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Field and Laboratory Studies of the Fungus *Mycoleptodiscus terrestris* as a Potential Agent for Management of the Submersed Aquatic Macrophyte *Hydrilla verticillata*

by Judy F. Shearer

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

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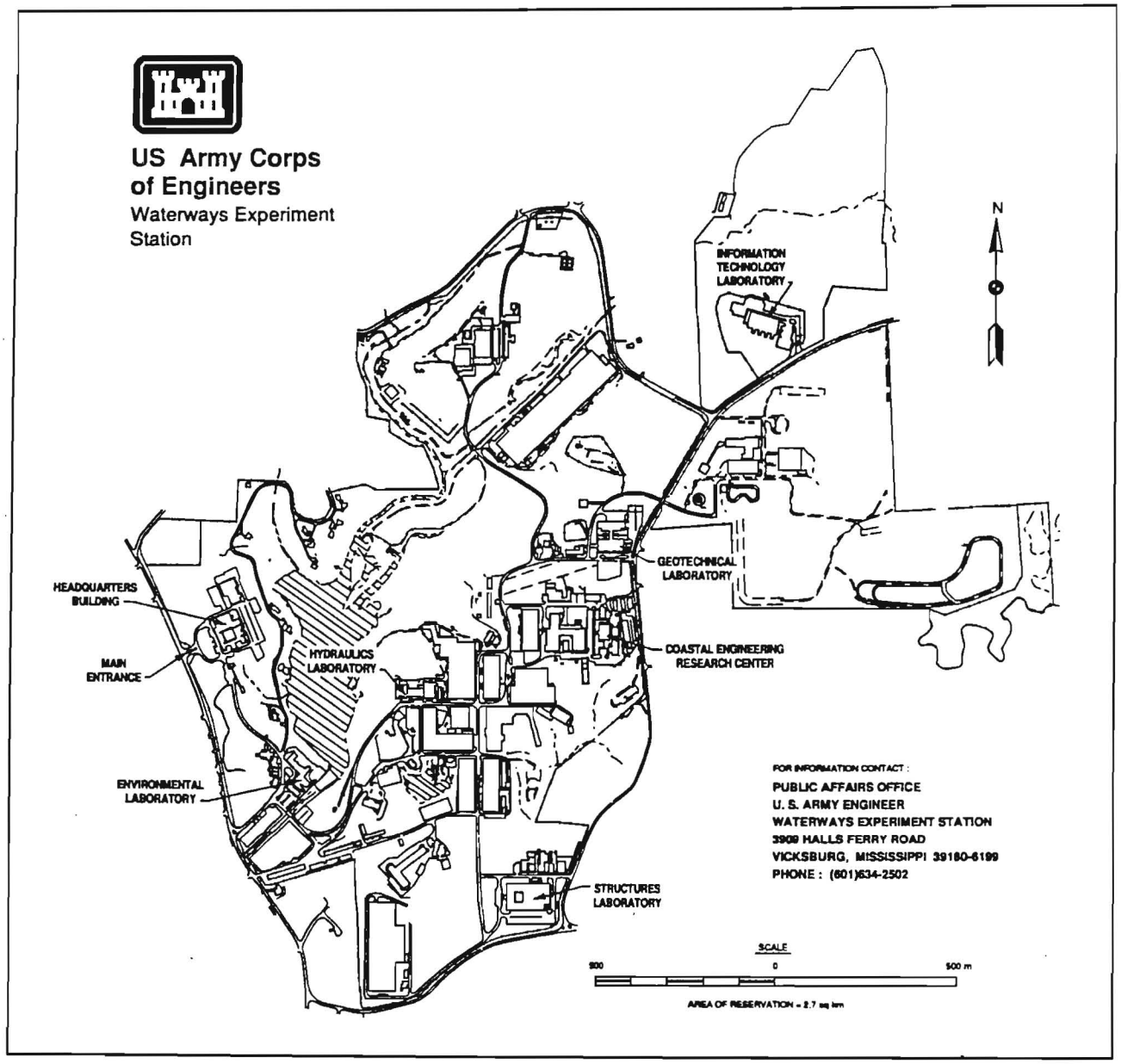

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Preface

The work reported herein was conducted as part of the Aquatic Plant Control Research Program (APCRP), Work Unit 32200. 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, Jr., was Assistant Manager, ERRAP, for the APCRP. Program Monitor during this study was Ms. Denise White, HQUSACE.

This report was prepared by Dr. Judy F. Shearer, Aquatic Ecology Branch (AEB), Ecological Research Division (ERD), EL, WES. Technical assistance at WES was provided by Ms. Janis Lanier and at the Lewisville Aquatic Ecosystem Research Facility by Messrs. Gary Dick, Steven Wood, and Joe Snow. The report was reviewed by Dr. Michael Grodowitz and Mr. Harvey Jones, AEB.

This investigation was performed under the general supervision of Dr. Alfred F. Cofrancesco, Chief, AEB, Dr. Conrad J. Kirby, Chief, ERD, and Dr. John W. Keeley, Director, EL.

At the time of publication of this report, Director of WES was Dr. Robert W. Whalin. Commander was COL Bruce K. Howard, EN.

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1 Introduction

As *Hydrilla verticillata* (L. fil.) Royle (hydrilla) became a severe weed problem throughout the southeastern United States, efforts were undertaken to research management procedures that offered alternative choices to the more traditional control methods of mechanical harvesting and herbicide applications. One of the foremost and cost-effective of these techniques is the use of biocontrol technology. Exotic species growing in their native ranges rarely reach problematic proportions because a wide array of natural organisms feed or predate on them. The strategy of classical biocontrol is to identify and utilize host-specific organisms isolated from the target or host plant within its native range and introduce them into areas where the plant has become a problem. For hydrilla management, two different types of biological agents have been implemented for hydrilla management. The grass carp, *Ctenopharyngodon idella*, has proven highly successful in reducing populations of hydrilla but has a severe drawback in that it is nonselective in its feeding and it impacts desirable native vegetation. Four exotic insects have undergone extensive testing both at overseas locations and at quarantine facilities and were approved for field release beginning in 1987 (Grodowitz et al. 1994). Initial releases of two of the insects, *Bagous affinis*, a tuber-feeding weevil, and *Hydrellia pakistanae*, a leaf-mining fly, began in the late 1980s in Florida (Center 1989; Center and Dray 1990). The fly has become successfully established throughout Florida and at sites in Texas and Alabama (Grodowitz et al. 1994). Because plant management is often not immediately realized following releases of biocontrol agents, analysis of the effects of insect releases is still under evaluation and will probably take several years for complete information to become available.

Another group of organisms that has good potential for biocontrol are microbial pathogens. Research in this area has lagged behind that of other biocontrol methods in part because of natural fears associated with microbials and a lack of definitive rules and regulations specifically governing the use and release of microbial agents. At the inception of hydrilla pathogen work in the 1980s at the U.S. Army Engineer Waterways Experiment Station (WES), the classical biocontrol approach was not a viable option because the importation of microbials from their country of origin was prohibited. Therefore, the only approach available was to search for indigenous pathogens of hydrilla and to develop the best candidate(s) for use as mycoherbicides. Extensive surveys were conducted throughout the range of hydrilla in the southeastern

United States (Joye and Cofrancesco 1991). Plant material was collected and microorganisms were isolated by plating them onto a variety of agar media selective for fungal and bacterial pathogens. In 1987, a promising candidate was isolated from hydrilla growing in Lake Houston, Texas (Joye 1988, 1989, 1990). Laboratory studies indicated that the pathogen, a fungus, could produce chlorosis of hydrilla leaf tissue, a complete loss of leaf color in 7 days, and plant disintegration within 10 days (Joye 1990). Electron microscopy studies confirmed that the fungus was effective because it destroyed the cellular integrity of plant tissues, resulting in collapse of the entire plant (Joye and Paul 1991). Because there was no sign of inward bending of the cell wall, it was hypothesized that action of the fungus was enzymatic.

