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The Potential Role of an Endophytic Fungus in the Decline of Stressed Eurasian Watermilfoil

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ABSTRACT

A greenhouse study was conducted to test effects of stress induced by simulated chemical runoff on endophyte-infected [*Mycoleptodiscus terrestris* (Gerd.) Ostazeski] and endophyte-free Eurasian watermilfoil (*Myriophyllum spicatum* L.). Simulated chemical runoff stress was induced by applying low rates of the herbicide endothall (dipotassium salt of 7-oxybicyclo {2,2,1}heptane-2, 3-dicarboxylic acid) for a 24-hour exposure time. Treatments included 0.5 and 1.0 mg L^{-1} endothall applied to endophyte-infected and endophyte-free Eurasian watermilfoil plants and untreated controls. Four weeks after herbicide application, shoot dry weight of Eurasian watermilfoil endophyte-infected plants was reduced by 75% and 72% when exposed to rates of 1.0 and 0.5 mg L^{-1} of endothall respectively compared to the endophyte-infected control plants. The shoot dry weight of endophyte-free plants was reduced by 58.2% and 30.8% respectively compared to the endophyte-free control plants. The presence of *M. terrestris* as an endophyte that has the potential to behave as a latent pathogen may lessen the ability of some Eurasian watermilfoil populations to survive stress conditions. Stress conditions appeared to weaken plants and render them susceptible to attack from an otherwise benign organism.

Key words: Myriophyllum spicatum, fungal endophyte, *Mycoleptodiscus terrestris.*

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INTRODUCTION

Since its introduction into the United States during the last century, Eurasian watermilfoil has spread and is now present in 43 of the 48 contiguous states and in Alaska (PMIS 2001). At scattered locations, established Eurasian watermilfoil populations have undergone major declines that varied in both rate and amount of decline. Smith and Barko (1990) reported that *M. spicatum* populations declined in Wisconsin, southern Ontario, the Chesapeake Bay, and the Okanagan Valley lakes of British Columbia. More recent declines have been known to occur in lakes in Vermont and Illinois and also declines have occurred in the Tennessee Valley Authority (TVA) reservoirs of Alabama, Kentucky and Tennessee (Smith and Barko 1996). Various causes proposed for the declines include factors such as nutrient depletion, shading by phytoplankton and algae, attack by parasites and pathogens, long-term effects of harvesting and/or herbicides, toxins, climate, competition, and insect herbivory, but none of these causes have been adequately explained (Smith and Barko 1990).

Following some of the more recent declines, plant materials were collected from the declining Eurasian watermilfoil populations and bioassayed for fungal pathogens. One common characteristic shared by all sites was the high frequency of occurrence of the fungus, *M. terrestris* in stem and leaf tissues of Eurasian watermilfoil plant material (Shearer 2001). The association of *M. terrestris* with Eurasian watermilfoil was first documented by Gunner (1983) following surveys of plant populations in Massachusetts in an effort to find microbial biological control agents for management of this aquatic weed. Although *M. terrestris* was studied as a potential biological control for Eurasian watermilfoil by EcoScience, Inc., Worcester, MA problems with formulation and unsuccessful field tests ceased further development of this fungus as a bioherbicide by the company (Smith and Winfield 1991; Shearer 1994).

Additional studies have documented that *M. terrestris* was consistently and repetitively isolated from healthy, stressed, asymptomatic, as well as senescing Eurasian watermilfoil populations (Shearer 2001). Such association of *M. terrestris* with Eurasian watermilfoil is consistent with fungi that are referred to as class-three endophytes or latent pathogens (Rodriguez and Redman 1997). They are so classified because plants remain asymptomatic of disease until changes in certain environmental or nutritional conditions and/or the state of host maturity alters the fungal state from that of a benign endophyte into a harmful pathogen (Agrios 1988).

Studies of endophytic fungi over the past 25 years indicate that they occupy a unique ecological niche, and are thought to influence plant distribution, ecology, physiology, and biochemistry (Sridhar and Raviraja 1995). The presence of *M. terrestris* as an endophyte in Eurasian watermilfoil tissues may have an indirect effect on health and survival of stressed plants. Once a Eurasian watermilfoil population is stressed, endophytic *M. terrestris* may behave as a pathogen and contribute to a major population decline in a short period of time. However, this hypothesis has not been tested. Therefore, this aquaria-based study presented herein was designed to provide a preliminary assessment of the effects of the endophytic fungus, *M. terrestris*, on the decline of stressed Eurasian watermilfoil populations.

