Mycoleptodiscus terrestris: An Endophyte Turned Latent Pathogen of Eurasian Watermilfoil

by Judy F. Shearer

PURPOSE: Plants that are stressed or weakened are more susceptible to disease than healthy plants. An endophytic fungus that has the capability of becoming a latent pathogen may trigger a host-fungus interaction resulting in disease development in Eurasian watermilfoil (*Myriophyllum spicatum* L.) (EWM) plants when they are subjected to stress. The purpose of the present study was to determine if endophyte infected (E+) plants that are weakened by nutrient stress decline more rapidly and to a greater degree than plants that are endophyte free (E-).

INTRODUCTION: At scattered locations in North America, EWM populations have undergone major declines that have varied in both rate and amount of decline. Smith and Barko (1990) reported EWM declines in Wisconsin, southern Ontario, the Chesapeake Bay, and the Okanogan Valley lakes of British Columbia. More recent declines have occurred in lakes in Vermont and Illinois as well as in Tennessee Valley Authority (TVA) reservoirs in Alabama, Kentucky, and Tennessee (Smith and Barko 1990). Among the causes that have been proposed as contributing to the declines are nutrient depletion, shading, disease, herbicide runoff, toxins, climate, competition, repeated mechanical harvesting, and insect herbivory, but none have adequately explained these declines (Smith and Barko 1990).

Past studies have documented that *Mycoleptodiscus terrestris* (Gerd.) Ostazeski (Mt) is commonly isolated as an endophyte from green and senescing EWM tissues (Shearer 2001). It is also found as an endophyte in Northern watermilfoil (*Myriophyllum sibiricum* Komarov) and in the hybrid *M. sibiricum* x *spicatum* (unpublished results). In all cases the plants in general appeared healthy and asymptomatic and lacked the distinctive spotting and browning typical of disease symptoms caused by Mt.

Previous greenhouse studies using E+ and E- plants have documented that E+ EWM plants that are stressed by simulated herbicide runoff decline to a greater extent than E- EWM plants (Shearer 2002). Under these conditions Mt would be classified as a class-three endophyte or latent pathogen (Rodriguez and Redman 1997) because the plants were asymptomatic until the stress-promoting conditions altered the fungal state from a benign endophyte to a harmful pathogen (Agrios 1988).

Nutrient-related stress factors could also lead to declines from E+ EWM. Sculthorpe (1985) reported that submerged plants absorb ions from both the substrate and from the water. If sediment nutrients become limiting, EWM plants would have to rely on uptake of nutrients from the surrounding waters. Being heterotrophic, a fungal endophyte in EWM tissues would be dependent on its host to supply it the necessary nutrients for growth and survival. By shunting nutrients from the host for its own growth, the fungus could potentially induce stress by competing for limited resources. To test this hypothesis, E+ and E- Eurasian watermilfoil plants were grown in fertilized and used sediments to determine if the endophyte could be induced to pathogenicity in plants that were potentially nutrient stressed.

MATERIALS AND METHODS: E- plants were obtained from the U.S. Army Corps of Engineers culture ponds located at the Lewisville Aquatic Ecosystem Research Facility (LAERF) located in Lewisville, TX. EWM plants were collected from LAERF because they were known to be free of the endophyte. Systematic plating of stem tissues over a number of years has never yielded an isolation of Mt from this site. E+ plants were obtained from culture stock at the U.S. Army Corps of Engineers Research and Development Center (USACE ERDC), Vicksburg, MS. Plating of stem pieces of EWM from the culture stock consistently yielded Mt from host tissues. In both cases the presence or absence of the endophyte was determined by surface disinfecting segments of EWM stems and leaves using a 10-percent commercial bleach followed by rinsing in sterile water and plating the pieces onto Martin's agar (dextrose, 10 g; agar, 20g; KH2PO4, 0.5 g; MGS O4, 0.5 g; K2 HPO4, 0.5 g; peptone, 0.5 g; yeast extract, 0.5 g; H2O, 1 L; rose Bengal, 0.05 g; streptomycin sulfate, 0.03 g). The streptomycin was added after the agar had been autoclaved and cooled to approximately 60 °C. After 7 days incubation at 28 °C, the plates were visually assessed to determine presence or absence of Mt growing from plant tissues.

