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Mesocosm Evaluation of Integrated Fluridone-Fungal Pathogen Treatment on Four Submersed Plants

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ABSTRACT

An outdoor mesocosm study was conducted to evaluate the efficacy and selectivity of the herbicide fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone) and the fungal pathogen *Mycoleptodiscus terrestris* (Gerd.) Ostazeski (*Mt*), applied alone and in combination with one another, against hydrilla (*Hydrilla verticillata* (L. f.) Royle), Eurasian watermilfoil (*Myriophyllum spicatum* L.), American pondweed (*Potamogeton nodosus* Poiret), and vallisneria (*Vallisneria americana* Michx.). Treatments included 5 µg/L fluridone, 100 and 200 colony forming units (CFU) per ml of *Mt*, integrated treatments of 5 µg/L fluridone + 100 or 200 CFU/ml *Mt*, and untreated controls. Treatment with either fluridone or 200 CFU/ml *Mt* alone was sufficient to reduce hydrilla growth by 40% 84 days after treatment (DAT); however, the combined application of *Mt* plus fluridone reduced biomass by 93% compared with untreated plants. Eurasian watermilfoil biomass was not affected by *Mt* alone and was equally inhibited with treatment of fluridone or fluridone with *Mt* (75% reduction at 84 DAT). Treatments did not inhibit biomass production of American pondweed or vallisneria. With the exception of American pondweed, all treatments that included fluridone significantly reduced total chlorophyll. Results show that integrating a low dose of fluridone (5 µg/L) with *Mt* can effectively and selectively reduce hydrilla biomass with minimal effect to non-target plant species such as vallisneria and American pondweed, but may not improve control of Eurasian watermilfoil over fluridone alone.

Key words: Hydrilla verticillata, integrated control, fungal pathogen, *Mycoleptodiscus terrestris*, *Myriophyllum spicatum*, *Potamogeton nodosus*, *Vallisneria americana.*

INTRODUCTION

Several investigators have reported that the efficacy of some plant pathogens can be enhanced by integration with chemical herbicides (Charudattan 1986, Hoagland 1996, Netherland and Shearer 1996, Rayachhetry and Elliot 1997). In a recent review, Hoagland (1996) stated that although many pathogens have been characterized as bioherbicidal, most lack sufficient aggressiveness to overcome weed defense mechanisms to achieve adequate control. However, some herbicides and plant growth regulators can act to weaken natural plant defense systems, rendering them more susceptible to pathogen attack (Hoagland 1996). Interactions between control agents may be antagonistic, synergistic, or additive, with additive and synergistic effects desirable for maximizing weed control. The potential advantages for implementing an integrated management strategy include: increased efficacy, reduced herbicide and pathogen levels required for weed control, expanded pathogen host range, and a more economically and environmentally acceptable method of nuisance plant management (Charudattan 1986, Hoagland 1996).

Using an integrated approach for managing the aquatic weeds waterhyacinth (*Eichhornia crassipes* (Mart.) Solms) and Eurasian watermilfoil, has been investigated by others (Charudattan 1986, Sorsa et al. 1988). Recently, Netherland and Shearer (1996) demonstrated that combining low doses of the systemic herbicide, fluridone, with a fungal pathogen, *Mt*, was effective for controlling the nuisance exotic plant hydrilla, in growth chamber trials. Applying a sublethal dose of fluridone (2 µg/L) with *Mt* at rates of 100 and 200 CFU/ml reduced hydrilla biomass by >90% and was more efficacious than applying either control agent alone. The integrated treatment provided the benefits of rapid biomass reduction exhibited by *Mt* and long-term prevention of hydrilla regrowth provided by fluridone. In addition, integrated treatments reduced fluridone exposure requirements by approximately 50 days, which may broaden the use of this herbicide in aquatic environments where high water exchange has limited its use in the past. Fluridone generally requires a contact time of 60-90 days to achieve satisfactory hydrilla control and thus has limited use in aquatic systems where high water exchange precludes long chemical-plant exposure periods (Netherland et al. 1993, Netherland and Getsinger 1995).

