Response of Eurasian Watermilfoil to Integrated Fluridone-Fungal Pathogen Treatment

by Linda S. Nelson and Judy F. Shearer

PURPOSE: This technical note describes laboratory investigations conducted to evaluate the effectiveness of the herbicide fluridone (1-methyl-3-phenyl-5[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone) and the fungal pathogen *Mt* (*Mycoleptodiscus terrestris* (Gerd.) Ostazeski), applied alone and in combination with one another, against Eurasian watermilfoil (*Myriophyllum spicatum* L.). Results of this research will demonstrate the potential for integrating chemical and biological control tactics to improve the long-term management of nuisance aquatic weed species.

BACKGROUND: Aquatic invasive weeds pose a recurrent threat to the health, productivity, and biological diversity of many U.S. water bodies. The associated losses and management costs due to invasive, alien plants exceed $30 billion annually in the United States (Schardt 2001). The state of Florida alone spent $60 million for management of nuisance aquatic vegetation in 1999 (Schardt 2001). Concerns over rising costs, harmful environmental effects, and long-term efficacy of current aquatic management practices have reinforced the need to investigate an "integrated" approach to weed management. The rationale for integrating control strategies is to combine the strengths of different technologies, thereby reducing inherent weaknesses of an individual technology when used alone. Often a beneficial synergistic or additive interaction will result from combining two different control agents.

Recent research has shown some positive, synergistic interactions between fungal pathogens and herbicides with relevance to aquatic weed control. Sorsa, Nordheim, and Andrews (1988) showed that combining low levels (0.65 to 1.29 ppm) of endothall (7-oxabicyclo[2.2.1] heptane-2,3-dicarboxylic acid) with the fungal pathogen *Colletotrichum gloeosporioides* ((Penz.) Penz. & Sacc. in Penz.), significantly enhanced control of Eurasian watermilfoil (hereafter referred to as milfoil). Netherland and Shearer (1996) demonstrated that combining low doses of the systemic herbicide fluridone with the endemic fungal pathogen *Mt* was effective for controlling the nuisance aquatic plant hydrilla (*Hydrilla verticillata* (L.f.) Royle). Applying sublethal doses (2 $\mu g\ L^{-1}$) of fluridone with either 100 or 200 colony-forming units (cfu) ml^{-1} *Mt* reduced hydrilla biomass more than 90 percent, and was more efficacious than applying either control agent alone. Similar results using fluridone and *Mt* were observed on hydrilla in outdoor mesocosm studies conducted by Nelson, Shearer, and Netherland (1998). In addition, these researchers observed that integrating low doses of fluridone with *Mt* minimized chemical injury to non-target plant species. Recent studies by Shearer and Nelson (2002) demonstrated that integrating sub-lethal doses of the contact herbicide endothall with *Mt* was effective for reducing hydrilla biomass while reducing the risk of damage to desirable, non-target species.

An endemic, pathogenic isolate of *Mt* has also been identified as a potential biocontrol agent for milfoil (Shearer 1996a). Like hydrilla, milfoil is an exotic aquatic plant that has invaded many lakes, rivers, and reservoirs in the United States. It is considered a nuisance because of its rapid growth rate, ability to form dense, monotypic stands with extensive surface canopies, and

aggressiveness in displacing desirable native vegetation. This submersed macrophyte is rooted in the sediment and can be found in waters from 1 to 10 m deep (Grace and Wetzel 1978, Smith and Barko 1990). Problems arise once an impenetrable surface canopy is formed and navigation and recreational activities are hindered.

The traditional control strategies currently used to manage submersed plants like milfoil include herbicides, biological control agents (insects, herbivorous fish) and mechanical harvesting. To date, fungal pathogens have not been used on an operational scale; however, *Mt* is under development as an aquatic bioherbicide and will likely be available as a management tool in the near future. The practice of integrating pathogen and chemical control technologies has not been widely used in aquatic environments, but deserves consideration as an alternate method that could improve weed control as well as reduce chemical inputs into surface waters.

The objective of this study was to evaluate the efficacy of integrating fluridone with *Mt* for control of the nuisance aquatic plant, milfoil.

