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Note

Integrated Use of Endothall and a Fungal Pathogen for Management of the Submersed Aquatic Macrophyte *Hydrilla verticillata*[1]

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Abstract: Laboratory experiments were conducted in 55-L aquaria to evaluate the efficacy of the aquatic herbicide endothall and the fungal pathogen *Mycoleptodiscus terrestris* (Gerd.) Ostazeski, applied alone and in combination against hydrilla. Treatments included 0.25, 0.50, and 1.25 mg ae/L endothall, 100, 200, and 400 colony-forming units (CFU)/ml *M. terrestris*, simultaneous integrated treatments of 0.25, 0.50, and 1.25 endothall + 100 or 200 CFU/ml *M. terrestris*, sequential integrated treatments of 100 and 200 CFU/ml *M. terrestris* + 0.25 and 0.50 mg ae/L endothall, and untreated controls. By 42 d after treatment (DAT), all treatments had significantly reduced shoot biomass levels of hydrilla compared with the untreated controls. Combining the two lowest herbicide rates with *M. terrestris* provided better hydrilla control than either treatment alone. Based on these results, an outdoor mesocosm study was conducted to evaluate the efficacy and selectivity of endothall and the pathogen applied alone and in combination against hydrilla, Illinois pondweed, American pondweed, and vallisneria. Treatments included 0.25 and 0.50 mg ae/L endothall, 100 and 200 CFU/ml *M. terrestris*, integrated treatments of 0.25 and 0.50 mg ae/L endothall + 100 and 200 CFU/ml *M. terrestris*, and untreated controls. Unlike the laboratory results, none of the treatments controlled hydrilla 100%. The combined treatments worked better than either treatment applied alone. By 42 DAT, all the combined treatments except 0.25 mg ae/L endothall + 100 CFU/ml *M. terrestris* had reduced above-ground hydrilla biomass by ≥ 90% compared with the untreated controls. All non-target species sustained varying amounts of injury from endothall and *M. terrestris* applied alone or in combination.

Nomenclature: Endothall; American pondweed, *Potamogeton nodosus* Poir. #[3] PTMNO; hydrilla, *Hydrilla verticillata* (L.f.) Royle # HYLLI; Illinois pondweed, *Potamogeton illinoensis* Morong # PTMIL; *Mycoleptodiscus terrestris* (Gerd.) Ostazeski; vallisneria, *Vallisneria americana* Michx.

Additional index words: Aquatic plant management, integrated pest management.

Abbreviations: CFU, colony-forming units; DAT, days after treatment; DW, dry weight; ERDC, Engineer Research and Development Center; LAERF, Lewisville Aquatic Ecosystem Research Facility.

INTRODUCTION

Hydrilla is ranked as one of the three most invasive aquatic weeds in the world (Soerjani 1986). Plant infestations can impede navigation, clog drainage or irrigation canals, affect water intake systems, interfere with recreational activities, and disrupt wildlife habitats. Hydrilla is well adapted both physiologically and morphologically to be an aggressive competitor in aquatic communities because it photosynthesizes at very low light levels, is insensitive to water quality, and produces several types of propagules (Holm et al. 1997).

Current management strategies for hydrilla control are generally based on the independent use of herbicides, biological organisms, mechanical harvesting, or habitat manipulation. Combining these strategies into an integrated approach has seen limited use in aquatic environments. As federal, state, and local governments are increasingly challenged to reevaluate restrictions and regulations concerning water use issues, the need for a multidisciplinary, integrated approach to the management of nuisance aquatic vegetation takes on greater importance.

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[3] Letters following this symbol are a WSSA-approved computer code from *Composite List of Weeds*, Revised 1989. Available only on computer disk from WSSA, 810 East 10th Street, Lawrence, KS 66044-8897.