Herbicide selectivity can often be achieved by applying lower than recommended dosages to sensitive vegetation. Selective removal of a nuisance plant species without damaging non-target plants is a desirable goal for many aquatic plant management situations. One advantage that may result from integrating fluridone with *Mt* is that lowering the fluridone concentration may allow increased species selectivity. Netherland et al. (1997) demonstrated in a mesocosm study that 60- and 90-day exposures of 5 µg/L fluridone were suffi-

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[2]Lilly Research Laboratory. 1980. Method AM-AA-CA-R005-AC-755: determination of fluridone in water by direct injection high pressure liquid chromatography. Eli Lilly and Co., Greenfiled, IN. 4 pp.

cient to significantly reduce Eurasian watermilfoil biomass with no effect on biomass production of non-target species (elodea (*Elodea canadensis* Mich.), American pondweed, sago pondweed (*Potamogeton pectinatus* L.), and vallisneria), whereas higher fluridone rates (10 µg/L) severely damaged all non-target species. Thus the potential exists to control the growth of noxious species with reduced rates of fluridone, without affecting desirable, native species.

The objectives of this study were: to verify laboratory efficacy of integrating fluridone with *Mt* for control of hydrilla, the target weed, under outdoor growing conditions; and to determine the specificity of fluridone-*Mt* treatment on other submersed plant species.

MATERIALS AND METHODS

This experiment was conducted in an outdoor mesocosm system at the Lewisville Aquatic Ecosystem Research Facility (LAERF), Lewisville, TX, that consists of large tanks (1.4 m tall by 2.6 m in diameter) which hold approximately 6500 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).

For this study, each mesocosm tank (18 total) was divided into four equal sections with netting to accommodate each of the four plant species. The netting allowed water flow between the divided areas but restricted plant growth to each section. Plants were grown in plastic pots (19.7 cm tall by 19.7 cm in diameter) filled with nutrient-enriched soil (1 Woodace briquette (14-3-3) plus 10 g ammonium sulfate per pot). Nine pots of each plant species (3 plants per pot) were placed in each tank section. Hydrilla (dioecious biotype) and Eurasian watermilfoil were propagated from 10-cm apical cuttings and planted 4 to 5 cm into the soil. American pondweed and vallisneria were initiated from pre-germinated tubers placed 4 to 5 cm into the soil. All plants and tubers used in this study were collected from pond-grown cultures at the LAERF. Plants were allowed to establish in the mesocosm tanks for 2 months prior to herbicide-pathogen treatment. At the time of treatment, hydrilla and Eurasian watermilfoil had grown to the water surface, American pondweed had formed a surface canopy of floating leaves, and vallisneria was well established.

Treatments were applied on 19 June 1996 and included 5 µg/L fluridone, 100 and 200 CFU/ml of *Mt*, integrated treatments of 5 µg/L fluridone + 100 or 200 CFU/ml *Mt*, and untreated controls. Fluridone stock solutions were prepared from the liquid commercial formulation Sonar^{R} AS (479 grams active ingredient per liter). *Mt* (isolated from hydrilla in TX) was applied as a thick slurry of live fungal mycelium. The *Mt* inoculum was prepared as described by Shearer (1996). Both fluridone and *Mt* were applied by pouring the chemical solution and the mycelial suspension evenly over the water surface. Integrated treatments were applied simultaneously to designated tanks.

Plant biomass was harvested at 21, 42, and 84 days after treatment (DAT). At each harvest, 3 randomly selected pots of each plant species were removed from each mesocosm tank. Aboveground biomass was clipped at the sediment surface, washed to remove algae and debris, and dried to a constant weight at 60 C. Plant biomass was recorded as g dry weight/pot.

Fresh tissue samples (4 samples per plant species per tank) were collected pretreatment and at each posttreatment harvest for chlorophyll analysis. The tissue selected for this procedure varied for each plant species and included 4-cm stem apices of hydrilla and Eurasian watermilfoil, floating leaves of American pondweed, and 4-cm leaf segments of vallisneria. Total chlorophyll (a and b) was measured using a DMSO extraction procedure (Hiscox and Israelstam 1979).

Water samples were collected from all fluridone-treated tanks at 1, 2, 3, and 7 DAT, weekly thereafter through 42 DAT, and at 63 and 84 DAT to confirm initial fluridone treatment rates and to determine herbicide dissipation. Samples were collected in 500-ml amber polyethylene bottles and frozen until analysis. Fluridone residues were detected using a high performance liquid chromatography (HPLC) procedure[2]. Residue data were subjected to linear regression procedures and the results obtained were used to determine the half life of fluridone under these experimental conditions.

Treatments were randomly assigned to mesocosm tanks and were replicated three times. At each sampling interval, biomass and chlorophyll data were subjected to analysis of variance and treatment means compared using Fisher's protected LSD test at the 0.05 level of significance.