Field tests conducted at Sheldon Reservoir in 1988 and 1989 showed that the fungus was capable of producing a significant reduction in aboveground biomass of hydrilla 21 days posttreatment in comparison with untreated plants (Joye 1990). Symptoms observed in the field were similar to those documented in laboratory and greenhouse studies.

The fungal pathogen of hydrilla was tentatively identified as *Macrophomina phaseolina* (Tassi) Goid (Joye 1990). Most fungal identifications are dependent in large part on spore characters and the method by which the spores are produced. Because the organism did not sporulate in pure culture, other less reliable characteristics such as colony color, hyphal growth and branching, and production of survival structures (sclerotia) were used for identification purposes.

Continued work in the laboratory in the early 1990s on plant/fungal interactions induced sporulation of the fungus, and it was determined that the hydrilla pathogen was not *M. phaseolina* but a strain of *Mycoleptodiscus terrestris* (Gerdemann) Ostazeski. The determination was confirmed by Dr. Bruce Sutton, a world authority on Coelomycetes at the International Commonwealth Institute, England. Because there was a concurrent project in the biocontrol laboratory in which formulated *M. terrestris* was being tested as a biocontrol for Eurasian watermilfoil, there was some concern that contamination might have been a factor. Visually, *M. terrestris* looks similar to *M. phaseolina* in pure culture; therefore, a reconfirmation of disease symptoms and disease-producing ability by the fungus now established as a species of *M. terrestris* was undertaken.

Because of the confusion surrounding the fungal isolate on hydrilla, it was deemed necessary to reassess the effect of the fungus on hydrilla in greenhouse and field tests. The objectives of the present study were to (a) assess disease-producing ability of the fungus on excised hydrilla tissue, (b) determine efficacy of *M. terrestris* on hydrilla in greenhouse aquaria and small tank studies, (c) determine efficacy on hydrilla in a small-scale field test, and (d) conduct preliminary epidemiological studies of the pathogen.

2 Materials and Methods

Plant/Fungus Interactions

The pathogenic fungus collected at Lake Houston (isolate designation FHY18) was retrieved from cryostorage and plated onto potato dextrose agar (PDA) (Difco Laboratories). The cultures were allowed to grow in the dark at 28 °C for 7 days. Sprigs of hydrilla approximately 15 cm in length were collected from greenhouse hydrilla stock cultures, thoroughly washed in tap water, and placed on moist filter paper in 15-cm-diam plastic petri dishes. Small triangular pieces of hyphal tissue approximately 1 by 1 by 1 mm were cut from the agar at the colony edge (i.e., the most actively growing vegetative portion of the fungal colony) and placed at alternate nodes along the length of the hydrilla sprig. The plates were incubated at room temperature and observed daily for approximately 10 days. Small pieces of hydrilla stem tissue were placed on slides, stained with cotton blue in lactophenol, and subjected to microscopic examination. Infection progression on plant tissues and fungal growth were recorded throughout the period.

Greenhouse Studies

Clear acrylic columns (76 by 13.7 cm) were used for the greenhouse studies. Thirty-two-ounce plastic cups filled three-fourths full with lake sediment amended with ammonium chloride (0.5 g/ℓ) and Esmigran (1.75 g/ℓ) were overlain with 5 cm of washed silica sand. Three 15-cm apical sprigs of hydrilla were planted in the sediment and the cup placed in the bottom of the column. Twelve liters of nutrient solution (Smart and Barko 1985) were added to each column. The columns were aerated and maintained at 25 °C in a 1,200-ℓ waterbath. Plants were allowed to grow approximately 3 weeks before testing was initiated.

The fungus was inoculated onto PDA plates and allowed to grow in an incubator at 28 °C for 7 days. Plugs 4 mm in diameter were cut from the leading edge of the fungal colony. Three plugs of the fungus were added to 250-ml Erlenmeyer flasks, each containing 150 ml of modified Richard's V8 juice broth (glucose, 10 g; KNO_3, 10 g; $CaCO_3$, 3 g; V8 juice (Campbells), 200 ml; H_2O, 800 ml). The flasks were placed on a platform shaker

(New Brunswick, Edison, NJ) set at 200 rpms. Flasks were swirled daily to prevent fungal buildup along the sides of the flask. After 6 days, the mycelial mat that developed in the flasks was filtered through four layers of cheesecloth and ground in a blender for 30 sec. Sterile water was added to the fungal slurry to give an inoculum concentration of approximately 1×10^4 colony-forming units (CFUs)/milliliter. To determine the specific propagule density of the inoculum, the slurry was serially diluted and plated onto Martin's agar (H_2O, 1 ℓ; agar, 17 g; KH_2PO_4, 0.5 g; K_2HPO_4, 0.5 g; $MgSO_4$ 7 H_2O, 0.5 g; peptone, 0.5 g; dextrose, 10 g; yeast extract, 0.5 g; rose bengal, 0.05 g; streptomycin, 0.03 g). Aliquots of 40, 20, and 10 ml of the fungal inoculum were added to the hydrilla columns. Each fungal and control treatment was replicated five times.

After 2 weeks, three stem pieces 2 cm in length were collected from plants or floating plant tissue in each column, surface sterilized in a 1.5-percent sodium hypochlorite solution for 1 min, rinsed in sterile water, and plated onto Martin's agar to determine retrievability of the fungus from the plant tissue. The remainder of the aboveground biomass from each column was harvested, dried in an oven at 60 °C for a minimum of 4 days, and weighed.

Tank Studies

Tanks 183 cm diam by 70 cm deep (approximately 2,200 ℓ) were used for testing efficacy of *M. terrestris* on a larger scale. Plastic containers 36 by 30 by 13 cm were filled with sediment to a depth of 8 cm and overlain with 4 cm of washed silica sand. Thirty apical tips of hydrilla 15 cm in length were planted in each container. Ten containers were placed in each of the seven tanks and the tanks filled with nutrient solution (Smart and Barko 1985). The plants were allowed to grow until they reached the water surface and formed a distinct canopy.

The inoculum was prepared as described above for the column studies. Treatments at low, medium, and high dosage levels of 500, 1,000, and 2,000 ml, respectively, rated at 1×10^5 CFUs/milliliter and a control were assigned randomly to the tanks. Disease symptoms were observed on the plants over time to assess the similarity to symptoms that had been documented in previous pathogen studies on hydrilla. After 2 weeks, photographs were taken of the tanks, and the amount of plant cover was estimated for both treated and control tanks.