MATERIALS AND METHODS: Studies were conducted in 55-L glass aquaria housed in a controlled-environment growth chamber located at the U.S. Army Engineer Research and Development Center (ERDC), Vicksburg, MS (Figure 1). The growth chamber was maintained at 22 ± 1 °C with a 14:10-hr light:dark photoperiod. Overhead lighting provided a mean photosynthetic photon flux density of 520 ± 50 µmol m^{-2} sec^{-1} at the water surface of each aquarium.

Milfoil was collected from ponds at the Lewisville Aquatic Ecosystem Research Facility in Lewisville, TX, and was shipped overnight to ERDC for use in these studies. It was previously determined that milfoil from this site was free of infection with *Mt*. Stem apices of milfoil (~ 20 cm) were planted into sediment-filled beakers (three plants per 300-ml beaker), which were then placed into each aquarium (10 planted beakers per aquaria). Each aquarium was filled with 52 L of a hard-water, culturing solution developed by Smart and Barko (1984). Air was gently bubbled through air stones placed in each aquaria to provide circulation of the culture solution. The culture solution was replaced in each aquaria every 3 days prior to treatment to minimize nuisance algal growth. The sediment used in these studies was collected from Brown's Lake, Vicksburg, MS, and was amended with ammonium chloride at a rate of 200 mg L^{-1} of sediment. Plants established under these conditions for 21 days prior to treatment.

A concentrated fluridone stock solution was prepared by mixing the aqueous suspension formulation Sonar™ AS (480 g ai L^{-1}) into glass-distilled water. The stock solution was mixed using a stir plate and magnetic stir bar and was prepared approximately 1 hr prior to treatment. The fungal pathogen was prepared as a slurry of live mycelium as described by Shearer (1996b). The strain of *Mt* used in these studies was isolated from infected milfoil growing in Alabama. Both fluridone and *Mt* were applied to each aquaria by pipetting the chemical solution and the mycelial suspension evenly over the water surface. Integrated treatments were applied simultaneously to designated tanks. Treatments included 2, 5, and 12 µg L^{-1} fluridone; 50, 100, 200, and 400 cfu ml^{-1} *Mt*; combined treatments of fluridone (all rates) with either 50, 100, or 200 cfu ml^{-1} *Mt*; and untreated controls.

Figure 1. Growth chamber facility and aquaria system used in studies

Shoot biomass was harvested 28, 56, and 84 days after treatment (DAT). For the first two harvests, three beakers of plants were randomly removed from each aquarium and the shoot biomass clipped at the sediment surface and dried at 70 °C for 48 hr. The remaining four beakers of plants were collected for the final harvest. Leaf chlorophyll was measured at 7, 28, 56, and 84 DAT using a dimethyl-sulfoxide extraction procedure followed by spectrophotometric quantification as described by Hiscox and Israelstam (1979). Two 5-cm stem tips were collected from each aquarium and extracted for chlorophyll analysis. Chlorophyll content was calculated following equations used by Arnon (1949) and is expressed as milligrams total chlorophyll (chlorophyll *a* and *b*) g^{-1} fresh weight.

Treatments were arranged in a completely randomized design with three replicates. Data were subjected to ANOVA procedures and significant differences from the untreated control were determined using Dunnett's two-tailed *t*-test at the 0.05 level of significance. Sigma Stat software (Jandel Scientific, San Rafael, CA) was used for data analysis.

RESULTS AND DISCUSSION: Compared with untreated plants, all integrated treatments of fluridone and *Mt* significantly reduced milfoil growth 84 DAT (Figure 2). Rates as low as 5 µg L^{-1} fluridone applied simultaneously with 100 cfu ml^{-1} *Mt* reduced milfoil biomass 92 percent, whereas either product applied alone at these rates was ineffective for significantly reducing plant growth. Combining higher rates of these two products resulted in similar effects on biomass (93 to 96 percent less than untreated plants). Significant reductions in plant biomass were detected as early

