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US Army Corps of Engineers
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Aquatic Plant Control Research Program

Field Test of First Commercial Formulation of *Mycoleptodiscus terrestris* (Gerd.) Ostazeski as a Biocontrol for Eurasian Watermilfoil

by Judy F. Shearer

Prepared for Headquarters, U.S. Army Corps of Engineers

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3909 Halls Ferry Road
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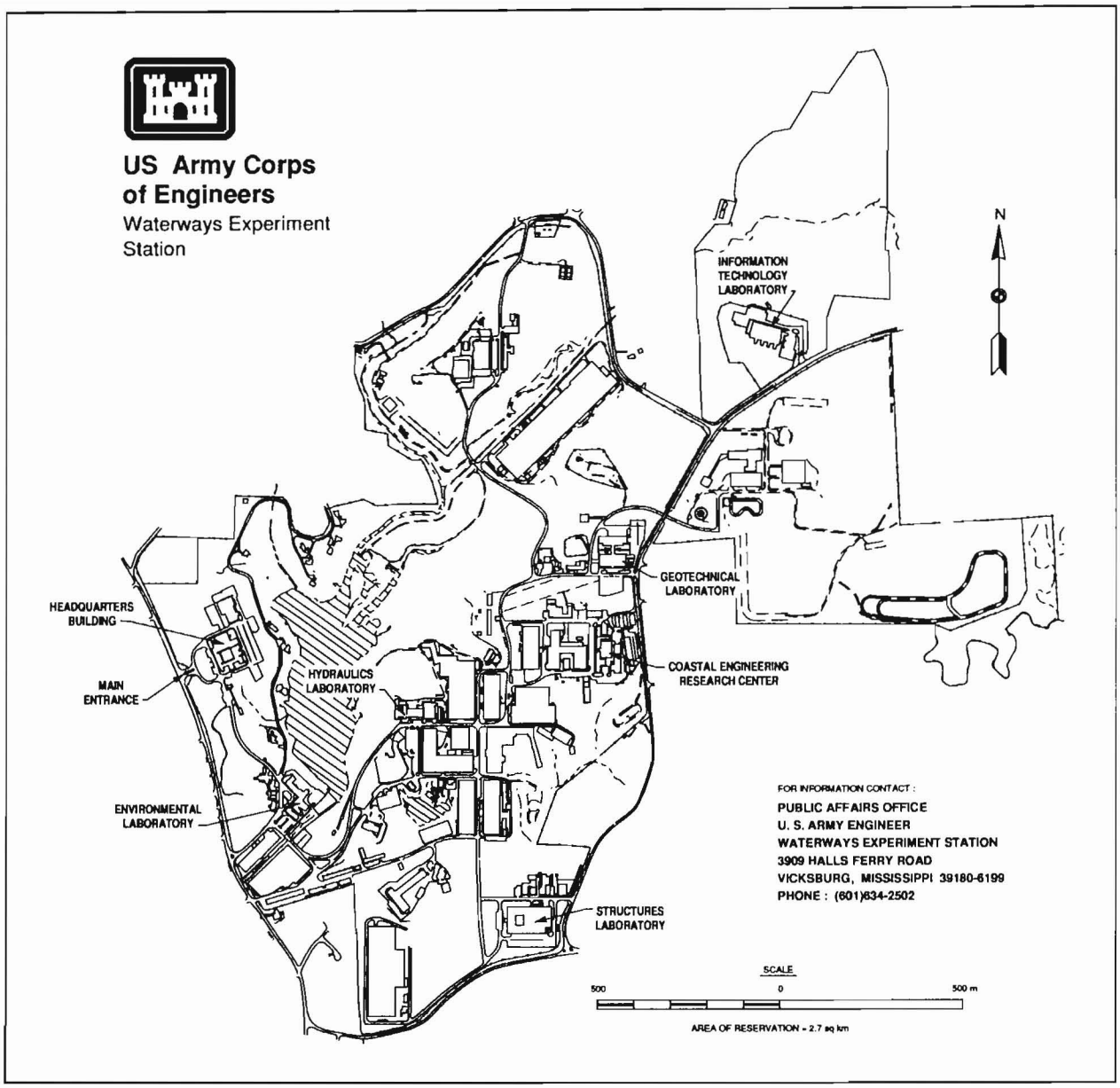

