.77$
5 + 200	0.9 cd	0.3 d	0.1 e	0.0 e	0.0 e	$r^2=0.61$
12 + 200	0.5 d	0.2 d	0.2 e	0.0 e	0.0 e	$r^2=0.68$
LSD	0.72	0.95	1.24	1.68	1.41	

¹ $\mu\text{g}/\text{L}$ = fluridone concentration and CFU = colony forming units of *Mt*

²Values followed by a different letter are significantly different within each sampling interval according to an LSD test at the 0.05 level of confidence.

³Test for linear response of biomass over exposure time within each treatment rate. R-square values based on simple linear regression. NS = not significant at the 0.05 level of confidence.

TABLE 2. NET PHOTOSYNTHESIS (PTS) OF HYDRILLA SHOOT APICES SAMPLED OVER TIME FOLLOWING APPLICATION OF FLURIDONE, MT, OR A COMBINATION OF FLURIDONE AND MT.

Treatment µg/L + CFU ¹	Hydrilla Net PTS (µg O ₂ g ⁻¹ fr. wt.)						Linear response ³
	Days after treatment ²						
	7	14	28	42	60	94	
Untreated	32 a	29 a	33 a	30 a	31 a	29 a	NS
2 + 0	29 a	24 b	17 c	15 b	11 b	8 c	r ² =0.86
5 + 0	21 b	16 c	12 d	5 c	-1 c	-3 d	r ² =0.89
12 + 0	19 b	14 c	4 e	1 d	-3 c	-5 d	r ² =0.82
0 + 25	31 a	30 a	30 a	27 a	28 a	30 a	NS
0 + 50	18 b	28 a	33 a	28 a	31 a	27 a	NS
0 + 100	9 cd	17 c	31 a	27 a	31 a	24 b	NS
0 + 200	8 cd	16 c	24 b	29 a	29 a	26 ab	NS
12 + 25	18 b	15 c	5 e	0 d	-4 c	-4 d	r ² =0.79
12 + 50	11 c	9 d	5 e	-1 d	-3 c	-6 d	r ² =0.86
2 + 100	9 cd	15 c	3 e	ND	-3 c	ND	NS
5 + 100	10 c	10 d	5 e	1 d	ND	ND	NS
12 + 100	5 d	7 de	2 e	ND	ND	ND	NS
2 + 200	6 d	9 d	5 e	2 cd	ND	NA	NS
5 + 200	4 d	4 e	3 e	ND	ND	ND	NS
12 + 200	3 d	5 e	-2 f	ND	ND	ND	NS
LSD	3.1	3.7	3.7	3.1	3.4	2.9	

¹µg/L = fluridone concentration and CFU = colony forming units of *Mt*

²Values followed by different letters are significantly different within each sampling interval (LSD at the 0.05 level). ND indicates no data was taken.

³Test for linear response of PTS rates over exposure time within each treatment rate. R-square values were determined based on regression analysis. NS indicates that no significant linear relationship existed.

TABLE 3. CHLOROPHYLL OF HYDRILLA SHOOT APICES SAMPLED OVER TIME FOLLOWING APPLICATION OF FLURIDONE, MT, OR A COMBINATION OF FLURIDONE AND MT.

Treatment µg/L + CFU ¹	Hydrilla chlorophyll content (mg g ⁻¹ fr. wt.)						Linear response ³
	Days after treatment ²						
	7	14	28	42	60	94	
Untreated	1.21 a	1.16 a	1.14 a	1.23 a	1.15 b	1.10 a	NS
2 + 0	1.18 a	1.07 b	0.93 c	0.77 c	0.62 c	0.48 b	r ² =0.94
5 + 0	1.04 b	0.71 d	0.42 d	0.27 d	0.20 d	0.08 c	r ² =0.82
12 + 0	0.68 d	0.43 gh	0.22 f	0.14 e	0.10 e	0.04 c	r ² =0.75
0 + 25	1.23 a	1.20 a	1.14 a	1.17 b	1.21 a	1.10 a	NS
0 + 50	0.97 b	1.10 ab	1.18 a	1.22 ab	1.13 b	1.08 a	NS
0 + 100	0.87 c	1.04 b	1.16 a	1.24 a	1.23 a	1.04 a	NS
0 + 200	0.59 e	0.92 c	1.07 b	1.26 a	1.13 b	1.10 a	NS
12 + 25	0.71 d	0.36 h	0.15 g	0.11 e	0.09 e	0.05 c	r ² =0.73
12 + 50	0.55 ef	0.35 h	0.17 fg	0.10 e	0.06 e	0.03 c	r ² =0.75
2 + 100	0.84 c	0.60 e	0.33 e	ND	0.01 f	ND	r ² =0.84
5 + 100	0.67 d	0.48 fg	0.21 f	0.02 f	ND	ND	r ² =0.88
12 + 100	0.52 ef	0.54 ef	0.18 fg	ND	ND	ND	r ² =0.80
2 + 200	0.83 c	0.53 cf	0.21 f	0.02 f	ND	ND	r ² =0.84
5 + 200	0.48 f	0.42 gh	0.13 g	ND	ND	ND	r ² =0.90
12 + 200	0.45 f	0.38 h	0.04 h	ND	ND	ND	r ² =0.85
LSD	.077	.072	.056	.052	.038	.066	

¹µg/L = fluridone concentration and CFU = colony forming units of *Mt*

²Values followed by different letters are significantly different within each sampling interval (LSD at the 0.05 level). ND indicates no data was taken.