Several investigators have proposed using sublethal herbicide rates to stress submersed vegetation or inhibit growth, thereby increasing susceptibility to pathogens (Charudattan 1986; Kerfoot 1989; Sorsa et al. 1988). Combining the systemic herbicide fluridone with the endemic fungal isolates on ceratophyllum (*Ceratophyllum demersum* L.), (Smit et al. 1990) or hydrilla (Nelson et al. 1998; Netherland and Shearer 1996) is reported to increase efficacy in comparison with either treatment used alone. Sorsa et al. (1988) suggested that pathogens may enable the application of herbicides at concentrations that would otherwise be ineffective after investigating the integrated use of the contact herbicide endothall with the endemic fungal pathogen *Colletotrichum* sp. for control of Eurasian watermilfoil (*Myriophyllum spicatum* L.).

Endothall is currently used for large-scale and spot treatments of hydrilla in hydrodynamic systems (Netherland et al. 1991). The severity of hydrilla injury that can be expected from a treatment is related to the herbicide concentration and exposure time (Netherland et al. 1991). Severe hydrilla injury (> 85% biomass reduction) occurred when plants were exposed to 2.0 mg ae/L for 48 h, or 3.0, 4.0, and 5.0 mg ae/L for 24 h. A lack of regrowth from rootcrowns and the destruction of root tissues also characterized these concentration and exposure times. Applied at 1.0 mg ae/L, endothall failed to produce significant hydrilla injury at a maximum exposure time of 72 h.

Any management plan that is used to control infestations of nuisance plant species such as hydrilla must also consider the treatment effects on nontarget plant species. Recent research has shown that although endothall was considered to have broad-spectrum contact activity on aquatic plants, it can be used selectively to control target weeds while reducing or preventing long-term damage to various desirable nontarget aquatic plant species (Skogerboe and Getsinger, personal communication). Treatments at 2 to 5 mg ae/L, which were dissipated with a 24-h half-life, were shown to control hydrilla but injured, slightly injured, or had no effect on 15 native species. At 8 wk after treatment, evaluations of the treated plants indicated that the injured species were beginning to recover.

The endemic fungal pathogen *Mycoleptodiscus terrestris* (Gerd.) Ostazeski was identified as a candidate for biological control of hydrilla (Joye 1990; Shearer 1993, 1998). Applied as a liquid suspension, the pathogen acts in a manner similar to a contact herbicide. Within 4 to 7 d after inoculation, disease symptoms appear as chlorosis followed rapidly by loss of structural integrity (Joye 1990). An electron microscopy study by Joye and Paul (1991) showed that host cell ultrastructure was affected by fungal invasion within 8 h after inoculation, although the plants were asymptomatic. Within 40 h colonization of host cells was observed, and by 196 h cellular integrity was destroyed, resulting in collapse of the entire plant. Although laboratory and field studies have shown that *M. terrestris* can provide a rapid knockdown of hydrilla when applied at a minimum rate of 400 colony-forming units (CFU)/ml, regrowth will commence within 4 wk after treatment if plant stems or root crowns are left uninjured (Netherland and Shearer 1996; Shearer 1997).

Initial host specificity testing of *M. terrestris* on a variety of aquatic and terrestrial plants was shown to induce disease in only one other closely related aquatic plant species, duck lettuce [*Ottelia alismoides* (L.) Pers.] (Joye and Cofrancesco 1991). More recent studies have indicated that the aquatic plant, American pondweed, was also susceptible to injury by applications of *M. terrestris*, but the effects were only temporary (Nelson et al. 1998).

Integrating a contact herbicide such as endothall with a contact microbial pathogen such as *M. terrestris* has the potential to control hydrilla while offering plant managers the desired effects of (1) reducing herbicide and pathogen application rates, (2) decreasing endothall exposure requirements, and (3) increasing species selectivity. The objectives of this study were to compare the efficacy of endothall, *M. terrestris*, and integrated (endothall + *M. terrestris*) treatments against dioecious hydrilla in laboratory and outdoor mesocosm conditions and to determine the selectivity of endothall + *M. terrestris* treatments on other submersed plant species.