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Preface

The work reported herein was conducted as part of the Aquatic Plant Control Research Program (APCRP), Work Unit 32202. The APCRP is sponsored by the Headquarters, U.S. Army Corps of Engineers (HQUSACE), and is assigned to the U.S. Army Engineer Waterways Experiment Station (WES) under the purview of the Environmental Laboratory (EL). Funding was provided under Department of the Army Appropriation No. 96X3122, Construction General. The APCRP is managed under the Environmental Resources Research and Assistance Programs (ERRAP), Mr. J. L. Decell, Manager. Mr. Robert C. Gunkel was Assistant Manager, ERRAP, for the APCRP. Technical Monitor during this study was Ms. Denise White, HQUSACE.

This report was prepared by Dr. Judy F. Shearer, Aquatic Ecology Branch (AEB), Environmental Resources Division (ERD), EL, WES. The study was supervised at WES by Dr. Ed Theriot, Chief, AEB, and Dr. Conrad J. Kirby, Chief, ERD. Dr. John Harrison was Director, EL.

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1 Introduction

Eurasian watermilfoil, *Myriophyllum spicatum* L., is a submersed aquatic plant that infests freshwater habitats throughout North America. Mechanical removal and herbicide treatment have been the most commonly used management methods to control nuisance populations. Management with biocontrol agents, with the possible exception of the grass carp or white amur, has not been an operational alternative. Although numerous organisms including snails, manatees, fish, insects, and fungi have been suggested for biocontrol against milfoil over the past 20 years, none have been developed for widespread field release.

That plant pathogenic fungi are effective biocontrol agents against noxious plants has been effectively demonstrated with the release of two products in the United States, DeVine and Collego, which are commercially available for control of stranglervine (Kenney 1986) and northern jointvetch (Bowers 1986), respectively. Several more fungal-based products are nearing registration (Te Beest, Yand, and Cisar 1992).

In the late 1970s, a weakly pathogenic fungus, *Mycoleptodiscus terrestris* (Gerd.) Ostazeski, was isolated from Eurasian watermilfoil collected in Massachusetts (Gunner 1983). Cellulolytic properties of *M. terrestris* made it a promising biocontrol candidate (Gunner 1983). Preliminary greenhouse and laboratory studies documented the effectiveness of the fungus in reducing milfoil biomass (Gunner et al. 1990; Stack 1990; Smith and Winfield 1991). A small field test using fungal mycelium in Stockbridge Bowl, MA, supported the laboratory findings by inducing a 16-fold reduction in stem-leaf biomass in treated versus control plots (Gunner 1987).

EcoScience Corporation, Worcester, MA, developed *M. terrestris* (Mt) into a mycoherbicide, Aqua-Fyte, for potential field release. The mycoherbicide strategy uses formulated pathogens to control nuisance plants in ways consistent with herbicide technology and equipment (Te Beest, Yang, and Cisar 1992). Eurasian watermilfoil control would be achieved by applying formulated Mt to a targeted field population at a dosage rate high enough to induce a disease epidemic.

Aqua-Fyte was effective in substantially reducing milfoil biomass in growth chamber studies when water temperatures were between 20 and 28 °C, the optimum disease-inducing range of the fungus (Shearer 1992). Preparatory to commercial release of the product, demonstrated efficacy of the mycoherbicide in field tests was necessary. A small-scale field release of Aqua-Fyte was undertaken at a U.S. Environmental Protection Agency/U.S. Department of Agriculture approved location in Alabama in July 1992.

2 Materials and Methods

The mycoherbicide Aqua-Fyte was prepared by growing the active ingredient (ai) Mt in large-scale fermentation equipment and incorporating it into a biodegradable medium of calcium alginate. The product for the field test was exuded into strings that were cut into 2- by 20-mm segments (Figure 1).