Field Test

Four paired plots were set up on two hydrilla-planted ponds at the Lewisville Aquatic Ecosystem Research Facility (LAERF), Lewisville, TX (Figure 1). The surface area of each plot measured 4 by 4 m. Water depth varied between pairs of plots, but paired plots were at similar water depths that

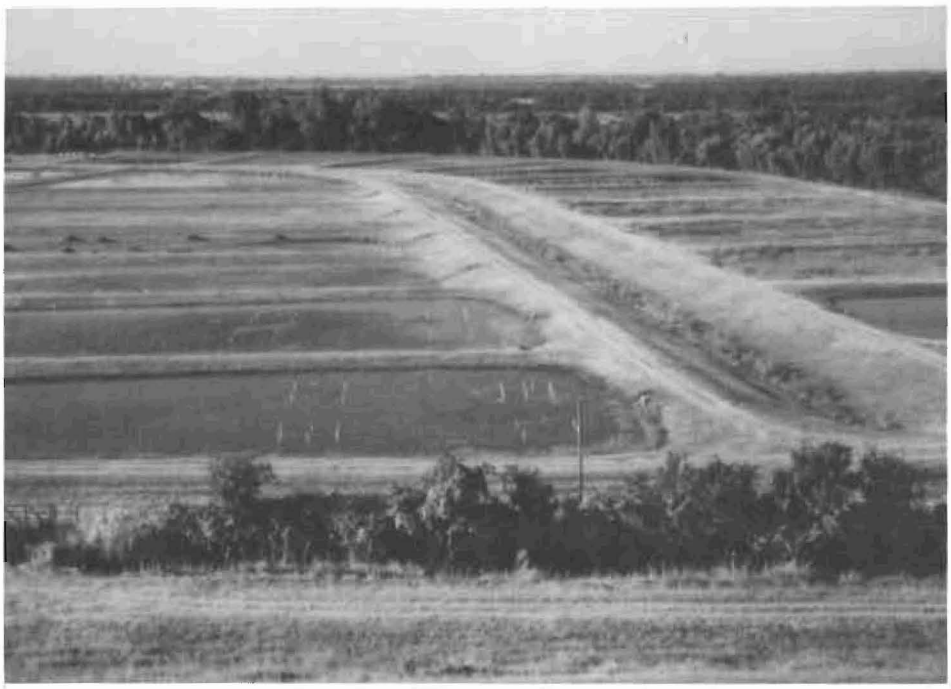

Figure 1. Two planted hydrilla ponds in foreground at Lewisville Aquatic Ecosystem Research Facility

averaged 1.24, 1.18, 1.05, and 0.96 m. Distance between plots was approximately 10 m.

To determine if *M. terrestris* was present on hydrilla tissue in the test ponds prior to initiating the experiment, plant samples were collected from 10 locations in each pond from an area between the four plots. The plant material was placed in sterile plastic bags, labeled, and kept cool in an ice-filled chest for transport back to the biocontrol laboratory at WES. The plants were washed to remove debris, wrapped in moist paper towels, and refrigerated at 4 °C until processed.

For each plant sample, 10 g of leaf and stem tissue were weighed in a sterile plastic weighing boat, surface sterilized in a 1.5-percent hypochlorite solution, rinsed with tap water, and ground in a blender with 100 ml of sterile water. One-milliliter aliquots of the resulting slurry were pipetted into sterile water blanks of 9 and 49 ml to give dilutions of 1/100 and 1/500, respectively. One-milliliter aliquots of the dilutions were dispensed onto Martin's agar plates (three plates/dilution). The plates were incubated in the dark at room temperature for 4 days, after which they were visually examined for presence/absence of *M. terrestris* colonies. The number of colonies on each plate was counted to determine the number of CFUs/gram wet weight hydrilla tissue.

Treatments consisted of one application rate of *M. terrestris* mycelium. Treatments were randomly assigned to the paired plots. Because the fungus was applied as an aqueous solution, the treated plots were enclosed in plastic

to prevent immediate dissipation of the mycelial matrix into the surrounding water (Figure 2).

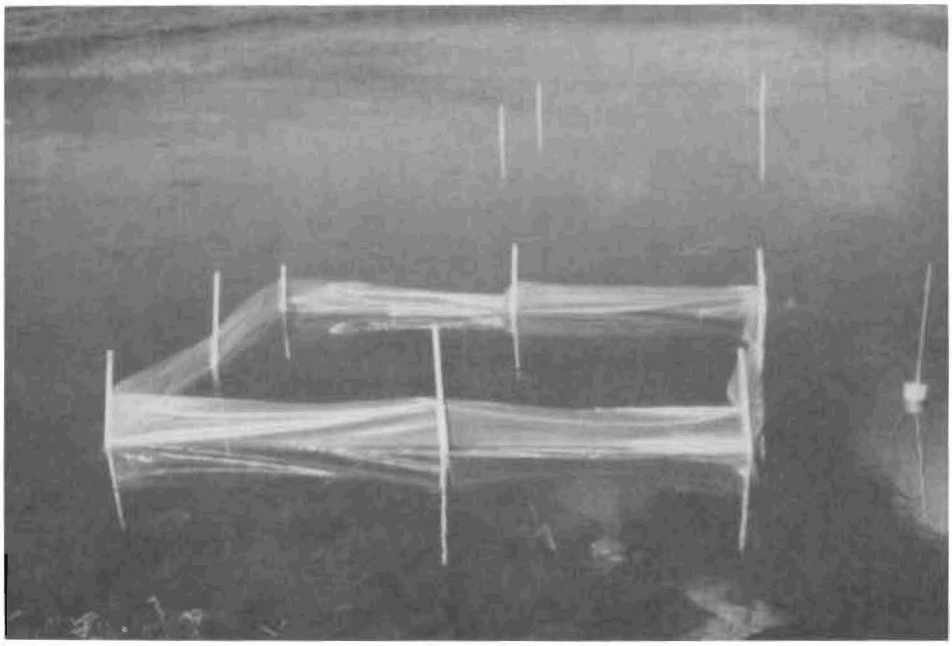

Figure 2. Plastic enclosed plot on hydrilla-planted pond at Lewisville Aquatic Ecosystem Research Facility

The strain of *M. terrestris* used for the field evaluation was an isolate (WES125693) obtained from hydrilla collected at Lake Sheldon, Texas. The inoculum was produced by Ricerca, Inc., Painesville, OH. A mycelial matrix was produced by growing the fungus in modified Richard's V-8 broth in large industrial fermentors under constant agitation at 300 rpms. After 6 days, the matrix was ground in a commercial blender for approximately 30 sec to macerate the mycelium. The resultant inoculum was rated at 3×10^4 CFUs/ milliliter.

The inoculum was applied by hand to the surface of the plots and allowed to naturally disperse through the plant mat within the enclosed plot. Plots 1.24 and 1.18 m deep each received 12 ℓ of mycelial broth; plots 1.05 and 0.96 m deep each received 10 ℓ of broth. After 2 weeks, the plastic was removed from the treated plots.

Four weeks postapplication, plant and water samples were collected from the periphery of treated plots at distances of 1, 3, and 5 m, respectively, extending outward from each side of the plot to determine if the fungus could be detected outside the plot boundary. The samples were kept cool in an ice-filled chest, transported to the laboratory, and kept at 4 °C until they could be processed.

Water samples were thoroughly shaken, dispensed onto Martin's agar plates in 1-ml aliquots, and evenly distributed over the medium surface (three plates/ sample). Plant samples were processed as described above for preinoculation evaluation of fungi in hydrilla tissue.

