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Evaluation of a New Biological Control Pathogen for Management of Eurasian Watermilfoil

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PURPOSE: This technical note describes the results of an aquarium study to evaluate the effectiveness of a potential fungal pathogen in managing the nuisance submersed plant Eurasian watermilfoil.

INTRODUCTION: *Myriophyllum spicatum* L. (Eurasian watermilfoil; hereafter called milfoil) was first documented in the United States in 1942 but its introduction could have taken place much earlier (Couch and Nelson 1985). It now occurs in lakes, ponds, reservoirs, or rivers in 48 states (excluding Wyoming and Hawaii) and in the Canadian provinces of British Columbia, Ontario, and Quebec. Herbarium records indicate that there could have been multiple introductions, as early reports came from widely separated locations including Washington DC, the Midwest, and Arizona and California (Smith and Barko 1990). Milfoil spreads naturally by fragmentation and stolons, and anthropogenically on boating equipment.

Like other aggressive invasive species, milfoil displaces native species, thereby reducing biodiversity. Its ability to grow at low temperatures allows it to quickly reach the water surface, forming a canopy that shades out other aquatic vegetation (Madsen et al. 1991). Excessive growth adversely affects recreational activities such as swimming, boating, and fishing and degrades the aesthetic appeal of a water body. Additionally, excessive growth results in clogged intakes of industrial and power-generating facilities, lowered dissolved oxygen, and increased mosquito breeding sites (Bates et al. 1985).

Traditionally milfoil has been controlled with mechanical removal or herbicide applications. According to Sorsa et al. (1988), the former is cost prohibitive and the latter potentially controversial due to real or perceived threats to human health and the environment. Biological control has been studied as an option for milfoil management for over 40 years. Although overseas studies have not yielded any classical agents, several native or naturalized insects and pathogens have been studied (Johnson and Blossey 2002). Three herbivores, a midge (*Cricoptopus myriophylli* Oliver), a weevil (*Euhrychiopsis lecontii* Dietz), and a pyralid moth (*Acentria ephemerella* Denis and Schiffermüller), have been implicated in milfoil declines in some lakes in North America (Johnson and Blossey 2002). Although milfoil is not a preferred consumptive plant species for grass carp (*Ctenopharyngodon idella* (Cuvier and Valenciennes)), it has been used for that purpose since 1963 (Julien and Griffiths 1998).

Surveys for pathogens of milfoil were first undertaken in the late 1970s. John Anderson and his students at the University of Wisconsin, Madison, researched the use of the fungal pathogens *Fusarium sporotrichioides* Sherbakoff (Andrews and Hecht 1981), *Acremonium curvulum* W. Gams (Andrews et al. 1981) and *Colletotrichum gloeosporioides* (Penz.) Penzig and Saccardo (Smith et al. 1989) for potential milfoil management. Although all three fungi caused some damage to milfoil in the laboratory, they were found not to be highly pathogenic and therefore were considered poor candidates

for further development as biocontrol agents. Concurrently, Gunner (1983) was also researching pathogenic agents for milfoil at the University of Massachusetts. *Mycoleptodiscus terrestris* (Gerd.) Ostazeski, a cellulolytic fungus, was found to be efficacious on milfoil in both laboratory and field trials (Gunner et al. 1988, 1990). As a result, a company, EcoScience, was formed to develop the organism into a commercial product, Aqua-Fyte. However, field trials undertaken at a pond located at the Tennessee Valley Authority Murphy Hill field station adjacent to Guntersville Reservoir yielded disappointing results and product development was discontinued (Shearer 1995).

The U.S. Army Corps of Engineers (USACE) began surveys for milfoil pathogens in the 1980s (Zattau 1988). Fifty milfoil-infested water bodies in 10 states yielded 462 bacterial and 330 fungal isolates. Based on lytic enzyme analysis, 14 bacterial and 22 fungal isolates were further evaluated for efficacy on milfoil. A few fungal isolates were efficacious but none were ever developed. All the bacterial isolates proved to be poor candidates in laboratory tests.

Although EcoScience discontinued mycoherbicide development of *M. terrestris*, USACE continued to examine the potential of a different *M. terrestris* milfoil isolate, combining it with aquatic herbicides in an integrated approach to milfoil management. *Mycoleptodiscus terrestris* was used in studies with fluridone (Nelson and Shearer 2002), 2,4-D (Nelson and Shearer 2005), and triclopyr (Nelson and Shearer 2008). In each case, combining the herbicide and the fungal pathogen at low dosage levels significantly reduced shoot biomass to a much greater degree than either product used alone.

New surveys for additional pathogenic agents of milfoil were conducted in 2009 (Shearer et al. 2011). Fungi were collected from 53 lakes located in different geographic regions of the United States, and 457 strains of fungi were isolated from these collections. Of these, 259 isolates were screened in a flask study for pathogenicity on milfoil (Shearer et al. 2011). The rest were not screened because they were known to be weak pathogens or saprophytes. A maximum disease rating of 4 was given to five isolates, four *M. terrestris* and one *Myrothecium roridum*. This technical note reports on the impact of *M. roridum* on milfoil in a larger scale study conducted in 55-liter aquariums.