MATERIALS AND METHODS

Fungal and Herbicide Preparation. The *M. terrestris* isolate used for inoculum was obtained from hydrilla collected in Texas (Shearer 1993). Starter cultures were initiated by inoculating potato dextrose agar[4] plates with mycelium from refrigerated stock cultures stored on half-strength corn meal agar[4] at 4 C. Following incubation in the dark for 7 d at 28 C, 4-mm plugs were cut with a cork borer from the leading edge of *M. terrestris* colonies and used to inoculate flasks containing 600 ml of modified sterile Richard's V-8 broth (glucose, 10 g; KNO_3, 10 g; $CaCO_3$, 3 g; V-8 juice (Campbell's), 200

[4] Difco Laboratories, Detroit, MI.

ml; H_2O, 800 ml). The flasks were incubated on a shaker at 200 rpm for 6 d at room temperature (22 to 25 C). The fungal biomass from each flask was filtered through four layers of cheesecloth, suspended in 100 ml of sterile water, and comminuted in a blender for 30 s. These procedures consistently yielded a fungal slurry containing 1×10^6 CFU/ml. Fungal viability was verified by dilution plating the slurry on Martin's agar (Tuite 1969), incubating the plates in the dark at 28 C for 4 d, and counting discrete colonies.

Endothall stock solutions were prepared from the commercial formulation of the aquatic herbicide Aquathol K®.[5] All treatment concentrations are reported as the acid equivalent of the endothall formulation.

Laboratory Study. Laboratory evaluations were conducted in a controlled-environmental room at the US Army Engineer Research and Development Center (ERDC), Waterways Experiment Station, Vicksburg, MS. Fifty-one 55-L aquaria filled with a culture solution (Smart and Barko 1985) were maintained at a temperature of 26 ± 2 C, a light intensity of 570 ± 60 mol/m²/s, and a photoperiod of 14 light:10 dark.

The dioecious biotype of hydrilla was field collected in Florida by Suwannee Lab Inc. (Lakeland, FL). Ten glass beakers (300 ml) filled with 250 ml of sediment (enriched with 200 mg NH_4Cl/L) 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 the aquariums to provide water circulation.

Hydrilla formed a surface canopy in each aquarium by 28 d. At the time of treatment, the flow-through water exchange system was deactivated. Endothall and *M. terrestris* were dispensed to the water surface using individual pipettors and were allowed to disperse through the water column. Pathogen and herbicide treatments included 0.25, 0.50, and 1.25 mg/L endothall, 100, 200, and 400 CFU/ml of *M. terrestris*, combined treatments of 0.25, 0.50, and 1.25 mg/L endothall + 100 or 200 CFU/ml *M. terrestris*, and untreated controls. Combined treatments were applied either simultaneously or sequentially, with the fungus being applied first followed by herbicide application 4 d later. Following a 96-h exposure time to endothall, each aquarium was emptied and refilled with fresh water three times to remove the endothall residues. After rinsing, the water exchange system was activated and continued to operate until termination of the experiment.

[5] Elf Atochem North America, Inc., Philadelphia, PA.

At 21 and 42 d after treatment (DAT), five beakers were removed from each aquarium to assay for above-ground hydrilla shoot mass. The harvested material was dried to a constant weight at 60 C for 4 d. Each treatment was replicated three times using a completely randomized design. At each sampling interval, biomass was subjected to analysis of variance and treatment means compared using Fisher's protected least significant difference (LSD) test at $P \leq 0.05$.

Mesocosm Study. Studies were conducted in an outdoor mesocosm system at the ERDC Lewisville Aquatic Ecosystem Research Facility (LAERF), Lewisville, TX, which consists of large above-ground tanks (1.4-m height by 2.6-m diameter) that hold approximately 6,700 L of water. Each tank was individually plumbed to regulate water flow as needed and was equipped with air flow for water circulation. Further description of this mesocosm system can be found in Dick et al. (1997).