Aboveground biomass samples were collected from the treated and control plots by a SCUBA diver. Ten plant samples were collected from each plot by placing a 0.1-m^2 quadrat on the sediment surface, clipping all the plant material within the quadrat, and placing it in a plastic bag. The samples were collected at 10 randomly assigned locations within each plot. Biomass was determined for both hydrilla and the other plant species. Each sample was thoroughly washed and hydrilla separated from the other plant species. A 25-g subsample of hydrilla from each sample was placed in a plastic bag, kept cool in an ice chest, and transported to the biocontrol laboratory for microbial processing. The rest of the sample was placed in a preweighed paper bag, dried at 60 °C for a minumum of 4 days, and weighed.

Hydrilla plant subsamples were processed as described previously. After 4 days incubation, the plates were visually examined for numbers of *M. terrestris* colonies.

3 Results and Discussion

Plant/Fungus Interactions

After 1 day, hyphal strands began to grow from the agar pieces. By Day 3, appresoria were observed developing on the stem and leaf surface (Figure 3). Once invasion of plant tissue began, sporodochia were observed developing on the surface of leaf and stem tissue (Figure 4). Sporulation of the fungus was apparent on the agar pieces and areas on the stem and leaves adjacent to the agar piece by Day 4 (Figure 5). The spores developed from phialidic cells of a sporodochium and were characteristic of the genus *Mycoleptodiscus*. They are two celled separated by a median septum with a unicellular appendage attached asymmetrically at each end of the spore. Following sporulation, plant tissue began to discolor and become flaccid. By Day 7, the sprig had lost cell integrity, and sclerotia were observed developing in and around the collapsed plant tissues.

Column Studies

Chlorosis of hydrilla leaves was detectable within 5 days after inoculation with the pathogen *M. terrestris*. The symptoms were similar to that described by Joye and Cofrancesco (1991). Chlorosis began at the tips of the leaves, progressed down the leaf as interveinal chlorosis, followed by a complete loss of leaf color. Disintegration of stem tissue was evident 10 days postinoculation; and by Day 14, little tissue remained in the columns treated with 20 ml of inoculum, and no green tissue was present in the columns treated with 40 ml of inoculum (Figure 6). Although some green tissue was present 14 days posttreatment in columns with a 10-ml inoculum level, the amount of aboveground biomass at this dosage also differed significantly from untreated controls (Figure 7). The amount of biomass reduction for dosage levels of 10, 20, and 40 ml of inoculum was 82, 95, and 100 percent, respectively.

Mycoleptodiscus terrestris was reisolated from at least one of three hydrilla stem pieces collected from all columns treated with the fungus. No fungal growth appeared on the stem pieces collected from untreated controls

Figure 3. Appresoria of *Mycoleptodiscus terrestris* on stem tissue of *Hydrilla verticillata*