MATERIALS AND METHODS: The pathogenicity of *M. roridum* to milfoil was evaluated in an aquarium study that was set up in a greenhouse at the Aquatic and Wetlands Ecosystems Research and Development Center at the Engineer Research and Development Center (ERDC) facility in Vicksburg, Mississippi. Milfoil was collected from culture tanks maintained in a biocontrol greenhouse at the center. Four apical stem cuttings of milfoil (approximately 15 cm in length) were planted in plastic deli cups (0.95 L) filled to three-fourths capacity with topsoil (Earthgro, Hyponex Corporation, Marysville, Ohio) amended with 2.4 g Osmocote (14-14-14) (Scotts-Sierra Horticultural Products Company, Marysville, Ohio) and overlain with silica sand to prevent soil resuspension and algal growth. Four cups were placed in each aquarium filled with a water-based culture solution (Smart and Barko 1985). Air was gently bubbled in each aquarium to provide circulation of the culture solution. Approximately 28 days after planting, the milfoil had reached the water surface and was ready for inoculation.

Myrothecium roridum was retrieved from storage and plated onto Potato Dextrose Agar (PDA) (Difco Inc, Detroit, Michigan) plates. The cultures were allowed to grow for 3 weeks under ambient light at room temperature (25 °C) in the biomanagement laboratory at ERDC. Each colony was cut into small pieces (1 mm x 1 mm). One half of the small pieces from each plate were added to a 250-ml baffled flask containing 100 ml of Richards's V-8 juice broth, which consisted of 10 g glucose; 10 g KNO₃; 3 g CaCO₃; 200 ml V-8 juice (Campbell's, Camden, New Jersey); and 800 ml H₂O. The flasks were placed

on a platform shaker (New Brunswick, Edison, New Jersey) set at 300 rpm. Flasks were swirled daily to prevent fungal buildup along the sides of the flasks. After 7 days, the contents were ground in a blender for 30 seconds to homogenize the culture. The number of colony-forming units (cfu) present in the inoculum was determined by dilution plating.

The milfoil treatments were *M. roridum* applied at rates of 5, 10, 15, and 20 ml per aquarium and an untreated control. Each treatment was replicated six times. The experiment was repeated twice. The treatments were evaluated 28 days post inoculation using a rating scale of 0-4 (0 = no disease, tissues green and healthy; 1 = slight chlorosis; 2 = general overall chlorosis; 3 = tissues discolored and stems beginning to fragment; 4 = total discoloration and tissues collapsed with no possibility of regrowth).

RESULTS AND DISCUSSION: The *M. roridum* inoculum used in the study was viable, yielding 1.16×10^8 cfu/ml. Although *M. roridum* was extremely efficacious in the flask study, resulting in total collapse of milfoil tissues (disease rating of 4), this was not the case in the aquarium studies. There were no visual differences between the treated and untreated aquariums; therefore, the biomass was not harvested for dry weight comparisons. The tissues remained mostly green; minimal browning was probably the result of age and canopy shading after approximately 2 months of growth that occurred between planting and experiment termination.

Several reasons may explain the apparent lack of efficacy on milfoil in the aquarium study. Some fungi can lose virulence in storage. For example, *M. terrestris* must periodically be inoculated onto hydrilla plants and reisolated to maintain its high level of virulence (author's personal experience). The *M. roridum* isolate had been in storage approximately seven months at the time of the flask study test, whereas it had been in storage for almost three years at the time of the aquarium study. The isolate grew slowly on PDA and in order to have enough biomass to chop up into pieces before adding it to the V-8 broth, it had to be grown three weeks rather than the usual two. Toxin production may be a factor in plant disease development (Agrios 2005). *Myrothecium roridum* toxin production has been suggested as contributing to lesion development in many plant species (Quezado Duval et al. 2010, Murakami et al. 1999). Many pathotypes of *M. roridum* have been reported, some more effective in inducing disease than others (Taneja et al. 1990). It is unknown if the isolate used in the present study was a toxin-producing strain. Finally *M. roridum* grows optimally at a pH range from 5.0 to 7.0 (Okunowo et al. 2010). The water in the aquariums was between pH 8.0 to 9.0 at the time of inoculation. Tissue lysing from the homogenization process was probably not a factor because cfu counts were high.

Species of *Myrothecium* have been reported to be potentially good biocontrol agents for a number of weeds including waterhyacinth [*Eichhornia crassipes* (Mart.) Solms] (Okunowo et al. 2010, Ponappa 1970, Liyanage and Gunasekera 1989) kudzu [*Pueraria lobata* (Willd.) Ohwi] (Hoagland et al. 2007), redvine [*Brunnichia ovata* (Walt.) Shinnery] and trumpetcreeper [*Campsis radicans* (L.) Seem. ex Bureau] (Boyette et al. 2008). Combined with glyphosate in an integrated weed management approach, *M. verrucaria* could control weeds in fields planted to glyphosate-resistant soybeans without adversely affecting the crop plant (Boyette et al. 2008).

FUTURE WORK: Since success has been documented using *M. roridum* as a biocontrol pathogen, other *Myrothecium* isolates in the biocontrol pathogen collection will be further evaluated. In addition, the isolate used in the present study will be reevaluated in flask studies as described in Shearer et al.

(2011). Additional sustainable control technologies will be necessary in the future to control invasive milfoil populations.

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