RESULTS AND DISCUSSION

Residue analyses at 1 DAT (data not shown) showed that the initial target fluridone concentration (5 µg/L) was achieved in all chemically-treated mesocosm tanks. Subsequent water residue data were used to determine fluridone dissipation over time. Regression analysis established that under these experimental conditions, the average half life of fluridone in herbicide-treated tanks was 49 days (r^2 = 0.93). Fluridone dissipation was comparable to dissipation rates reported by Netherland et al. (1997) under similar experimental conditions.

Treatment effects on dry weight biomass varied greatly among plant species (Figure 1A-D). The greatest response was observed on the target plant, hydrilla (Figure 1A). At 21 DAT, treatment with either fluridone alone or 200 CFU/ml *Mt* was sufficient to reduce hydrilla biomass by an average of 36%. However, the combined application of *Mt* plus fluridone reduced biomass up to 75% compared with untreated plants. By 84 DAT, the combined treatments resulted in a 93% reduction in hydrilla biomass. Both fluridone alone and 200 CFU/ml *Mt* reduced hydrilla biomass by 40% at the final harvest. Statistically, there were no differences between the two rates of *Mt* or between fluridone alone and *Mt* at 200 CFU/ml on hydrilla.

Characteristic injury symptoms of fluridone and *Mt* were observed on hydrilla. Successful fungal infection was noted on all *Mt*-treated tanks 10 DAT and was identified by leaf tip chlorosis and stem defoliation. Although biomass was not significantly different between the two rates of *Mt*, disease symptoms were visibly more abundant on tanks treated with the higher than the lower rate of *Mt*. At the first posttreatment harvest, new and healthy hydrilla growth (lateral shoots from viable stems) also was present in all tanks treated with *Mt* by