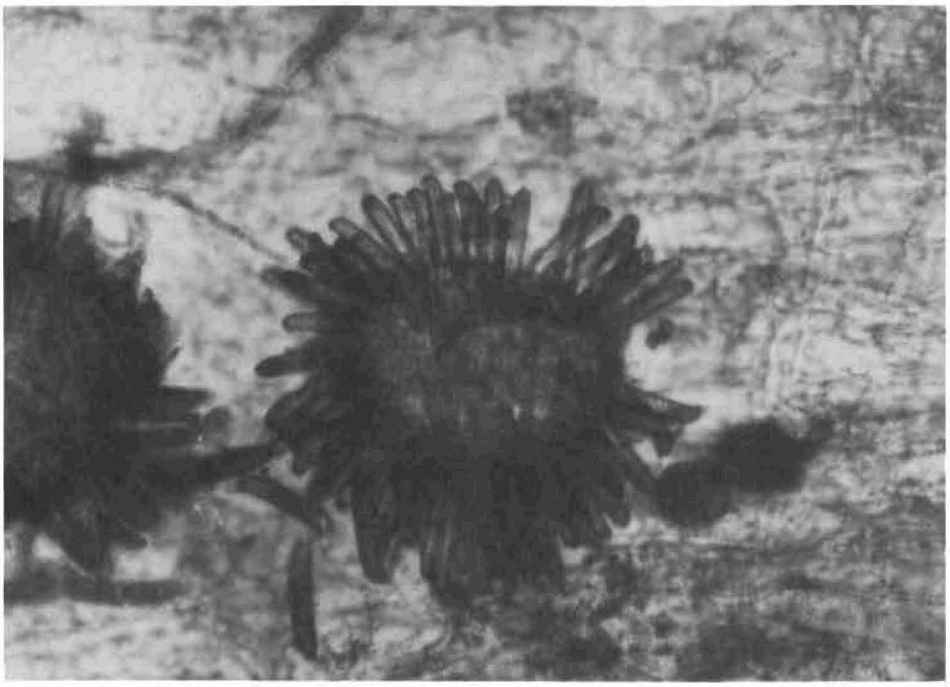

Figure 4. Sporodochia of *Mycoleptodiscus terrestris*

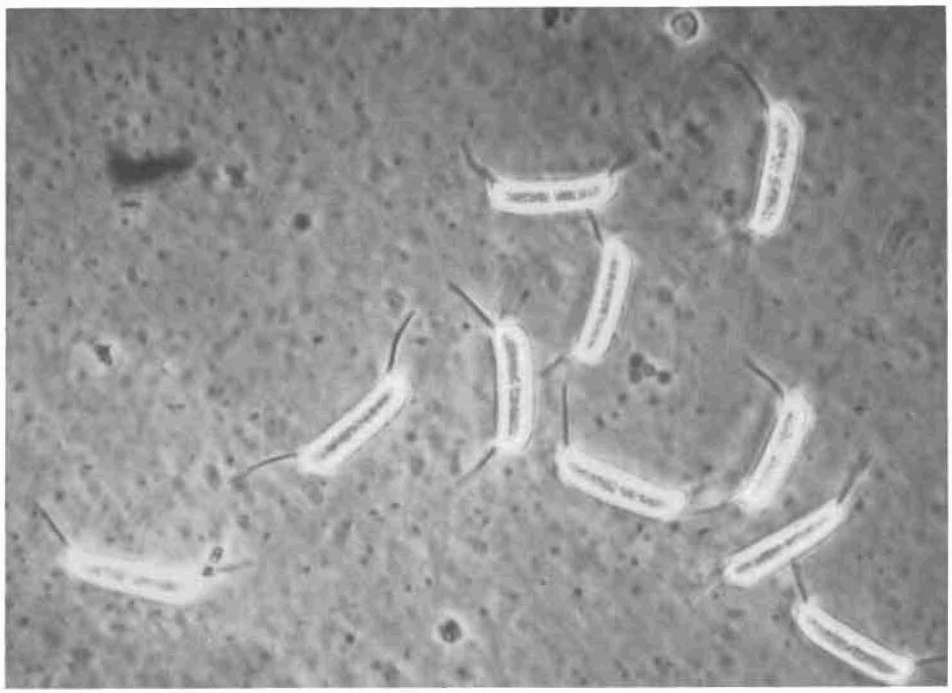

Figure 5. Spores of fungus *Mycoleptodiscus terrestris*

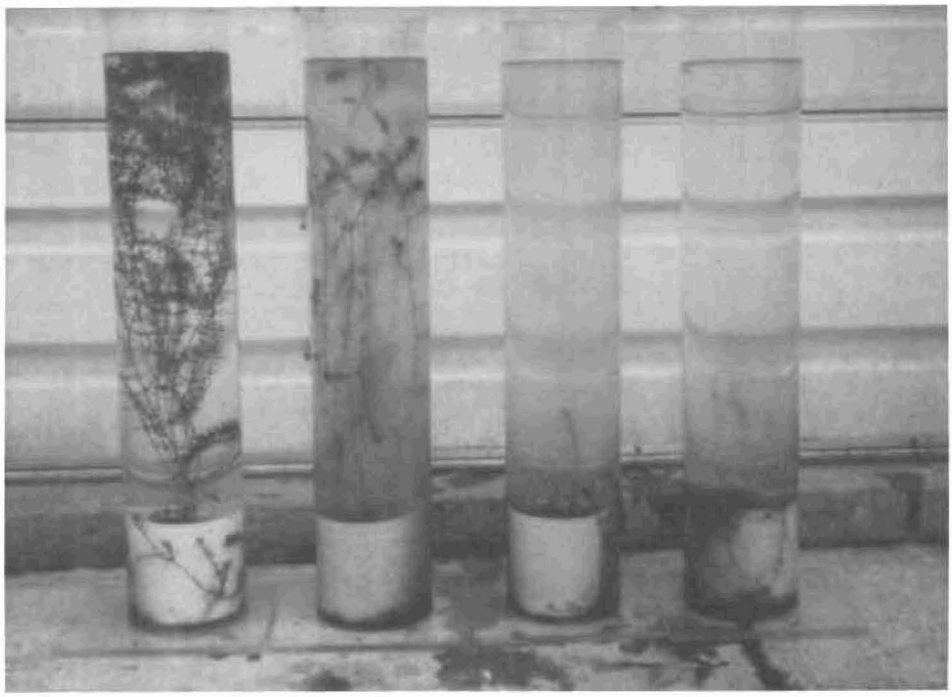

Figure 6. Control and dosage levels of 10, 20, and 40 ml of *Mycoleptodiscus terrestris* inoculum applied to hydrilla grown in 12-ℓ columns at 14 days postinoculation

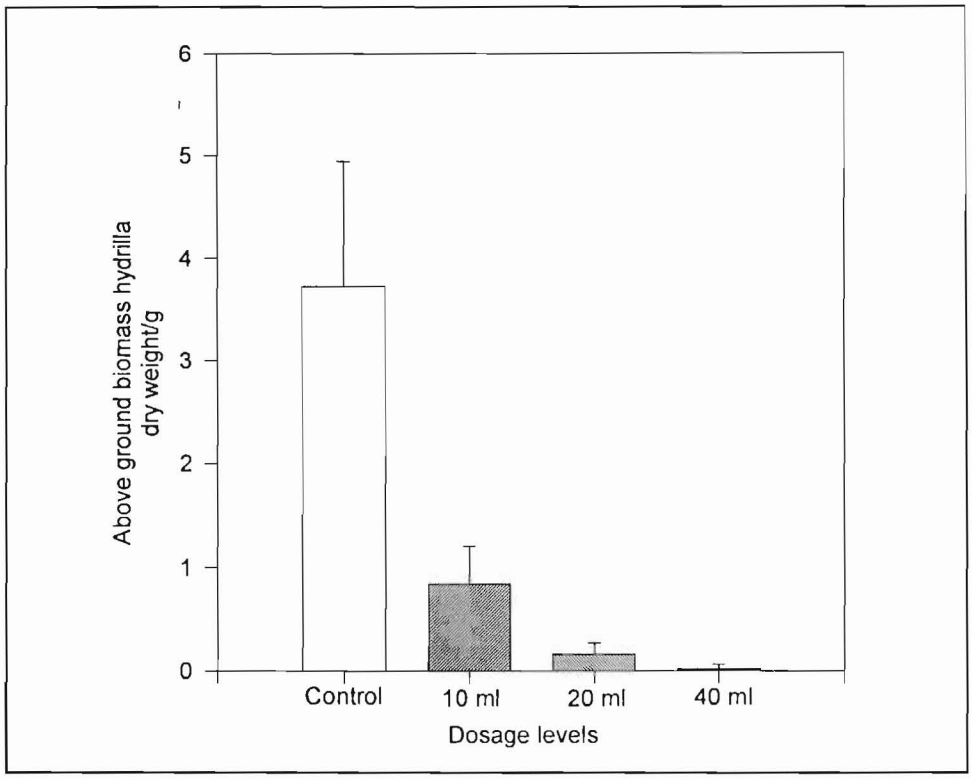

Figure 7. Mean dry weights of aboveground biomass of hydrilla collected from 12-ℓ columns 14 days postinoculation with fungus *Mycoleptodiscus terrestris*

(Table 1). Untreated plants remained green and healthy throughout the experiment.

Table 1
Frequency of Hydrilla Stem Pieces Infected with the Fungus *Mycoleptodiscus terrestris* 2 Weeks Postinoculation

Treatment	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
Control, ml	0/3	0/3	0/3	0/3	0/3
10	2/3	3/3	3/3	2/3	2/3
20	3/3	3/3	1/3	2/3	3/3
40	2/3	3/3	3/3	3/3	1/3

Tank Studies

Chlorosis was evident on leaves within 5 days postinoculation. Although the leaves at the plant apex remained green, those on the rest of the stem showed symptoms of disease (Figure 8) and soon began to disintegrate. As the cells in the stem became infected, disarticulation of the stems began, and stem remnants were observed floating on the surface of the water (Figure 9). Two weeks postinoculation, percent cover of hydrilla at the surface of the tanks was estimated to be 91, 75, 55, and 25 percent for control, low, medium, and high dose treatments, respectively (Figure 10). The fungus was capable of reducing aboveground biomass of hydrilla within 2 weeks of inoculation (Figure 11). However, because a complete kill was not achieved with any of the dosage rates, the hydrilla began to grow back within 4 weeks postinoculation.

Figure 8. Chlorosis symptoms on leaves of hydrilla 5 days postinoculation with fungus *Mycoleptodiscus terrestris*

Field Test

Preinoculation fungal colony counts in hydrilla plant tissue were very low. This was expected because the plant material appeared green and healthy. An average of 12.2 CFUs/gram wet weight plant tissue were detected on the dilution plates. The species count at 39 was somewhat higher than expected, but was the result of many single species isolates. *Mycoleptodiscus terrestris* was not found in the plant tissue and, therefore, was assumed not to be present in the pond prior to inoculation.