Each mesocosm tank (27 in all) was partitioned into quadrants to accommodate four plant species. Plastic pots (19.7-cm height by 19.7-cm diameter) filled with nutrient-enriched soil (one Woodace briquette (14-3-3) [N-P_2O_5-K_2O] plus 10 g ammonium sulfate per pot) were placed 11 per quadrant in the mesocosms. Apical cuttings (15 to 20 cm) of dioecious hydrilla, American pondweed, and Illinois pondweed were collected from cultures maintained at the LAERF. Vallisneria was acquired from Suwannee Lab Inc. Five plants per pot of each species were positioned 4 to 5 cm into the soil and allowed to grow for 1 mo. The flow-through water system was activated and remained on until treatment time.

After 1 mo, hydrilla had grown to approximately 15 cm below the water surface, the pondweeds had begun to form floating leaves, and vallisneria plants were approximately up to half-tank height. One pot of each species was harvested from each mesocosm to provide an estimate of pretreatment shoot biomass. The plant material was dried to a constant weight at 60 C for 4 d. Pretreatment dry weight (DW) of shoot biomass for hydrilla, American pondweed, Illinois pondweed, and vallisneria was 29.6 ± 11.8 g, 5.1 ± 3.8 g, 6.2 ± 2.8 g, and 4.2 ± 2.6 g DW, respectively.

Just prior to treatment, the flow-through water system was deactivated. Treatments were applied on August 27, 1999 and included 0.25 and 0.50 mg/L endothall, 100 and 200 CFU/ml *M. terrestris*, integrated treatments of 0.25 and 0.50 mg/L endothall + 100 or 200 CFU/ml *M. terrestris*, and untreated controls. Herbicide stock solutions and fungal inoculum were prepared as described earlier. Herbicide and pathogen treatments were applied

by pouring the chemical solution and the mycelial suspension evenly over the water surface. Integrated treatments were applied simultaneously to the designated tanks. After 4 d exposure time, the flow-through water system was reactivated and remained on for the duration of the study.

Plant biomass was harvested 21 and 42 DAT. At 21 DAT, five randomly selected pots of each plant species were removed from each mesocosm. Shoot biomass was clipped at the sediment surface, washed to remove algae and debris, and dried to a constant weight at 60 C for 4 d. Plant biomass was recorded as grams DW per pot. At 42 DAT the remaining pots were removed from each mesocosm and harvested as described earlier.

Treatments were randomly assigned to mesocosm tanks and were replicated three times. At each sampling interval, biomass was subjected to analysis of variance and treatment means compared using the Scheffé test at $P \leq 0.05$.[6]

RESULTS AND DISCUSSION

Laboratory Study. Laboratory results indicated that hydrilla control improved with increasing concentrations of endothall, *M. terrestris*, and combinations of endothall and *M. terrestris*. At 21 DAT the 0.25 mg/L endothall treatment showed a significant increase in shoot biomass compared with untreated plants (Table 1). All other treatments resulted in a significant reduction of shoot biomass levels compared with untreated plants, except endothall at 0.5 mg/L. These two endothall treatments applied at one-fourth to one-eighth the rate recommended on the herbicide label were not expected to reduce hydrilla biomass significantly by 21 DAT. However, a 4-d exposure of 1.25 mg/L endothall reduced hydrilla biomass by 77% compared with untreated plants, which agreed with previous studies that indicated that a minimum of 3 d exposure to 1.0 mg/L endothall was required to provide significant hydrilla control of 76 to 80% (Netherland et al. 1991; Van and Conant 1988). Combinations of endothall and the fungal pathogen were equally efficacious whether they were applied simultaneously or sequentially. Biomass reductions of 100% were achieved with simultaneous treatments of 1.25 mg/L combined with either 100 or 200 CFU/ml *M. terrestris* and the sequential treatment of 200 CFU/ml *M. terrestris* followed 4 d later by 0.5 mg/L endothall.