MATERIALS AND METHODS

Plants free of the endophyte *M. terrestris* were obtained from Kirk Pond located at the U.S. Army Corps of Engineers Portland District Fern Ridge Park, Junction City, OR. Eurasian watermilfoil plants were collected from this site because surveys conducted in early 1990's had shown that the endophyte *M. terrestris* was not present in the populations in Kirk Pond (Madsen et al. 1992). Eurasian watermilfoil plants infected with the endophytic *M. terrestris* were purchased from Suwannee Laboratories, Inc. (Lakeland, FL). Presence or absence of the endophyte was determined by surface disinfecting segments of Eurasian watermilfoil stems and leaves using a 20% commercial bleach followed by rinsing in sterile water, and plating the pieces onto Martin's agar (dextrose, 10 g; KH_2PO_4, 0.5 g; $MgSO_4 \cdot 7\ H_2O$, 0.5 g; K_2HPO_4, 0.5 g; peptone, 0.5 g; yeast extract, 0.5 g; H_2O, 1L; rose Bengal, 0.05 g; streptomycin sulfate, 0.03 g). After 7 days incubation at 28C, the plates were visually assessed to determine presence or absence of *M. terrestris* growing from plant tissues.

Eighteen 55 L aquaria (0.9 m tall by 0.09 m^2) were filled with a water based culture solution recommended for aquatic plant growth (Smart and Barko 1984). Lake sediment collected from Brown's Lake at the U.S. Army Engineer Research and Development Center Waterways Experiment Station (ERDC-WES), Vicksburg, MS, was amended with ammonium chloride (0.5 g L^{-1}) and Esmigran (1.7 g L^{-1}). Five plastic cups (0.95 L) filled three-fourths with lake sediment that contained five 20-cm apical cuttings from either endophyte-infected or endophyte-free Eurasian watermilfoil were overlaid with silica-sand, and were placed in each aquarium. The plants were then allowed to grow 28 days by which time they had formed surface canopies.

The study consisted of untreated reference aquaria and two concentration exposure time treatments of endothall (1.0 mg L^{-1} and 0.5 mg L^{-1} ae for 24 hours) to endophyte-infected and endophyte-free Eurasian watermilfoil plants. To simulate induced stress from chemical runoff, both endophyte-infected and endophyte-free Eurasian watermilfoil plants were treated with low rates of endothall (the dipotassium salt of 7-oxybicyclo {2,2,1}heptane-2,3-dicarboxylic acid) for a 24 hour exposure time. Endothall was chosen because previous concentration exposure time studies indicated that the herbicide applied at rates of 1.0 mg L^{-1} for 24 hours would injure Eurasian watermilfoil but within 1 week post-treatment it had a high probability of regrowth (Netherland et al. 1991). Each treatment was replicated three times and randomly assigned to a test aquarium. After the 24-hour exposure to endothall, the aquaria were drained and refilled with nutrient solution. The experiment was repeated twice.

The plants were allowed to respond to the simulated chemical runoff treatments for 4 weeks. Previous studies indicated a 4-week time period was sufficient to determine if Eurasian watermilfoil would recover from the chemical treatments (Netherland et al. 1991). From the Eurasian watermilfoil shoot biomass in each aquarium, six stem pieces (ca. 2 cm long) were collected to bioassay for presence or absence of *M. terrestris* in the plant tissues. The stem pieces were plated onto Martin's agar and incubated for 7 days at 28C. The plates were then visually assessed for presence or absence of *M. terrestris* colonies growing from the stem pieces. The remaining shoots were harvested and oven-dried at 60C to a constant weight.

Biomass data were statistically evaluated using analysis of variance (ANOVA) (Statistica, StatSoft, Tulsa, OK). Mean separations were accomplished using Tukey's Honest Significant Difference (HSD) test. Test of significance was conducted at $P \leq 0.05$.

RESULTS AND DISCUSSION

Assessment of Eurasian watermilfoil stem and leaf segments confirmed that the endophyte, *M. terrestris*, was present in the Eurasian watermilfoil populations from Florida (87% recovery rate) but was absent from plants collected from Oregon. The apical tips that were selected for planting from both populations appeared green, healthy, and asymptomatic of endophytic infection. The plants from both populations grew equally well and formed a canopy within 28 days after planting.