Sediment used in the study was collected from Brown's Lake, ERDC, and was processed in the greenhouse to obtain two sediment treatments. A nutrient-poor sediment (N-) was obtained by utilizing sediments that had previously been used to grow EWM plants. A nutrient-rich sediment (N+) was obtained by amending fresh sediment with ammonium chloride (0.5 g L-1) and Esmigran (1.7 g L-1) (Scotts, Marysville, OH). The sediments were placed in plastic cups (0.95 L) to within 5 cm of the top of the cup. Five 20-cm apical cuttings from either E+ or E- EWM were placed in a cup containing either N- or N+ sediment and overlain with silica-sand to reduce sediment resuspension and algal growth. The cups were placed four to an aquarium (55 L) that had been filled with a water-based culture solution recommended for aquatic plant growth (Smart and Barko 1984). Each treatment was replicated five times.

The plants were allowed to respond to the sediment treatments for 4 weeks. Shoot biomass was then harvested and oven-dried at 60 °C 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 Significance Difference (HSD) test. Test of significance was conducted at $P \leq 0.05$.

RESULTS AND DISCUSSION: Assessment of EWM stem and leaf segments confirmed that the endophyte Mt was present in EWM from greenhouse culture tanks but was absent from plants collected at LAERF. The apical tips that were selected for planting from both collections appeared green and healthy and were asymptomatic of endophytic infection.

Within two weeks following planting, small dark spots began appearing on the stems of E+ EWM plants rooted in N- sediments. Many plants in response to ingress by a fungal pathogen produce phytoalexins to try to inhibit microbial growth and expansion (Agrios 1988). The production of these phenolic compounds is often associated with spotting on plant surfaces where the plant tries to wall off the infection. Disease onset can still occur, however, if the pathogen becomes more aggressive or host defenses are compromised. After three weeks growth, disease symptoms became obvious on E+ EWM plants in N- sediments. Stem and leaf segments were turning brown and becoming flaccid. By four weeks following planting, EWM plants were continuing to decline and the study was terminated. In contrast, aboveground biomass of EWM in all other treatments remained asymptomatic and disease free although it was apparent that the E- EWM plants grown in N- sediments exhibited spindly growth and shoot biomass appeared reduced compared to plants growing in N+ sediments.

Shoot biomass of plants that were grown in N+ sediments was significantly greater at four weeks following planting than shoot biomass of plants grown in N- sediments (F (1,15); p = 0.00145) (Figure 1). Although nutrients in the water column were equally available to all EWM plants in the study, biomass accumulation was much reduced in plants that were grown in the N- sediments suggesting that nutrients in the water column alone were not sufficient for maintaining good plant growth.