By 42 DAT, all treatments had significantly reduced shoot biomass levels of hydrilla compared with the untreated controls (Table 1). In some cases where hydrilla injury was severe, shoot fragments were found floating in the aquaria. Because hydrilla can regrow from plant fragments, all potentially viable tissue was included for the determination of final biomass. Plants treated with *M. terrestris* alone were recovering and beginning to regrow, as evidenced by the increase in plant biomass from 21 to 42 DAT. This is consistent with previous laboratory studies (Netherland and Shearer 1996). Treatments of 1.25 mg/L endothall, 400 CFU/ml *M. terrestris*, and all herbicide pathogen combinations provided 95 to 100% hydrilla control. Combining the two lowest herbicide rates with *M. terrestris* provided better hydrilla control than either treatment alone. Combination treatments may offer the potential for selective hydrilla control if the rates could be shown not to affect adversely the nontarget aquatic species.

Table 1. Mean DW biomass of hydrilla shoot tissue at two time intervals following application of endothall, *M. terrestris*, or a combination of endothall and *M. terrestris*.

Treatment[b] (E + MT)	Hydrilla shoot mass[a]	
	21 DAT	42 DAT
	g DW per pot	
Untreated	7.07 b	12.66 a
Endothall (E)		
0.25 + 0	8.51 a	6.23 b
0.50 + 0	6.17 b	3.78 c
1.25 + 0	1.61 cd	0.08 d
M. terrestris (MT)		
0 + 100	2.85 c	5.74 bc
0 + 200	1.51 cde	4.37 bc
0 + 400	0.57 def	0.65 d
Simultaneous treatments		
0.25 + 100	0.98 def	0.42 d
0.50 + 100	0.22 ef	0.00 d
1.25 + 100	0.00 f	0.00 d
0.25 + 200	0.56 def	0.24 d
0.50 + 200	0.16 f	0.21 d
1.25 + 200	0.00 f	0.00 d
Sequential treatments[c]		
100 + 0.25	1.04 def	0.29 d
100 + 0.50	0.23 ef	0.39 d
200 + 0.25	0.41 def	0.12 d
200 + 0.50	0.00 f	0.00 d

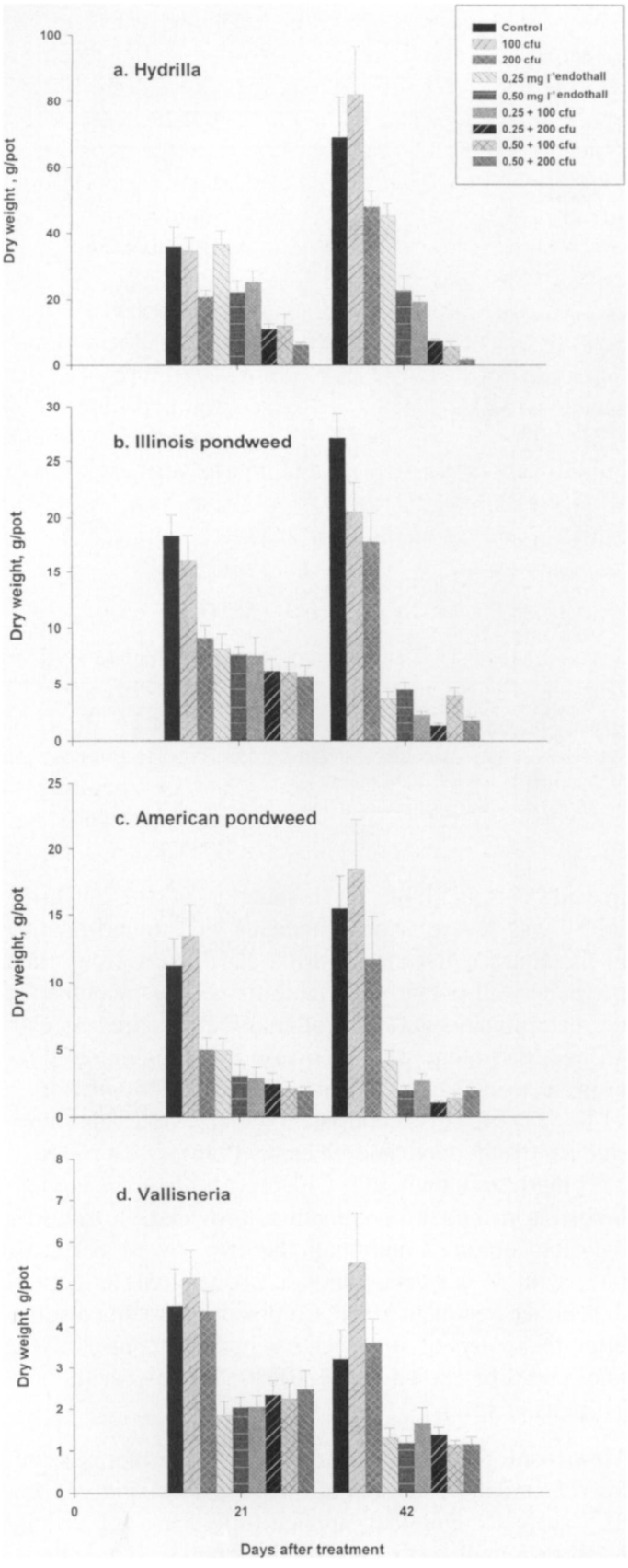
[a] Values followed by a different letter are significantly different within each sampling interval according to Fisher's protected LSD test at $P \leq 0.05$.