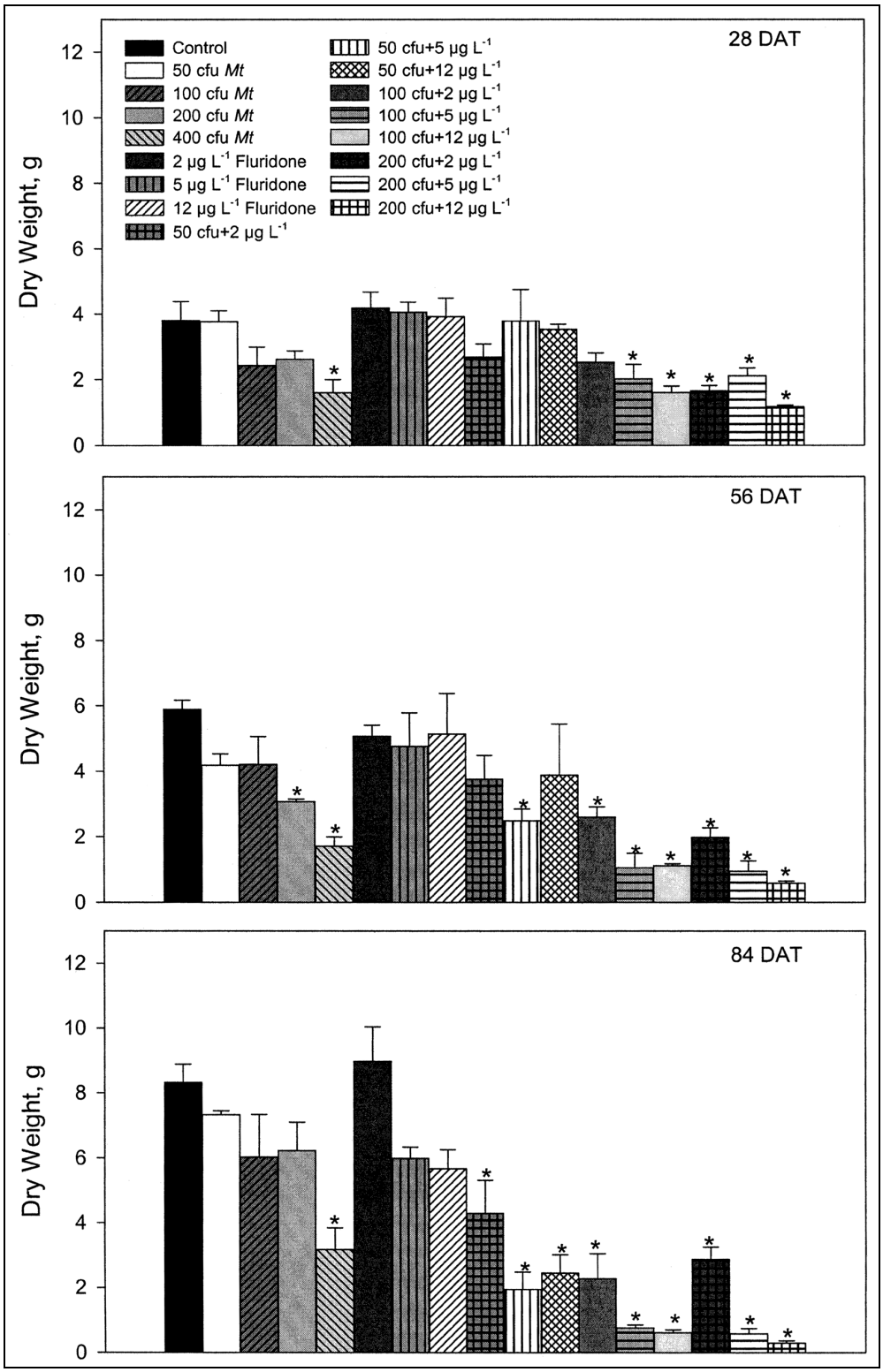

Figure 2. Response of milfoil shoot biomass 28, 56, and 84 days after treatment (DAT) with fluridone, *Mt*, and fluridone + *Mt*. Bars above histograms represent standard errors of the mean. Asterisk (*) indicates significant differences from the untreated control according to Dunnett's two-tailed *t*-test (P=0.05)