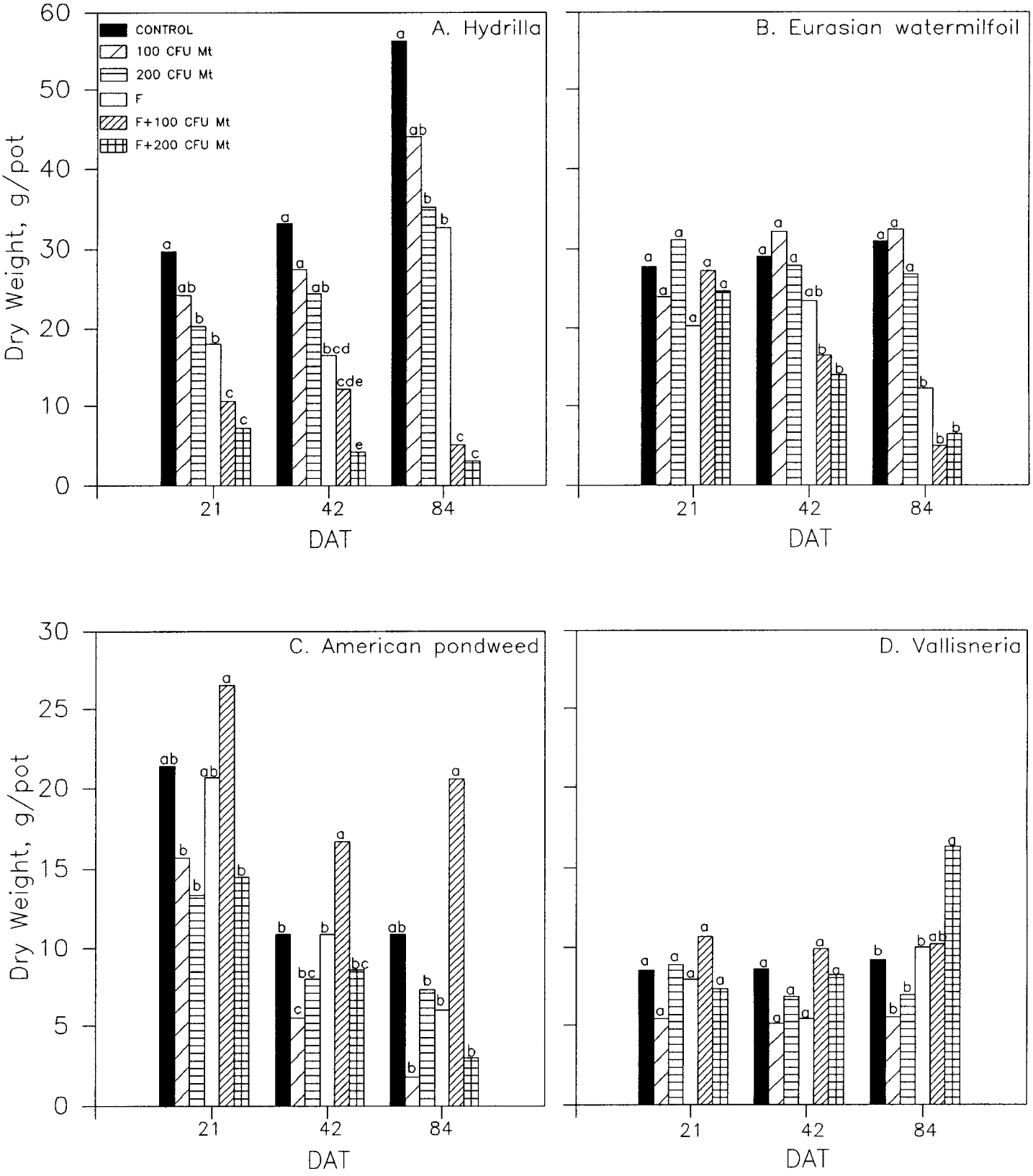

Figure 1. Mean dry weight biomass of hydrilla (A), Eurasian watermilfoil (B), American pondweed (C), and vallisneria (D) at 21, 42, and 84 days after treatment (DAT) following application of *Mt* at 100 and 200 colony forming units (CFU) per ml, fluridone (F = 5 µg/L fluridone), and integrated treatments of fluridone + *Mt*. Within each sample time, means followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's protected LSD test.

TABLE 1. EFFECT OF FLURIDONE, *MT*, AND FLURIDONE + *MT* TREATMENTS ON TOTAL CHLOROPHYLL CONTENT OF FOUR SUBMERSED PLANT SPECIES.

Species	Treatment µg/L + CFU[1]	Total chlorophyll content (mg g^{-1} fr wt)			
		Days after treatment[2]			
		Pretrt	21 DAT	42 DAT	84 DAT
Hydrilla	Untreated	1.17	1.11 a	1.09 a	1.12 a
	0 + 100	1.04	0.95 a	1.15 a	1.14 a
	0 + 200	1.02	0.97 a	1.03 a	1.22 a
	5 + 0	1.21	0.20 c	0.50 b	0.44 b
	5 + 100	1.14	0.30 bc	0.44 b	0.54 b
	5 + 200	1.16	0.39 b	0.52 b	0.56 b
	(LSD)	NS	(0.19)	(0.25)	(0.23)
E. Watermilfoil	Untreated	1.44	1.56 a	1.73 a	1.35 a
	0 + 100	1.58	1.53 a	1.70 a	1.51 a
	0 + 200	1.47	1.65 a	1.77 a	1.49 a
	5 + 0	1.40	1.05 b	1.03 b	0.98 b
	5 + 100	1.45	1.09 b	1.00 b	0.81 b
	5 + 200	1.50	1.06 b	1.14 b	0.92 b
	(LSD)	NS	(0.25)	(0.20)	(0.26)
American Pondweed	Untreated	1.42	1.10	1.40	1.43 b
	0 + 100	1.53	0.86	1.30	1.41 b
	0 + 200	1.74	0.97	1.40	1.51 b
	5 + 0	1.54	1.19	1.39	1.32 b
	5 + 100	1.70	1.11	1.30	1.27 b
	5 + 200	1.63	0.95	1.15	1.83 a
	(LSD)	NS	NS	NS	(0.32)
Vallisneria	Untreated	0.86	0.78 b	0.85 ab	0.68
	0 + 100	0.86	0.97 a	0.78 abc	1.35
	0 + 200	0.87	0.78 b	0.93 a	0.78
	5 + 0	0.87	0.52 c	0.66 bc	0.50
	5 + 100	0.92	0.62 c	0.64 bc	0.46
	5 + 200	0.74	0.51 c	0.58 c	0.63
	(LSD)	NS	(0.13)	(0.22)	NS

[1]µg/L = fluridone concentration and CFU = colony forming units of *Mt*.
[2]Within columns, means followed by different letters are significantly different (LSD, $P \leq 0.05$); DAT, days after treatment; NS = not significant.

itself. Fluridone effects on hydrilla, pink stem coloration and bleached leaves on new tissues, were observed 21 DAT. Fluridone, but not *Mt*, symptomology was also observed on Eurasian watermilfoil. Neither vallisneria nor American pondweed displayed visible symptoms of fungal infection or fluridone leaf bleaching.