[b] Treatments rates used were endothall at 0.25, 0.50, and 1.25 mg ae/L and *M. terrestris* at 100 and 200 CFU/ml.

[c] *M. terrestris* was applied first followed by endothall 4 d later.

Mesocosm Study. Treatment effects on biomass followed similar trends among plant species (Figures 1a–d). Except for endothall applied to hydrilla at 0.25 mg/L, all endothall treatments and the combined endothall–pathogen treatments significantly reduced shoot mass compared with untreated controls by 42 DAT. Although

[6] Statistica, StatSoft, Tulsa, OK.



re-isolations from the four species indicated *M. terrestris* presence in plant tissues 21 DAT, any sustained injury did not prevent recovery, and by 42 DAT biomass was not significantly different from the controls.

Based on laboratory studies, reduction in hydrilla shoot mass in the mesocosms was less than expected for all treatments except endothall applied alone at 0.25 and 0.5 mg/L 21 DAT (Figure 1a). At 42 DAT, percent hydrilla biomass reduction was less than expected for all treatments. The combined treatment of 0.5 mg/L endothall with 200 CFU/ml *M. terrestris* produced the highest percent control of hydrilla at 97% compared with 100% control with this treatment under laboratory conditions. The results using endothall alone were within the range of hydrilla control expected using low rates and long exposure times (Netherland et al. 1991). Netherland et al. (1991) predicted that application rates of 1 mg/L or less at a 72-h contact time of endothall would provide $< 70\%$ hydrilla control with a high potential for regrowth following treatment. By 42 DAT, endothall applied alone at 0.25 and 0.5 mg/L for a 96-h contact time in this study reduced hydrilla biomass by 34 and 67%, respectively.

The application rates of *M. terrestris* used in the mesocosm study were not expected to significantly reduce hydrilla shoot mass. Previous studies have shown that a minimum of 400 CFU/ml is required for significant hydrilla control using the pathogen alone (Shearer 1997). By 21 DAT, hydrilla was beginning to recover from treatments at both 100 and 200 CFU/ml. These results are consistent with the work of Netherland and Shearer (1996). They found that low rates of *M. terrestris* initially impact hydrilla, but by 14 DAT plants had begun to show signs of recovery, and by 28 DAT biomass levels of the treated plants increased threefold. Between 21 and 42 DAT, hydrilla biomass in the mesocosms increased approximately 2.3 times for both the 100- and 200-CFU/ml treatment.