Efficacy testing of Aqua-Fyte on Eurasian watermilfoil under natural conditions was undertaken on a 62-acre (25-ha) milfoil-infested pond located at the Guntersville Reservoir Aquatic Ecosystem Facility in Guntersville, AL (Figure 2). Treatments consisted of one application rate of the mycoherbicide at 70 lb (dry weight) ai/acre and a control. Four replicate paired plots separated by 100-m buffers were set up in dense stands of milfoil vegetation. Each 20- by 10-m plot was subdivided into two 10- by 10-m subplots. Subdivision of the plots was necessary to allow for the destructive nature of the biomass sampling. Preinoculation biomass samples were collected in one 10- by 10-m subplot. The treatments were applied to the second subplot, followed by harvesting of postapplication biomass samples 4 weeks later. The paired plots were separated by 15-m buffers to prevent inoculum from entering and contaminating control plots. Treatments were randomly assigned to the paired plots.

The mycoherbicide was applied in early July when water temperatures were within the 20- to 28-°C range and milfoil plants had topped out at the water surface. The string formulation was suspended in water and hand applied (Figure 3). Even coverage of the treated plots was assured by slowly moving a boat back and forth while the applicator carefully distributed the formulated fungus over the plant mat (Figure 4).

For biomass sampling, each subplot was divided into 25 sections. Biomass samples were collected inside 10 randomly selected 2- by 2-m grid sections 1 day prior to the mycoherbicide application and 4 weeks post application. Plant samples were collected by a scuba diver using a 0.1-m^2 quadrat placed on the sediment surface. All plant material rooted within the quadrat was clipped at the soil surface, bagged, and labeled.