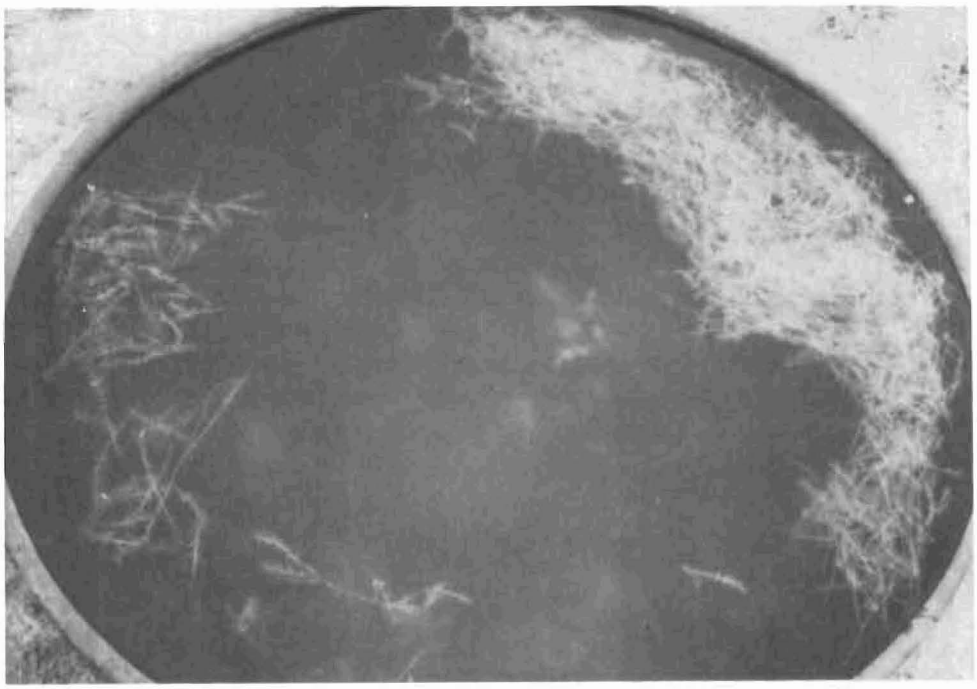

Figure 9. Stem fragments of hydrilla floating on surface of inoculated tank with a high dose of *Mycoleptodiscus terrestris*

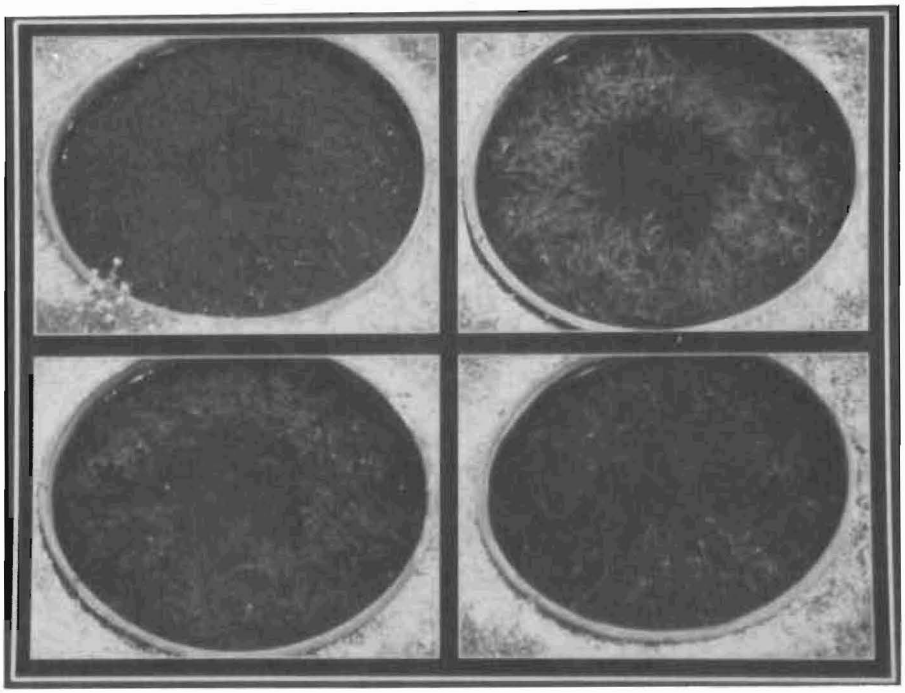

Figure 10. Dosage response experiment in hydrilla-planted tanks 2 weeks postinoculation with fungus *Mycoleptodiscus terrestris*: a. control (upper left); b. low dose (upper right); c. medium dose (lower left); d. high dose (lower right)

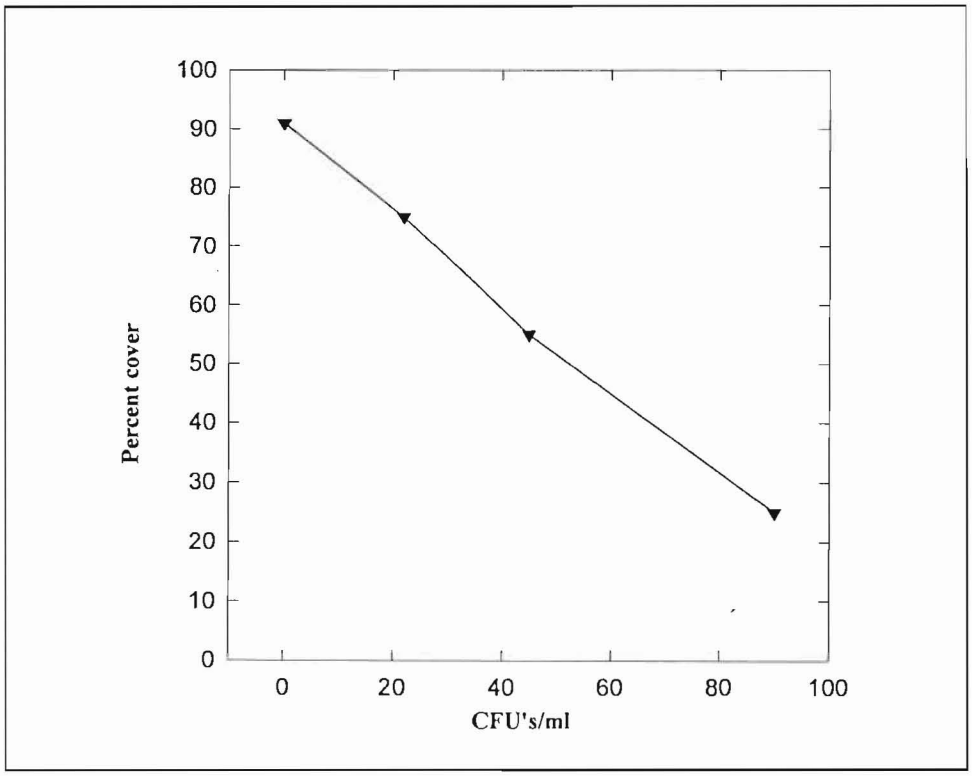

Figure 11. Percent cover of hydrilla in control and treated tanks 2 weeks post-inoculation with *Mycoleptodiscus terrestris* at high, medium, and low dosage rates

Because the hydrilla ponds had been planted in late fall 1992, coverage in the pond by early summer 1993 was patchy. While pairs of plots were constructed to reflect similar depths, plant coverage varied somewhat from plot to plot, even though some supplemental planting within the plots was undertaken prior to test initiation (Table 2).

Table 2
Hydrilla Coverage in Control and Treated Plots in Ponds 20 and 21 at Lewisville Aquatic Ecosystem Research Facility

Plot	Control	Treated
1	++++	++++
2	+++	++++
3	+++	+++
4	++	+

Note: Coverage: ++++ = excellent; +++ = good; ++ = fair; + = poor.