as 28 DAT with combined treatments of 100 cfu ml^{-1} *Mt* with either 5 or 12 µg L^{-1} fluridone and 200 cfu ml^{-1} *Mt* with all rates of fluridone. Only the highest rate of *Mt* (400 cfu ml^{-1}) applied by itself consistently reduced milfoil biomass (61-percent reduction at 84 DAT) during the course of this study; however, healthy regrowth from surviving root crowns was evident by the final harvest. Although herbicide injury was visibly noted on milfoil treated with 12 µg L^{-1} fluridone alone, many viable plant fragments remained and contributed to final biomass.

It should be noted that other researchers have reported excellent control of milfoil following an extended exposure (90 days) to12 µg L^{-1} fluridone (Netherland, Getsinger, and Turner 1993). Under these experimental conditions, a similar treatment (84-day exposure to12 µg L^{-1} fluridone) was not effective for suppressing milfoil biomass. This reduced efficacy may be attributed to a slow rate of milfoil growth at the time of treatment. For many herbicides including fluridone, rapid plant growth is essential for maximum herbicidal activity. The Sonar™ AS product label specifically states that best results will occur when fluridone is applied prior to initiation of weed growth or when weeds begin active growth. Although timing of application is critical when using fluridone alone, the data here suggest that timing of application may be less critical when integrating fluridone with *Mt*.

Effects on leaf chlorophyll content were noted following several treatments (Figure 3). Initially (7 DAT), most of the treatments (except 50 and 100 cfu ml^{-1} *Mt*, 2 µg L^{-1} fluridone, and 50 cfu ml^{-1} *Mt* + 2 µg L^{-1} fluridone) reduced chlorophyll levels 28 to 56 percent compared to untreated plants. As milfoil recovered, chlorophyll content approached levels found in untreated plants. By 84 DAT, only five treatments had significantly lower chlorophyll levels compared to control plants. Reduced leaf chlorophyll has been reported by others (Netherland, Getsinger, and Turner 1993; Netherland and Shearer 1996; Nelson, Shearer, and Netherland 1998) and is a characteristic response following fluridone or fluridone and *Mt* applications.

One advantage often cited for implementing integrated management strategies is increased selectivity as a result of using less herbicide. Results of outdoor mesocosm evaluations showed there was a significant difference in the species-selective properties of fluridone between application rates of 5 and 10 µg L^{-1} (Netherland, Getsinger, and Skogerboe 1997). However, it was also reported that sustained (>60 days) fluridone concentrations of 12 µg L^{-1} were required for successful control of milfoil in laboratory studies (Netherland, Getsinger, and Turner 1993). Therefore, the range of fluridone concentrations for selective control of milfoil in a mixed plant community is quite narrow (Netherland, Getsinger, and Skogerboe 1997). Results reported here suggest that combining 100 to 200 cfu ml^{-1} *Mt* with 5 µg L^{-1} fluridone will provide control of milfoil while lowering chemical use rates and the risk of herbicide injury to non-target plants.

The presence of vegetation plays an important role in aquatic ecosystems, therefore the ability to selectively remove invasive plant species while minimizing effects to desirable species is a worthy goal for many aquatic plant managers. Furthermore, the recent discovery of herbicide-resistant hydrilla in several Florida lakes will likely elevate the need to identify new technologies that minimize repetitive chemical use. Integrating sub-lethal doses of herbicides with fungal pathogens is a promising technology that will play an important role as an alternative aquatic plant management tool in the near future.