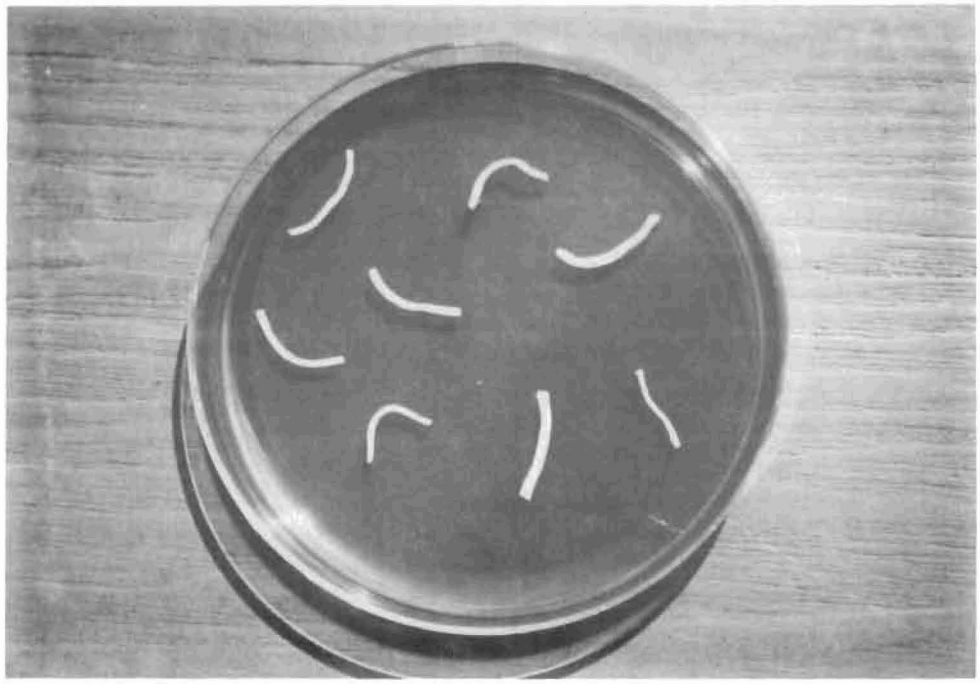

Figure 1. String formulation of *M. terrestris*

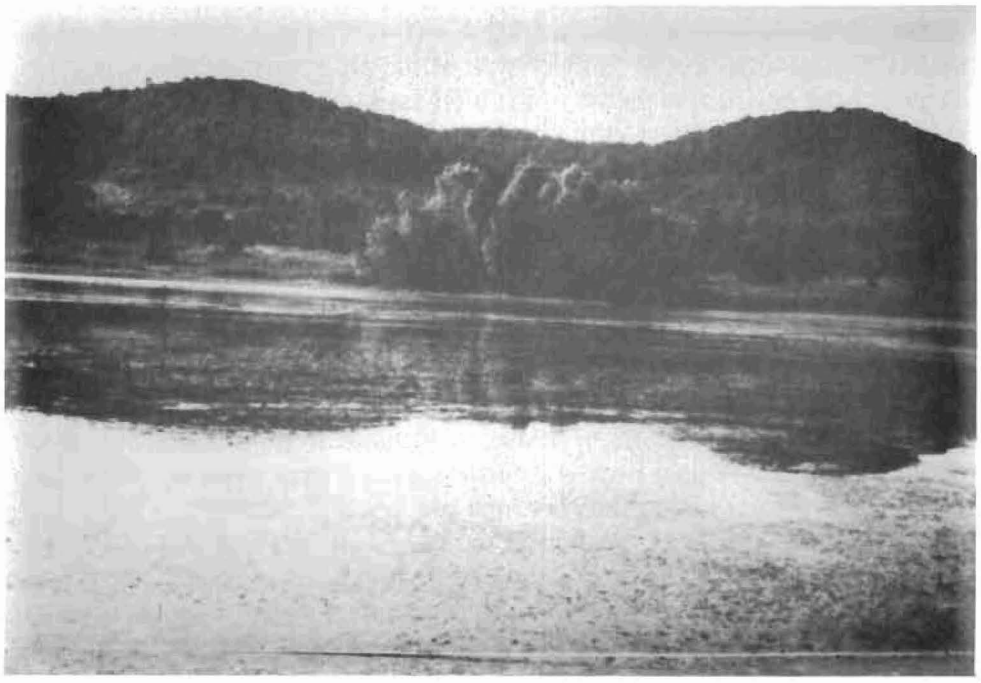

Figure 2. Milfoil-infested pond at Guntersville Reservoir Aquatic Ecosytem Facility, Guntersville, AL