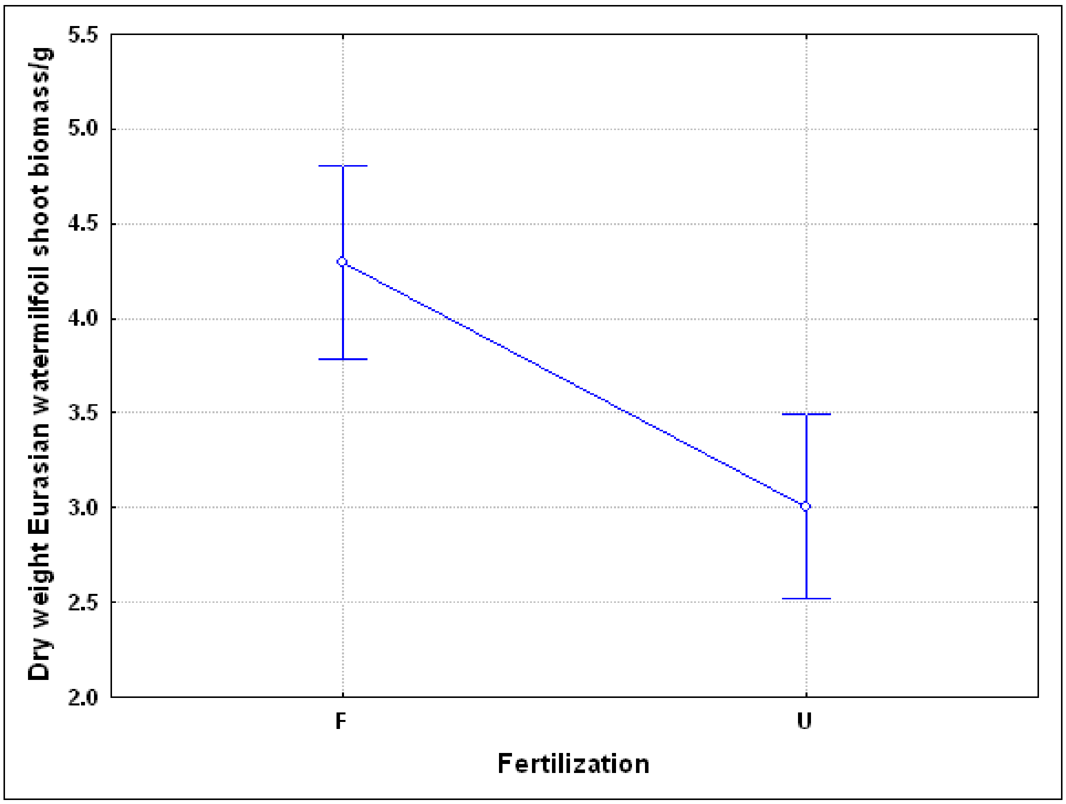

Figure 1. Dry weight of Eurasian watermilfoil shoot biomass harvested four weeks following planting in fertilized (F) and used (U) sediments.

The endophytic condition of the plants was not a significant factor in shoot biomass accumulation. The dry weights were not significantly different F (1.15), p = 0.11054. However the interaction between endophytism and fertilization was significant; F (1,15), p = 0.01128 (Figure 2). The biomass of E+ EWM plants that were grown in N- sediments was significantly lower than any other treatment. Although not significantly different, the biomass of E+ EWM plants was slightly greater than E- EWM plants indicating that under non-stress conditions, endophytic infection does not appear to adversely affect the host.

