



US Army Corps
of Engineers



JOHN D. MADSEN

AQUATIC PLANT CONTROL
RESEARCH PROGRAM

TECHNICAL REPORT A-91-4

STUDIES ON THE USE OF FUNGAL PLANT
PATHOGENS FOR CONTROL
OF *HYDRILLA VERTICILLATA* (L.f.) ROYLE

by

Gary F. Joye, Alfred F. Cofrancesco, Jr.

Environmental Laboratory

DEPARTMENT OF THE ARMY
Waterways Experiment Station, Corps of Engineers
3909 Halls Ferry Road, Vicksburg, Mississippi 39180-6199



June 1991

Final Report

Approved For Public Release; Distribution Unlimited

Prepared for DEPARTMENT OF THE ARMY
US Army Corps of Engineers
Washington, DC 20314-1000

Destroy this report when no longer needed. Do not return it to the originator.

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

The contents of this report are not to be used for advertising, publication, or promotional purposes. Citation of trade names does not constitute an official endorsement or approval of the use of such commercial products.

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.				
1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE June 1991	3. REPORT TYPE AND DATES COVERED Final report		
4. TITLE AND SUBTITLE Studies on the Use of Fungal Plant Pathogens for Control of <i>Hydrilla verticillata</i> (L.f.) Royle			5. FUNDING NUMBERS	
6. AUTHOR(S) Gary F. Joye, Alfred F. Cofrancesco, Jr.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) USAE Waterways Experiment Station, Environmental Laboratory, 3909 Halls Ferry Road, Vicksburg, MS 39180-6199			8. PERFORMING ORGANIZATION REPORT NUMBER Technical Report A-91-4	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) US Army Corps of Engineers, Washington, DC 20314-1000			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES Available from National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words) A survey was conducted in populations of <i>Hydrilla verticillata</i> (L.f.) Royle growing in lakes and rivers of the southeastern United States for the purpose of identifying plant pathogens with potential biocontrol use. An isolate of the fungus identified as <i>Macrophomina phaseolina</i> (Tassi) Goid. was collected from the foliage of hydrilla growing in Lake Houston, Texas. In repeated laboratory, greenhouse, and field tests, this fungus was pathogenic to hydrilla. Inoculum concentrations of between 1×10^4 and 1×10^6 colony forming units per milliliter were sufficient to kill hydrilla test plants over a 3- to 4-week period. In a field of 46 species and subspecific taxa within 22 families, this fungus was pathogenic only to hydrilla and duck lettuce (<i>Ottelia alismoides</i> (L.) Pers.). This fungus may be useful as a biocontrol agent of hydrilla.				
14. SUBJECT TERMS Biocontrol Pathogen Fungal pathogen Submersed aquatic plant <i>Macrophomina phaseolina</i>			15. NUMBER OF PAGES 26	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT UNCLASSIFIED	18. SECURITY CLASSIFICATION OF THIS PAGE UNCLASSIFIED	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT	

Preface

This study was conducted by personnel of the US Army Engineer Waterways Experiment Station (WES) as part of the Aquatic Plant Control Research Program (APCRP). The APCRP is sponsored by the Headquarters, US Army Corps of Engineers (HQUSACE), and is assigned to the 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 Mr. James W. Wolcott, HQUSACE.

The principal investigator for this work was Dr. Gary F. Joye of the Aquatic Habitat Group (AHG), Environmental Resources Division (ERD), EL. The work was conducted under the direct supervision of Dr. Alfred F. Cofrancesco, Jr., and under the general supervision of Mr. Edwin A. Theriot, Chief, AHG, Dr. Conrad J. Kirby, Chief, ERD, and Dr. John Harrison, Chief, EL.

Appreciation is expressed to Dr. R. T. Hanlin of the University of Georgia for confirming the identity of *Macrophomina phaseolina*. The authors thank Mr. Robert Comstock of the Texas Parks and Wildlife Department for permission to establish field tests at Sheldon Reservoir, Texas. Appreciation is also expressed to Dr. James K. Mitchell, Mses. Jan Freedman, Ramona Warren, and Laura Bailey, and Mr. Harvey Jones for their assistance in this work. The report was edited by Ms. Jessica S. Ruff of the WES Information Technology Laboratory.

COL Larry B. Fulton, EN, was Commander and Director of WES.
Dr. Robert W. Whalin was Technical Director.

This report should be cited as follows:

Joye, Gary F., and Cofrancesco, Alfred F., Jr. 1991. "Studies on the Use of Fungal Plant Pathogens for Control of *Hydrilla verticillata* (L.f.) Royle," Technical Report A-91-4, US Army Engineer Waterways Experiment Station, Vicksburg, MS.