Figure 3. Hand applying Aqua-Fyte to test plots of Eurasian watermilfoil

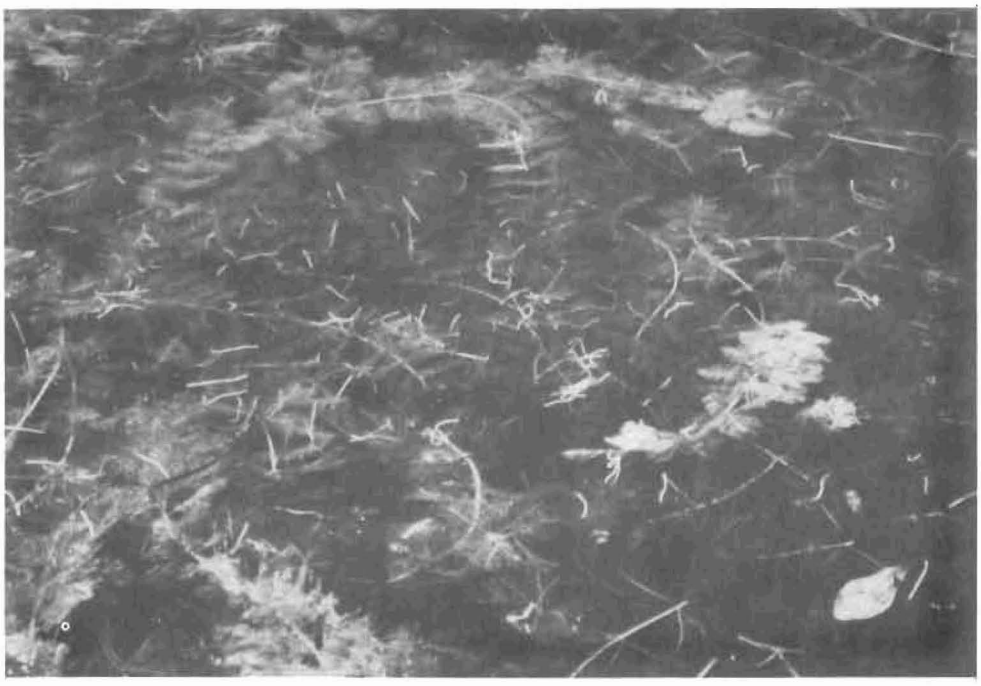

Figure 4. String formulation evenly distributed over milfoil mat in test plots

Plant samples were thoroughly washed, spun dry in a washing machine set on a 6-min spin cycle, and weighed. All biomass calculations were determined from plant wet weights. From each sample, an approximate 100-g subsample was placed in a sterile plastic bag, kept cool in an ice chest, and returned to the U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS, for microbial analysis. In the laboratory, the subsamples were placed in a refrigeration unit and kept at 4 °C until processed.

Dilution plating was used to determine microbial counts and frequencies in milfoil stem tissue. From each biomass subsample, 10 g of stem tissue was weighed in a sterile plastic boat, submerged in a 1.5-percent hypochlorite solution for 1 min to eliminate surface contaminants, and rinsed in sterile water for 3 min. The tissue was ground for 30 sec in a sterile blender containing 100 ml of water. Aliquots of the slurry were pipetted into sterile water blanks to give dilutions of 1/100 and 1/500. After thorough shaking, 1-ml aliquots of the dilutions were distributed over the surface of Martin's agar plates (three plates per dilution). The plates were incubated in the dark at room temperature for 5 days. Total colony counts and the number of Mt colonies in each sample were determined by visual examination.

Thirty colonies were picked at random onto potato dextrose agar slants to determine frequencies and densities of fungal species on field-collected milfoil. The slants were incubated at room temperature for 1 week. Counts were made of the number of morphologically similar isolates in each sample.

3 Results

Four weeks postapplication of Aqua-Fyte, visual differences were not observed in the plant canopy between the treated and control plots. Plants were topped out at the water surface and looked green and healthy.

A plant disease epidemic did not develop in the Eurasian watermilfoil plots treated with Aqua-Fyte. The application rate of 70 lb (dry weight) ai/acre was the maximum allowable under the experimental use permit. While water temperatures at the surface of the plant mat reached the maximum limit (28 °C) for fungal infectivity, temperatures below the surface were well within the range for optimum fungal effectiveness in producing disease in milfoil tissue.

Although the fungal formulation was carefully applied by hand, there was some concern about even coverage over the plot. Because plants near the plot perimetry appeared to have received fewer strings of the formulated fungus than the plot center, the 4-week postapplication biomass samples were collected from within randomly assigned grids from the center of both treated and control plots.

Significant differences were not observed between wet weights of preapplication and postapplication plant biomass samples in plots treated with Aqua-Fyte (Figure 5). Significant differences were not observed between wet weights of treated and control samples 4 weeks postapplication. Differences in wet weights resulted from differences in plant densities rather than treatment effects or changes in growth pattern of milfoil during the 4-week testing period.

Eurasian watermilfoil plants at the test site have a naturally occurring population of *M. terrestris*. The background levels of the endemic Mt monitored prior to the field study remained at a low level and did not change appreciably during the period (Table 1).

A strain specific marker had not been placed in the fungal active ingredient in Aqua-Fyte to readily distinguish it from other isolates of Mt collected from field material. Strains of Mt are almost impossible to distinguish without such a marker; therefore, no attempt was made during the study to separate applied from naturally occurring Mt. It was assumed

that any large increases of Mt in plant tissue following mycoherbicide application would be due to the introduced fungus.