One week postinoculation with *M. terrestris*, leaves at the apex of the plants remained green, but those on the rest of the plant became chlorotic (Figure 12). This occurred because the fungus required contact with the plant for 36 to 48 hr before penetration of tissue could occur, and hydrilla still continued to metabolize and grow rapidly. Four weeks postinoculation, there was a significant reduction in aboveground biomass in three of the four treated plots compared with the untreated controls (Figure 13). In paired Plot 4, the coverage of hydrilla was rated as fair and poor for control and treated subplots, respectively. The uneven coverage of hydrilla combined with a high incidence of other plant species may have masked the effects of the fungus in this plot. It appears that the fungus has little impact on other plant species in the hydrilla pond because they were recovered from all treated plots (Figure 14).

Figure 12. Leaves of hydrilla showing chlorosis at 7 days postinoculation with fungus *Mycoleptodiscus terrestris*

Four weeks postinoculation, *M. terrestris* was detected in one plant sample collected from a treated plot (Appendix A). This was rather unexpected because the fungus is recovered with regularity from greenhouse inoculated material. It appears that a secondary cycle of infection did not take place beyond the initial infection process. Average colony counts postinoculation were similar to those recorded preinoculation (12.8 versus 12.2, respectively). The 90 fungal species isolated postinoculation was considerably higher than the 39 isolated preinoculation but was not unusual because the number of samples examined increased 4-fold.

Examination of the area surrounding the plots did not provide any visual evidence of plant disease (i.e., the hydrilla appeared green and healthy).

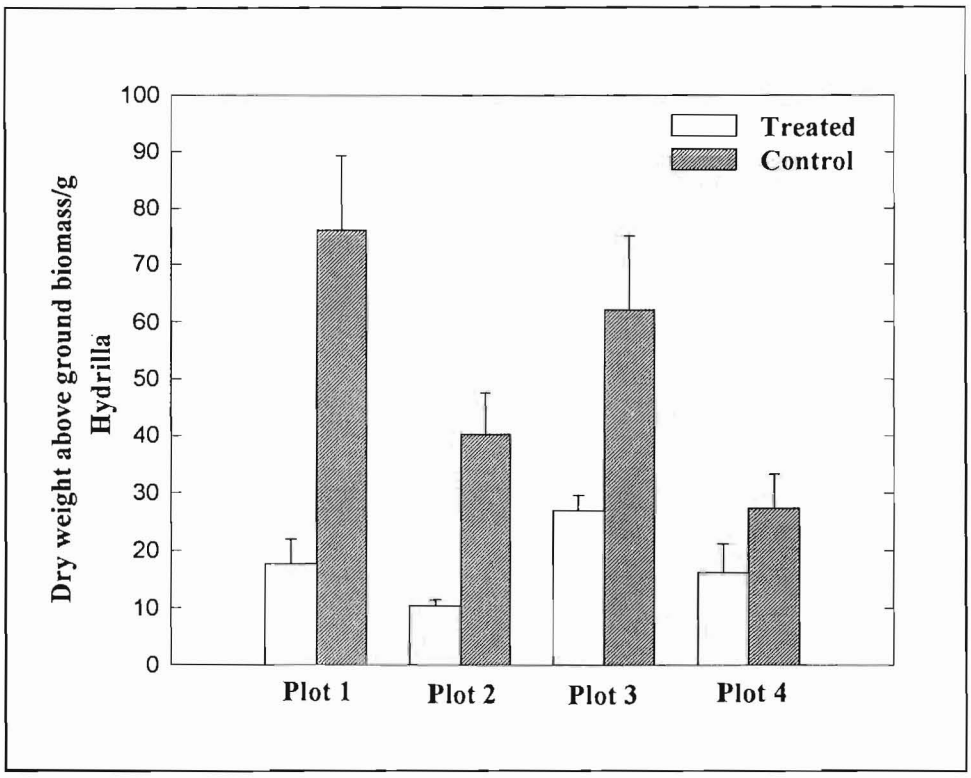

Figure 13. Mean dry weights of aboveground biomass of hydrilla collected 4 weeks postinoculation with fungus *Mycoleptodiscus terrestris*

Mycoleptodiscus terrestris was not detected in water or plant samples collected from around the periphery of the treated plots 1 month postapplication. Lack of recovery of the fungus combined with visual examination would indicate that a secondary cycle of infection did not occur, and the fungus did not advance and invade plants that were not inoculated (i.e., a disease epidemic did not ensue).

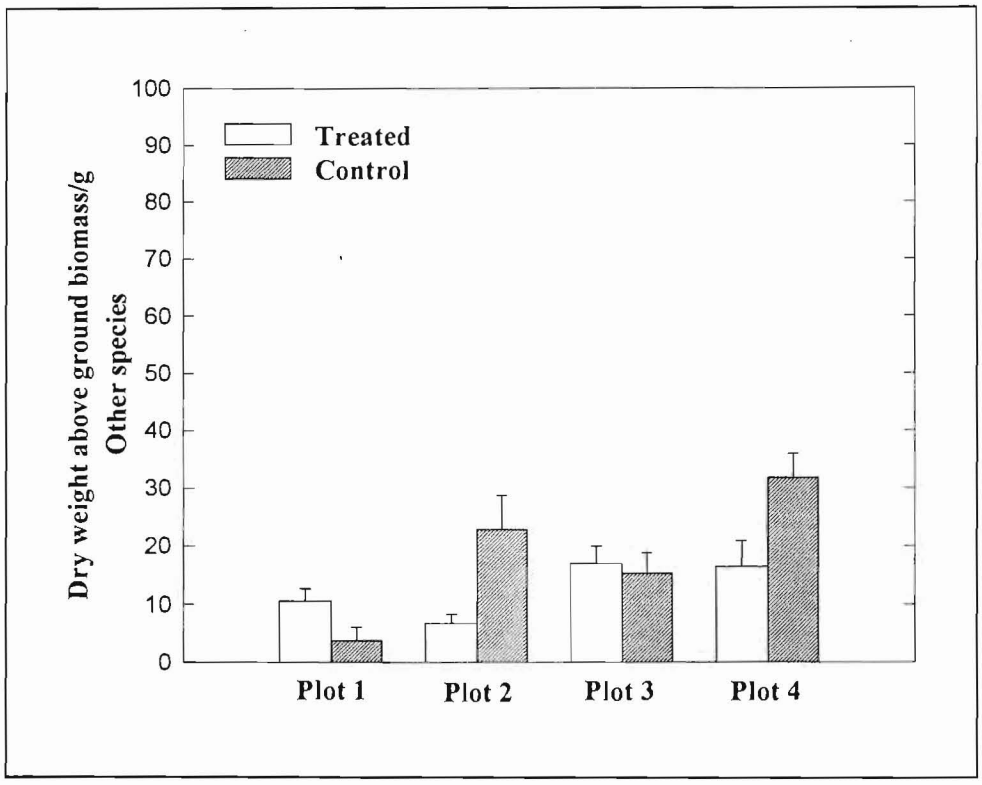

Figure 14. Mean dry weights of aboveground biomass of other species collected 4 weeks postinoculation with fungus *Mycoleptodiscus terrestris*

4 Conclusions

The fungus identified as *M. terrestris* when placed on excised hydrilla tissue induced leaf chlorosis and stem degradation. Within 4 days following inoculation, the fungus began sporulating on the surface of exposed plant tissues. Within 7 days, cell walls had lost their integrity, and the plant stem became flaccid and incapable of supporting the plant. With a reconfirmation of disease symptoms on hydrilla combined with the definitive identification of the pathogen through production of spores, the fungus previously reported as *M. phaseolina* can now be reported with accuracy to be a strain of *M. terrestris*.