Although Eurasian watermilfoil was not the target plant in this study, treatment with fluridone alone and fluridone + either 100 or 200 CFU/ml *Mt* reduced Eurasian watermilfoil biomass by 75% at 84 DAT (Figure 1B). Unlike the synergistic effect observed on hydrilla, the response on Eurasian watermilfoil was likely due to fluridone itself as there were no statistical differences between treatments of fluridone alone and those integrated with *Mt*. The fact that effects on biomass were not observed until late in the study (42 DAT) further implies fluridone activity as the main source of efficacy. Fluridone is a slow acting herbicide in comparison to the quick infection response observed with *Mt* (Netherland and Shearer 1996). Results are consistent with other outdoor mesocosm studies in which fluridone at a rate of 5 µg/L was sufficient to reduce Eurasian watermilfoil biomass (Netherland et al. 1997). Strains of *Mt* (other than that used in this study) have been isolated for activity on Eurasian watermilfoil and were found to be effective in greenhouse trials (Gunner et al. 1990). Combining milfoil-specific strains of *Mt* with fluridone may have potential as an integrated approach for management of Eurasian watermilfoil, and should be evaluated.

Non-target species were less affected by fluridone and *Mt*. Compared with untreated plants, none of the treatments inhibited biomass of American pondweed at 21 DAT (Figure 1C). Results were variable at subsequent harvests. For example, *Mt* at 100 CFU/ml significantly reduced biomass by 50% 42 DAT while treatment with fluridone + 100 CFU/ml *Mt* resulted in a significant increase (35%) in biomass. By the end of the study, none of the treatments were statistically different than controls however, fluridone + 100 CFU/ml *Mt* showed significantly higher biomass when compared with other fluridone or *Mt* treatments. Some of the observed variation in biomass data can be attributed to insect damage. At 21 DAT, floating leaves of American pondweed had been severely decimated by an unidentified species of whitefly (*Trialeurodes* sp.) and a common aquatic insect identified as the larva of the waterlily leafcutter (*Synclita obliteralis* (Walker)). Infestation was not evenly distributed among tanks (some tanks were not infested at all) and may account

for the variability in biomass data observed on this plant species. American pondweed in two of the three replicate tanks treated with fluridone + 100 CFU/ml *Mt* did not show insect damage, which may explain the high biomass levels recorded for this treatment.

Vallisneria biomass was not inhibited by any of the applied treatments (Figure 1D). There were no statistical differences among treatments at 21 and 42 DAT, and by the final harvest only fluridone + 200 CFU/ml *Mt* was significantly different than untreated plants. For reasons unknown, this treatment showed a 44% increase in biomass compared with untreated plants.

With the exception of American pondweed, all treatments that included fluridone significantly reduced total chlorophyll content in sampled tissues (Table 1). Hydrilla was most sensitive, with chlorophyll decreases of >70% measured at 21 DAT and a >50% decrease recorded thereafter. For Eurasian watermilfoil, chlorophyll content in fluridone-treated plants was 32 to 39% less that of untreated plants throughout the study. Initially, vallisneria showed reduced leaf chlorophyll (by 29% at 21 DAT), however, at 84 DAT there were no differences among treatments, indicating plant recovery. For all plant species, *Mt* alone did not affect total chlorophyll at the times sampled. Netherland and Shearer (1996) showed reduced chlorophyll content in hydrilla at 7 and 14 days following treatment with 100 and 200 CFU/ml *Mt*, but the effects dissipated by 28 DAT.

The results of this study confirm those observed in growth chamber studies by Netherland and Shearer (1996). For hydrilla, a beneficial synergistic interaction was observed with combined applications of 5 µg/L fluridone with either 100 or 200 CFU/ml *Mt*. Neither control agent alone provided adequate hydrilla control. For Eurasian watermilfoil, 5 µg/L fluridone was sufficient to significantly reduce biomass, which was consistent with reports that maintenance of low doses of fluridone over time can significantly inhibit biomass production (Netherland et al. 1997). There was no advantage to integrating fluridone with *Mt* on Eurasian watermilfoil. At the rates applied, the strain of *Mt* utilized in this study was ineffective on Eurasian watermilfoil. Other strains of *Mt* have been isolated for pathogenicity on this plant species and may be potential candidates for integrating with fluridone.

The desired level of selectivity was achieved with the integrated treatments applied in this study. Biomass of American pondweed and vallisneria was not severely impacted by treatment rates sufficient to control the target species, hydrilla. The results demonstrated that by integrating fluridone and *Mt*, a low herbicide rate that reduced the likelihood of chemical damage to non-target species could be used. The potential for selectivity gives further merit to the concept of integrated weed management.

Future research will focus on larger-scale field testing of fluridone-*Mt* treatments for controlling hydrilla, as well as evaluating other potential herbicide-pathogen combinations for aquatic plant management. Development of a granular *Mt* formulation to provide an easier and more uniform means of application also has been initiated.

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