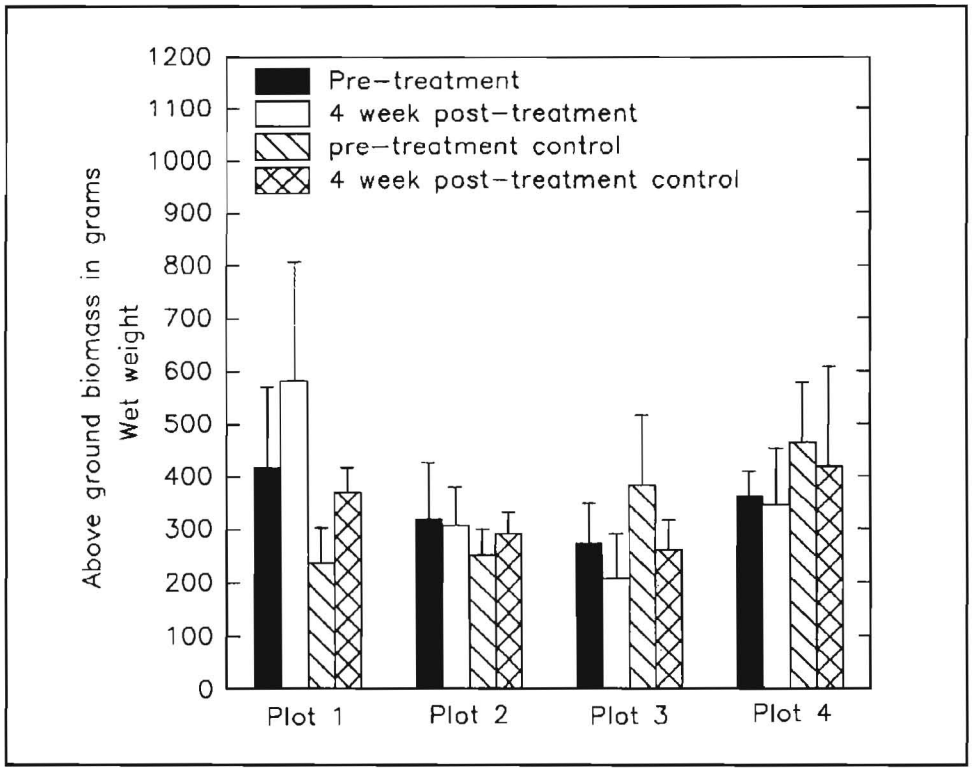

Figure 5. Mean wet weights of aboveground biomass samples of Eurasian watermilfoil from Aqua-Fyte-treated and control plots preapplication and 4-week postapplication

Table 1
Number of Fungal Colony Forming Units per Gram Wet Weight of Milfoil Stem Tissue in Treated and Control Plots Preapplication and 4-Week Postapplication of Aqua-Fyte

	Aqua-Fyte				Control			
	Preapplication		Postapplication		Preapplication		Postapplication	
Plot	Total CFUs	% Mt	Total CFUs	% Mt	Total CFUs	% Mt	Total CFUs	% Mt
1	79.4	1.0	75.6	4.3	90.3	0.3	96.7	4.6
2	52.5	3.7	47.4	4.2	41.4	3.1	73.0	4.8
3	27.7	0.0	49.5	0.3	47.6	1.8	70.0	5.1
4	68.8	0.6	85.0	1.0	58.9	0.0	43.1	0.0

Note: Amount of *M. terrestris* in milfoil tissue is expressed as a percent of total CFUs.

The number of fungal colony forming units (CFUs) in milfoil stem tissue ranged from 27.7 to 96.7 CFUs/gram wet weight of plant tissue (Table 1). The number of colonies of Mt expressed as a percent of total CFUs ranged from 0 to 5.1 percent. *Mycoleptodiscus terrestris* made up a very small proportion of the total fungal flora found in milfoil stem tissue.

A significant increase in amount of *M. terrestris* was not detected in milfoil stem tissue following inoculation with the mycoherbicide. The small numbers of Mt colonies isolated from stem tissue in all plots was consistent with expected background levels of endemic Mt (see Appendix A).