³Test for linear response of PTS rates over exposure time within each treatment rate. R² values were determined based on regression analysis. NS indicates that no significant linear relationship existed.

also noted at several sampling times (Tables 1, 2, and 3). The inability to completely control hydrilla following extended laboratory exposure periods, and the initial dose response to low fluridone treatment rates has been noted in previous studies (Netherland et al. 1993, Netherland and Getsinger 1995 a,b). Furthermore, evidence from earlier studies suggests that hydrilla would have recovered had fluridone been removed from the water column during the course of the study (Netherland et al. 1993, Netherland and Getsinger 1995b).

The 25 CFU/ml *Mt* treatment produced no visual injury symptoms and remained comparable to the untreated reference throughout the study. While the 50 CFU/ml treatment resulted in some initial leaf discoloration, biomass was only reduced by 20% and PTS and chlorophyll were not different from untreated controls by 14 DAT (Tables 1, 2, and 3).

Following *Mt* treatments of 100 and 200 CFU/ml, hydrilla leaves became translucent, and stem degradation and defoliation occurred within 5 DAT. PTS and chlorophyll content were significantly reduced by 45 to 72% at 7 DAT (Tables 2 and 3). Although biomass was reduced 75 to 89% at 14 DAT, PTS and chlorophyll data suggested plants were recovering (Tables 1, 2 and 3). Biomass levels increased 3 fold between 14 and 28 DAT.

As *Mt* concentrations were increased, initial injury symptoms were more severe and biomass and physiological recovery were delayed. This dose-response was somewhat masked by the ability of all treatments to recover in a linear fashion over time (Table 1). Moreover, physiological data indicated that *Mt* produced no long-term stress (Tables 2 and 3). Initial injury followed by rapid regrowth has been observed during pilot trials with *Mt* conducted in mesocosms and ponds (J. Shearer personal communication).

Combining the 25 and 50 CFU/ml *Mt* treatments with fluridone at a rate of 12 µg/L showed symptoms and results that were very similar to the 12 µg/L fluridone treatment alone (Tables 1, 2, and 3). These results further suggest that *Mt* at rates lower than 100 CFU/ml were ineffective.

The fluridone + *Mt* treatments at 100 and 200 CFU/ml manifested symptoms similar to the *Mt* treatments alone as leaves became translucent and stems were defoliated within 5 DAT. Biomass was reduced by 67 to 89% at 14 DAT. Through 28 DAT, new shoots were sprouting from remaining stems and rootcrowns; however, these tips quickly manifested fluridone symptoms and PTS and chlorophyll content decreased by 50 to 100% (Tables 2 and 3). Biomass was reduced 92 to 98% at 28 DAT and continued to decrease throughout the remainder of the study (Table 1). Physiological sampling from 42 through 94 DAT was not conducted to the lack of viable plant tissue (Tables 2 and 3).

Although the combination of higher fluridone and *Mt* rates generally produced more severe initial injury symptoms, all treatment combinations were highly effective with few significant differences between treatments. By 94 DAT the 12 µg/L fluridone treatment alone had reduced biomass to a level that was not statistically different from the integrated treatments. However, differences in the quality of remaining plant tissue was noticed when comparing integrated and fluridone treatments.

In general, the integrated treatments resulted in a notable loss of tissue turgidity compared to plants treated with fluridone alone. This loss of turgidity (plants are described as mushy) has often been observed in hydrilla following field applications of fluridone. As noted earlier, the ability of fluridone (or other systemic herbicides) to inhibit growth and stress hydrilla may increase its susceptibility to endemic pathogenic attack.

While *Mt* applied alone as a live fungal mycelium resulted in rapid tissue injury at rates of 100 and 200 CFU/ml, lack of residual control resulted in the rapid recovery of hydrilla. Although *Mt* application rates were much lower (two to three orders of magnitude) than previous reports for pathogens controlling submersed macrophytes (Andrews et al. 1982, Sorsa et al. 1988, Charadattan 1990), work on formulating *Mt* in an appropriate delivery system and determining the feasibility of applying these rates in the field must be developed. The specificity of *Mt* for other plants also requires further testing. Gunner et al. (1990) reported *Mt* pathogenicity to *Myriophyllum spicatum* L. as rates were increased from 330 to 1650 CFU/ml, whereas no effects were observed on *Valisneria spp.*, *Elodea*, or *Sagittaria* at rates exceeding 1,000,000 CFU/ml.

Integrating fluridone and *Mt* provided the benefits of excellent initial biomass reduction exhibited by *Mt* along with long-term hydrilla control provided by fluridone. Fluridone exposure requirements were reduced by approximately 50 days when it was applied in combination with *Mt* at rates greater than 100 CFU/ml. Of significant note was the ability of the 2 µg/L treatment in conjunction with both rates of *Mt* to provide complete control of hydrilla. Future work will focus on repeating these results at the mesocosm and pond scale, and expanding studies to include beneficial native species in addition to hydrilla.

ACKNOWLEDGEMENTS

This research was conducted under the US Army Corps of Engineers Aquatic Plant Control Program, Environmental Laboratory, US Army Engineer Waterways Experiment Station. Permission was granted by the Chief of Engineers to publish this information. The authors would like to thank Anne Stewart, Janis Lanier, and Margaret Richmond for technical assistance. The cooperation of DowElanco for providing herbicide for this study is also greatly appreciated.

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