Similar to the laboratory results, the combined endothall–pathogen treatments were more efficacious than either treatment applied alone. By 42 DAT, all combined treatments except 0.25 mg/L with 100 CFU/ml *M. terrestris* had reduced hydrilla shoot mass $\geq 90\%$ compared with untreated controls. In order to achieve similar

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Figure 1. Mean DW biomass of hydrilla (a), Illinois pondweed (b), American pondweed (c), and vallisneria (d) at 21 and 42 d after treatment following application of *M. terrestris* at 100 and 200 CFU/ml, endothall at 0.25 and 0.50 mg ae/L, and integrated treatments of endothall + *M. terrestris*. Vertical bars represent the means of three replicates. Error bars represent one standard error of the mean.

results with endothall alone, application rates and exposure times might have to be as great as 2.0 mg/L for 48 h, or 3.0, 4.0, or 5.0 mg/L for 24 h (Netherland et al. 1991). Application rates for the pathogen might have to double or triple to achieve similar results (Shearer 1998).

All nontarget species sustained varying amounts of injury from endothall and *M. terrestris* treatments applied alone or in combination (Figure 1). Biomass reductions were less from the pathogen treatments than from endothall alone or in combination with the pathogen (Figures 1b–d). *M. terrestris* applied at 100 CFU/ml induced no measurable damage to American pondweed or vallisneria. By 42 DAT, vallisneria treated with 200 CFU/ml *M. terrestris* was recovering, and biomass exceeded that of the untreated controls. Illinois pondweed biomass was reduced 25 to 35% by treatments of 100 and 200 CFU/ml *M. terrestris*, respectively, compared with the untreated controls by 42 DAT. However, between 21 and 42 DAT, biomass of Illinois pondweed treated with *M. terrestris* increased 1.3- to 1.9-fold, indicating that recovery was occurring. Likewise, biomass of American pondweed plants treated with *M. terrestris* increased 1.4- to 2.4-fold over the same time period. Based on the results of this mesocosm study, plant species susceptible to injury from the pathogen should be expanded. Neither the pondweeds nor vallisneria were included in the species list used by Joye and Cofrancesco (1991) in preliminary host-specificity testing of *M. terrestris*.

Previous selectivity studies suggested that endothall applied at 0.5 to 1 mg/L for 24 h induced significant injury to American pondweed, Illinois pondweed, and vallisneria (Skogerboe and Getsinger, personal communication). By 8 wk after application, all three species were reported to show signs of recovery and the potential to regrow. Although the endothall rates applied in the present study were less than or equal to those used by Skogerboe and Getsinger (personal communication), the contact time was much longer (96 compared with 24 h). All treatments of endothall alone and endothall combined with *M. terrestris* significantly reduced shoot mass of Illinois pondweed, American pondweed, and vallisneria by 42 DAT compared with untreated controls. Visual observation of plants at harvest time indicated that new stems were emerging from the root crowns and new leaves developing on the remaining intact stems, suggesting that, given time, recovery would occur.

A 96-h exposure time in both laboratory and mesocosm studies was used to ensure sufficient time for pathogen cells to contact and invade hydrilla plant tissues. The long exposure time in all likelihood enhanced the effect of enodothall and the combined endothall pathogen treatments on nontarget species. Recent greenhouse studies using *M. terrestris* alone have indicated that significant hydrilla biomass reductions can occur with an exposure time $\leq$ 24 h (unpublished data). By decreasing the exposure time, hydrilla control would seemingly not be compromised, but injury to nontarget species may be considerably reduced.

The results of these laboratory and mesocosm studies have demonstrated that integrating endothall and *M. terrestris* has potential for combining low rates of a herbicide with an endemic pathogen for hydrilla control while reducing the risk of damage to nontarget species. Additional studies will be required to verify if reduced exposure times can result in effective hydrilla control while increasing nontarget selectivity.

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