4 Discussion

The mycoherbicide Aqua-Fyte was ineffective in reducing aboveground biomass of Eurasian watermilfoil under natural conditions in the field. Poor field performance of the mycoherbicide is most likely based on fungus/formulation problems rather than biological, chemical, or physical factors encountered in the field. Fungal viability or virulence was excluded as a problem because Mt was easily reisolated from the string formulation, and mycelia from cultures obtained from reisolates when reapplied to greenhouse-grown milfoil produced typical disease symptoms on test plants. That the fungus is a weak pathogen may make it a poor competitor when introduced into the myriad of organisms that exist naturally on milfoil. The strategy of using mycoherbicides, however, is to overcome those problems with high dosage rates.

Formulation was the most likely cause of poor field performance. The strings did not seem to provide enough contact points nor enough contact time for fungal entry into the plant. Redesign of the formulation into a more gel-like consistency might result in more complete coverage of plant stems providing multiple points for fungal invasion.

Future research must confirm the effectiveness of *M. terrestris* on milfoil in the field. An application of fungal mycelium without incorporation into a formulation medium would verify if the fungus alone is capable of producing a disease epidemic in milfoil populations. If successful, then formulation redesign will need to be considered.

If field tests confirm that the fungal isolate is ineffective, several options are available. Virulence of the test isolate could be enhanced in the laboratory; different isolates of the fungus from cryostorage could be tested; searches could be undertaken to find new and better isolates; or searches for new endemic pathogens of milfoil could be initiated.

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Appendix A: Number of Colonies of Most Frequently Isolated Fungi from 10-g Wet Weight Milfoil Stem and Leaf Tissue per Sample Collected from Test Plots on Murphy Hill North Pond, Guntersville, AL, 8 May 1992

Plot 1-A

Species	1	2	3	4	5	6	7	8	9	10	Total
Mycoleptodiscus terrestris			1					1	3		5
Dematiaceous I	1	2	3	7	3	10	4	3			33
Cylindrocarpon destructans	11	3	4	15	8	7	8	10	11	26	103
Acremonium sp.			2		1	1					4
Polyscytalum sp.	2	6	2	1	2	5	2	3	1	1	25
Dematiaceous II				2							2
Cladosporium sphaerospermum		6		1	1	1					9
Tetracladium setigerum	2			1			1				4
Pythium sp.											0
Polyscytalum sp.											0
Moniliaceous I							1				1
Tuberculariaceae I											0
Pythium sp.	2		1								3

Plot 2-B

Species	1	2	3	4	5	6	7	8	9	10	Total
Mycoleptodiscus terrestris		1	11				1			1	14
Dematiaceous I	2	5	1	4	1	3	5		9	2	32
Cylindrocarpon destructans	19	9	15	5	2	12	11		8	10	91
Acremonium sp.	3	6	1				2				12
Polyscytalum sp.	1	2	1	2			4		1	3	14
Dematiaceous II											0
Cladosporium sphaerospermum	1	1		1			1		1		5
Tetracladium setigerum		1					1		1		3
Pythium sp.				1		1					2
Polyscytalum sp.						2	3				5
Moniliaceous I		1								2	3
Tuberculariaceae I											0
Pythium sp.											0

Plot 1-D

Species	1	2	3	4	5	6	7	8	9	10	Total
Mycoleptodiscus terrestris	1	1			2		1	1		7	13
Dematiaceous I	2	2	5	4	4	4	6	4	4	3	38
Cylindrocarpon destructans	12	6	3	7	15	12	7	3	15	1	81
Acremonium sp.					1	1	4		1	2	9
Polyscytalum sp.	4	3	6	1	5	1	1	5	4	4	34
Dematiaceous II			1		1				1		3
Cladosporium sphaerospermum				1					2		3
Tetracladium setigerum				1	2						3
Pythium sp.											0
Polyscytalum sp.				3				1			4
Moniliaceous I	1										1
Tuberculariaceae I				1							1
Pythium sp.									1		1