The pathogen was capable of severely impacting hydrilla in controlled greenhouse experiments. Significant reductions in aboveground biomass were achieved with applications where only 10 ml of inoculum rated at 1×10^4 CFUs/milliliter were added to hydrilla grown in small columns. Larger tank experiments produced similar results. It was noted that if a complete kill of hydrilla was not achieved, the plant can regrow. Timing of the fungal application may be of extreme importance when managing hydrilla using pathogens delivered as mycoherbicides. If hydrilla is rapidly growing and metabolizing, the plant may be able to outgrow the effects of a fungal application. Multiple treatments may be necessary to achieve control.

A successful field test of *M. terrestris* applied to hydrilla planted in ponds was conducted at LAERF. A significant reduction in aboveground biomass was realized 4 weeks postinoculation with the pathogen. The fungus did not appear to impact other plant species growing in the plots with hydrilla. In cases where a monoculture of hydrilla is not present, fungal application may be of use in reducing hydrilla populations to the point where native species may be able to compete. Because hydrilla has the ability to regrow after fungal application, additional testing will be necessary to determine rates that will be lethal or significantly stress the plants to the point that regrowth is curtailed.

Initial observations would indicate that treatment of hydrilla with the pathogen *M. terrestris* did not create a disease epidemic. The fact that the fungus was not recovered beyond the boundaries of treated plots suggests that a secondary infection cycle did not occur. Secondary infection cycles are usually induced by abundant sporulation of a fungus on the host following initial

application. The plant/fungus interaction studies have shown that the fungus readily sporulates on the surface of plant material that is exposed. It is unknown if the fungus sporulates on hydrilla under submerged conditions in the field. Additional epidemiological research will be necessary to elucidate action of the fungus under natural conditions in the field.

Continued studies with the hydrilla pathogen will be focused on how to optimize CFU production through fungal fermentation and milling processes. Once optimum yields are realized, the fungus will be formulated into a medium that will adhere to the plant and be effective over a relatively longer period of time. Studies on fungal development need to be continued to determine how to optimize *M. terrestris* survivability at room temperature. This has importance because a formulated biological product (i.e., a mycoherbicide) must have a shelf life if it is to be readily used by applicators. Knowing more about fungal sporulation under field conditions will aid in the ability to predict disease development and spread following a fungal application.

References

Center, T. D. (1989). "Release and establishment of insect biocontrol on *Hydrilla*." Proceedings, 23rd Annual Meeting, Aquatic Plant Control Research Program. Miscellaneous Paper A-89-1, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS, 34-40.

Center, T. D., and Dray, F. A. (1990). "Release, establishment, and evaluation of insect biocontrol agents for aquatic plant control." Proceedings, 24th Annual Meeting, Aquatic Plant Control Research Program. Miscellaneous Paper A-90-3, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS, 39-49.

Grodowitz, M. J., Center, T. D., Snoddy, E., and Dray, F. A. (1994). "Release and establishment of insect biocontrol agents for the management of hydrilla." Proceedings, 28th Annual Meeting, Aquatic Plant Control Research Program. Miscellaneous Paper A-94-2, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS, 181-201.

Joye, G. F. (1988). "Biological control of *Hydrilla verticillata* (L.f.) Royle with an endemic fungal disease." (Abstract) *Phytopathology* 78, 1593.

_____. (1989). "Biological control of hydrilla with an endemic plant pathogen." Proceedings, 23rd Annual Meeting, Aquatic Plant Control Research Program. Miscellaneous Paper A-90-1, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS, 41-51.

_____. (1990). "Biocontrol of *Hydrilla* with the endemic fungus *Macrophomina phaseolina*." *Plant Dis.* 74, 1035-1036.

Joye, G. F., and Cofrancesco, A. F., Jr. (1991). "Studies on the use of fungal plant pathogens for control of *Hydrilla verticillata* (L.f.) Royle," Technical Report A-91-4, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Joye, G. F., and Paul, R. (1991). "Histology of infection of *Hydrilla verticillata* by *Macrophomina phaseolina*," Technical Report A-91-6, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Smart, M., and Barko, J. W. (1985). "Laboratory culture of submersed freshwater macrophytes on natural sediment," *Aquatic Bot.* 21, 251-263.

Appendix A
Fungal Colony-Forming Units (CFUs) Isolated From Hydrilla Plant Samples Collected From Treated and Control Plots

Plot 1	Treated		Control	
Sample Number	CFUs/g	Colonies, Mt	CFUs/g	Colonies, Mt
1	10.0	0	0	0
2	6.7	0	0	0
3	3.3	0	183.3	0
4	3.3	0	6.7	0
5	0	0	6.7	0
6	3.3	0	13.3	0
7	0	0	0	0
8	0	0	3.3	0
9	0	0	3.3	0
10	150.0	0	3.3	0

Plot 2	Treated		Control	
Sample Number	CFUs/g	Colonies, Mt	CFUs/g	Colonies, Mt
1	10.0	0	10	0
2	3.3	0	0	0
3	6.7	0	6.7	0
4	3.3	0	3.3	0
5	10.0	0	3.3	0
6	6.7	0	3.3	0
7	0	0	6.7	0
8	0	0	30.0	0
9	6.7	0	13.3	0
10	3.3	0	6.7	0

Plot 3	Treated		Control	
Sample Number	CFUs/g	Colonies, Mt	CFUs/g	Colonies, Mt
1	3.3	0	6.7	0
2	3.3	0	10	0
3	0	0	0	0
4	3.3	0	10	0
5	3.3	0	43.3	0
6	6.7	0	10	0
7	10	0	16.7	0
8	73.3	0	13.3	0
9	33.3	0	13.3	0
10	13.3	0	16.7	0

Plot 4	Treated		Control	
Sample Number	CFUs/g	Colonies, Mt	CFUs/g	Colonies, Mt
1	33.3	0	6.7	0
2	3.3	0	0	0
3	0	0	6.7	0
4	0	0	6.7	0
5	3.3	0	0	0
6	3.3	0	6.6	0
7	0	0	10	0
8	0	0	10	0
9	0	0	6.7	0
10	3.3	1	13.3	0

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13. ABSTRACT *(Maximum 200 words)*

Laboratory and field studies were undertaken to assess the potential of a fungal pathogen as a biocontrol agent for the management of *Hydrilla verticillata* (L. fil.) Royle. The pathogen previously reported to be *Macrophomina phaseolina* (Tassi) Goid was positively identified as a strain of *Mycoleptodiscus terrestris* (Gerd.) Ostazeski. Laboratory tests reconfirmed that *M. terrestris* produced characteristic disease symptoms on hydrilla tissues followed by a significant reduction in aboveground biomass. Assessment of *M. terrestris* in a field situation was conducted on hydrilla-planted ponds at the Lewisville Aquatic Ecosystem Research Facility. Aboveground biomass of the host plant was significantly reduced 4 weeks postinoculation with a mycelial matrix of the fungus.

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