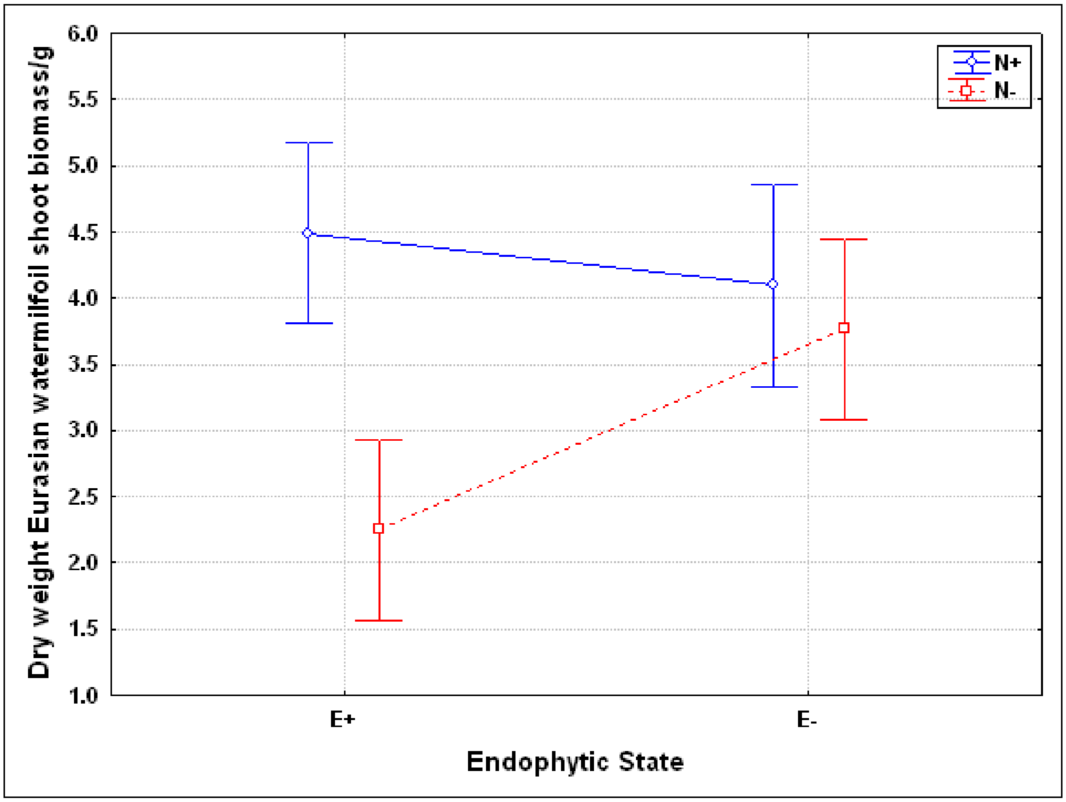

Figure 2. Dry weight of E+ and E- Eurasian watermilfoil plants harvested four weeks following planting in fertilized (N+) and used (N-) sediments.

It is unknown at the present time if endophytism in EWM offers any advantages to the plant. Studies of other plant species have provided mixed results as to the importance of endophytes in excluding or reducing herbivory and pathogen ingress. When some grass and sedge species are infected by fungal endophytes in the Clavicipitaceae, they have been reported to have an increased resistance to insect pests (Clay 1988). Grass seeds infected with endophytes tend to have higher alkaloid concentrations and thus deter feeding by both insects and birds (Clay 1991). In contrast, Faeth and Hammon (1997a) found no evidence that Emory oak endophytes reduced herbivory by leafminers nor did they find any antagonistic interaction between endophytes and leafminers (Faeth and Hammon 1997b). Populations of EWM with and without the presence of the milfoil weevil, *Euhrychiopsis lecontii*, yield similar endophyte assemblages suggesting that they do not deter or promote feeding (personal observation). Indirectly through its feeding activity the insect may be an important vector in movement of the endophytes from infected to uninfected plants.

Some fungal endophytes have also been reported to inhibit plant pathogens. Fewer lesions have been reported on *Panicum agrostoides* when the endophyte *Balansia henningsiana* is present (Clay et al. 1989). Disease severity of crown rust was reported to be reduced in tall fescue plants that were endophyte infected compared to plants that were noninfected (Ford and Kirkpatrick 1989). It is unknown if endophytes of EWM inhibit other pathogens but it is interesting to note that the fungus *Acremonium curvulum* that has been reported to be a weak pathogen of EWM (Andrews et al. 1981) has never been isolated from plants that were infected with the endophyte Mt.

FUTURE WORK: At present there is no information about the role that endophytes might play in promoting or deterring insect feeding on EWM. Studies at the University of Minnesota used the milfoil weevil in tests to determine its preference for feeding on EWM and Northern watermilfoil, its native host. Similar studies could be conducted using E+ and E- EWM. Such studies would be contingent on a ready supply of weevils. The ability to rear weevils at the mass rearing facility located at ERDC's Aquatic and Wetland Research Facility is currently being studied.

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> Shearer, J. F. 2009. *Mycoleptodiscus terrestris: An endophyte turned latent pathogen of Eurasian watermilfoil*. APCRP Technical Notes Collection. ERDC/TN APCRP-BC-11. Vicksburg, MS: U.S. Army Engineer Research and Development Center. *http://el.erdc.usace.army.mil/aqua/aqua.html*.

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