Four weeks following application of low levels of the herbicide endothall to simulate stress, dry weight of shoot biomass of the endophyte-infected Eurasian watermilfoil plants was reduced by 75% and 72% by rates of 1.0 mg L^{-1} and 0.5 mg L^{-1} respectively compared to the endophyte-infected control plants. The biomass of endophyte-free plants was reduced by 58% and 31% respectively, compared to the endophyte-free control plants (Figure 1). A significant reduction in shoot biomass occurred only with exposure to the higher rate of endothall. By the end of the experiment the endophyte-free plants were recovering and beginning to actively regrow in both rates of endothall treatments. On the other hand biomass of endophyte-infected Eurasian watermilfoil was significantly reduced in both rates of endothall treatment. At the time of harvest, vigorous regrowth had not initiated from the plant material remaining in the aquaria although some newly emerging green tips were evident. The fact that both levels of herbicide had nearly equal effects on endophyte-infected Eurasian watermil-

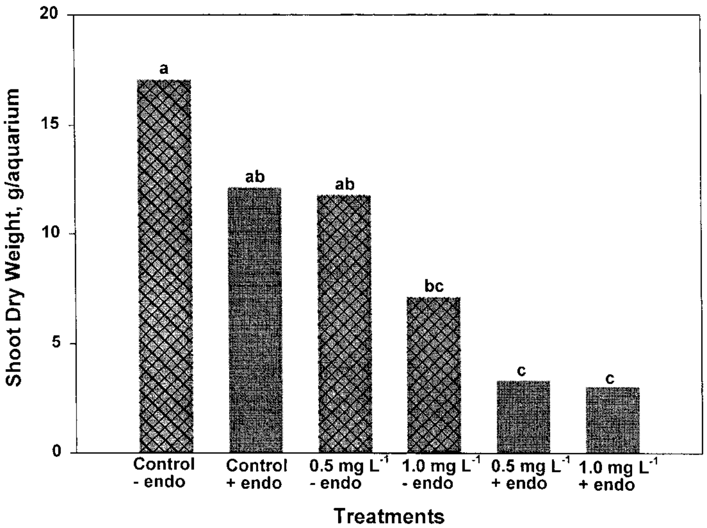

Figure 1. Dry weight of Eurasian watermilfoil shoot biomass 28 days after simulated chemical runoff stress induced by treatments with endothall at 1.0 mg L^{-1} and 0.5 mg L^{-1} for 24 hours and untreated controls (- endo = without *M. terrestris* endophyte; + endo = with *M. terrestris* endophyte). Means followed by the same letter are not significantly different at $P \leq 0.05$ according to Tukey's Honest Significant Difference (HSD) test.

foil suggested that there may be a threshold of stress above which Eurasian watermilfoil plants could not ward-off an endophyte from being activated to the pathogenic state. Internal defense mechanisms normally used to ward off disease were in all likelihood compromised by the weakened condition of the plant. Studies of latent infections by endophytes in soybeans have yielded similar results (Sinclair and Cerkauskas 1996). Symptomless pods and stems of soybeans harboring the fungus *Cercospora kikuchii* developed more lesions when tissues were treated with paraquat than when tissues were not treated thereby implicating latent infection. In addition, soybeans infected with the latent pathogen, *Colletotrichum truncatum*, only developed severe symptoms after prolonged periods of high humidity, as plants senesced, or when they became stressed.

Shoot biomass of the endophyte-infected control plants was ca. 30% less than the endophyte-free plants at the conclusion of the experiment. Several factors could account for the differences. Firstly, the amount of photosynthates that would normally go to support plant growth in endophyte infected plants is in all likelihood reduced by sequestration by the fungal endophyte for its own growth and maintenance. Secondly, populations of Eurasian watermilfoil collected from two geographically separated regions may be inherently different in their ability to produce biomass although they may show similar growth patterns.

During experimentation, cross-contamination may occur between two host populations. In this study the purity of the population was assessed at the end of the experimentation period. *M. terrestris* remained absent from aquaria containing the endophyte-free Eurasian watermilfoil. By contrast, a 100 percent recovery rate was recorded for *M. terrestris* from stem pieces collected from aquaria containing endophyte-infected Eurasian watermilfoil.

These results may offer some insight into why some seemingly healthy Eurasian watermilfoil populations have declined unexpectedly in a short period of time. The presence of an endophytic fungus such as *M. terrestris* may lessen the ability of some Eurasian watermilfoil populations to survive stress conditions that first weaken plants and then render them susceptible to attack from an otherwise benign organism. Also of consideration would be the particular strain of the fungus inhabiting the host. Fungal strains differ in their ability to induce disease and some endophytes may remain avirulent even under stress-induced conditions whereas others may become highly virulent and attack a host plant. The research may also offer some considerations for aquatic managers in controlling infestations of Eurasian watermilfoil. The possibility exists that the presence of a class-three endophyte in a Eurasian watermilfoil population might mean the use of less herbicide if the additive effect of the endophyte could be factored in with chemical treatment application rates.

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