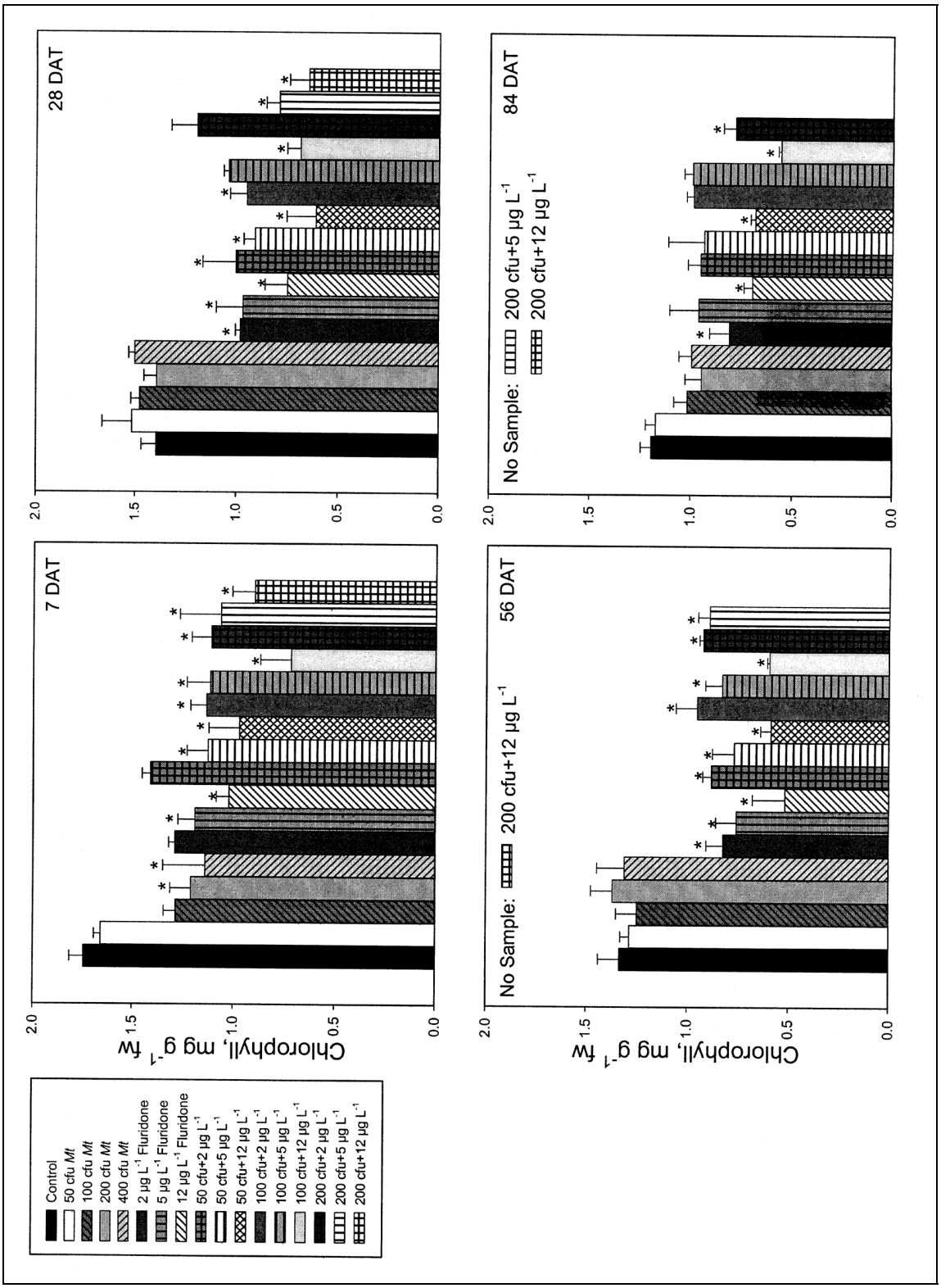

Figure 3. Response of milfoil leaf chlorophyll content (chlorophyll *a* and *b*) following treatment with fluridone, Mt, and fluridone + Mt. Bars above histograms represent standard errors of the mean. Asterisk (*) indicates significant differences from the untreated control according to Dunnett's two-tailed *t*-test (P=0.05). "No sample" indicates those treatments with insufficient leaf tissue remaining for collection and analysis

FUTURE WORK: Future research will evaluate additional herbicide-*Mt* combinations for control of hydrilla and milfoil. Once *Mt* is formulated for use in large-scale field applications, studies will be conducted to demonstrate integrated chemical and biological control techniques on an operational scale.

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