Plot 2-D

Species	1	2	3	4	5	6	7	8	9	10	Total
Mycoleptodiscus terrestris	1			2		2	2	2		2	11
Dematiaceous I	5	7	2	2	5	2	6	4	2	2	37
Cylindrocarpon destructans	13	4	4	8	12	4	5	19	16	5	90
Acremonium sp.				2		1		2	2	1	8
Polyscytalum sp.	3	6		3	4	4	1	1		2	24
Dematiaceous II		1				1					2
Cladosporium sphaerospermum					1	1					2
Tetracladium setigerum		1			1						2
Pythium sp.											0
Polyscytalum sp.		1									1
Moniliaceous I					1				2		3
Tuberculariaceae I	1						1		1		3
Pythium sp.											0

Plot 3-A

Species	1	2	3	4	5	6	7	8	9	10	Total
Mycoleptodiscus terrestris								1			1
Dematiaceous I	4	1	1		1	1		1		1	10
Cylindrocarpon destructans	6	1	2							3	12
Acremonium sp.							1		1		2
Polyscytalum sp.	4							1			5
Dematiaceous II								4			4
Cladosporium sphaerospermum		1	2		3	1		3	1	6	17
Tetracladium setigerum			1				3				4
Pythium sp.				1							1
Polyscytalum sp.			1							3	4
Moniliaceous I	1										1
Tuberculariaceae I											0
Pythium sp.			1			2					3

Plot 3-D

Species	1	2	3	4	5	6	7	8	9	10	Total
Mycoleptodiscus terrestris	1			1	2	3	5	1	5	1	19
Dematiaceous I	6	5	4	4	8	4	3	1	1	3	39
Cylindrocarpon destructans	8	6	12	8	12	3	9	8	7	11	84
Acremonium sp.			1					1	1		3
Polyscytalum sp.	4	6	1		1	3	1	3			19
Dematiaceous II					2	6				4	12
Cladosporium sphaerospermum	1		1			1		8			11
Tetracladium setigerum				1	1	2					4
Pythium sp.											0
Polyscytalum sp.									2		2
Moniliaceous I				1							1
Tuberculariaceae I							1				1
Pythium sp.											0

Plot 4-A

Species	1	2	3	4	5	6	7	8	9	10	Total
Mycoleptodiscus terrestris											0
Dematiaceous I	1	4	7	7	3	1	9	9	9	2	52
Cylindrocarpon destructans	1	4	7	3	1	1	2	2		1	22
Acremonium sp.			1			1				2	4
Polyscytalum sp.		4	2	5	1		1	3	1	1	18
Dematiaceous II											0
Cladosporium sphaerospermum	1	1		1		1		1			5
Tetracladium setigerum	1				1					1	3
Pythium sp.					1						1
Polyscytalum sp.			3	3				2			8
Moniliaceous I	1								1	1	3
Tuberculariaceae I								1			1
Pythium sp.				1				1	1		3

Plot 4-C

Species	1	2	3	4	5	6	7	8	9	10	Total
Mycoleptodiscus terrestris			1				2				3
Dematiaceous I	1	3	7	4	5	9	0	6	5	18	58
Cylindrocarpon destructans	9	13	7	11	2	3	3	6	9	4	67
Acremonium sp.		2				1	2				5
Polyscytalum sp.	2	3	3	6	8	3	1	4	3	3	36
Dematiaceous II			5							1	6
Cladosporium sphaerospermum					1						1
Tetracladium setigerum		1	1	2				2	1		7
Pythium sp.		1						3			4
Polyscytalum sp.	1			4					1		6
Moniliaceous I					3	1					4
Tuberculariaceae I											0
Pythium sp.										3	3

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13. ABSTRACT *(Maximum 200 words)*

The plant pathogenic fungus *Mycoleptodiscus terrestris* (Gerd.) Ostazeski has been identified as a potential biocontrol agent for the submersed aquatic macrophyte *Myriophyllum spicatum* L. Successful tests in laboratory, pool, and pond experiments led to the development of the organism into the mycoherbicide Aqua-Fyte. Efficacy of the commercial formulation was tested on field populations of Eurasian watermilfoil in Guntersville, AL, July 1992. The mycoherbicide was ineffective in reducing aboveground biomass of milfoil under natural conditions in the field. A reevaluation of the formulation must be undertaken to understand differences in performance of the mycoherbicide between laboratory and field trials.

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