Contents

	<u>Page</u>
Preface	1
Introduction	3
Background	3
Control of hydrilla	3
Materials and Methods	5
Microbial isolation and culture	5
Test tube bioassays	6
Greenhouse bioassays	6
Host specificity	7
Field tests	8
Results and Discussion	8
Microbial isolation and culture	8
Test tube bioassays	9
Greenhouse bioassays	9
Host specificity	10
Field tests	10
Conclusions	10
References	11
Tables 1-4	
Figures 1-9	

STUDIES ON THE USE OF FUNGAL PLANT PATHOGENS FOR
CONTROL OF *HYDRILLA VERTICILLATA* (L.f.) ROYLE

Introduction

Background

1. *Hydrilla verticillata* (L.f.) Royle, common name hydrilla, Hydrocharitaceae, is a submersed aquatic plant and one of the most problematic pests of waterways in the tropical and subtropical regions of the world (Robson 1976). Under severe infestations, hydrilla will impede navigation, clog drainage and irrigation canals, reduce recreational activity, and disrupt wildlife habitat (Environmental Laboratory 1985). Hydrilla is well fitted to survive unfavorable conditions and to outcompete other species (Pieterse 1981). It has the ability to rapidly grow under low light intensities (Bowes et al. 1977) and is able to reproduce vegetatively from stem fragments, tubers, and turions (Van, Haller, and Garrard 1978).

2. Tubers and turions enable the species to survive adverse conditions such as cold and drought (Van, Haller, and Garrard 1978). These structures are anatomically and morphologically identical (Yeo, Falk, and Thurston 1984). Tubers are produced during the late summer and early fall (Sculthorpe 1967) at the tips of branches that grow in the hydrosol; turions are produced in the axis of leaves. Upon maturity, these structures detach from the parent plant. These vegetative propagules are also rich in starch and minerals that supply nutrients for regeneration the next growing season (Miller, Garrard, and Haller 1976) (Figure 1).

Control of hydrilla

3. Mechanical removal of hydrilla is relatively expensive but, in many cases, is required because of the restriction of chemical herbicides in some states. In addition, certain chemical herbicides promote eutrophication and decrease oxygen levels caused by the rapid decay of organic matter (Pieterse 1981). However, manual control cannot cope with extensive vegetation, especially in deep water. Also, the cuttings of hydrilla, if not removed from the water, will help to spread the plant infestations (Blanchard 1967, Langeland and Sutton 1980). Sobal (1987) reported that if hydrilla fragments were chopped small enough, the percentage of regenerative stem fragments may be significantly reduced.

4. Application of chemical herbicides is the most common method of control for hydrilla. The combination of diquat and copper is commonly used to control hydrilla (Vandiver 1978). It has been hypothesized that a synergistic effect causes a reduction of phosphorus and an accumulation of copper in the plant (Sutton, Weldon, and Blackburn 1970). The application of diquat/copper is generally thought to be safe to fish; however, a decrease in the numbers of invertebrates occurs (May 1973). This could affect the early life stages of fish that feed on these organisms.

5. The cost of chemical and mechanical control, as well as the concern over the use of chemical herbicides, has increased the interest in biological control (Blanchard 1967). One of the most promising biological control agents is a plant-eating fish, the grass carp (*Ctenopharyngodon idella* Val.). This fish feeds on many aquatic plants, especially submersed species. Since special conditions are required for the spawning of the grass carp, it was presumed that this species would not become a nuisance in waterways outside its native habitat of China and Siberia. However, artificial reproduction is possible in fishery stations (Pieterse 1981).

6. Some controversy has arisen in Florida, based on the possibility of the fish reproducing naturally in sufficient quantities to interfere with fisheries and waterfowl populations (Burkhalter 1975). To reduce the potential for an ecological disaster, a sterile triploid grass carp was developed from a cross between a female grass carp and a bighead carp (*Hypohthalmichthys nobilis* Rich. (Stanley 1976). This sterile grass carp is routinely released in waterways infested with submersed aquatic plants (Cassani 1981).

7. In recent years, progress has been achieved in the use of insects for biocontrol of hydrilla (Bennett 1977). A moth, *Paraoynx diminatalis* Snellen, which is a native of Southeast Asia, was released in Florida for control of hydrilla (Pieterse 1981). However, predation by fish limited the natural population of the larvae of the moth. In 1987, a weevil, *Bagous affinis*, was released in Florida. This weevil, unfortunately, is not aquatic, although the adults feed on the hydrilla at the water surface. The larvae feed on the subterranean tubers in dry soil (Center 1989). In 1987, a fly, *Hydrellia pakistanae*, was released in Florida. The impact of these insects is presently being monitored (Center 1989).

8. During the late 1960's and early 1970's, researchers in Florida began to look at plant pathogens for control of aquatic plants (Charudattan 1973, 1975; Charudattan et al. 1985). In 1974, the fungus *Fusarium roseum*

'Culmorum' was isolated from diseased *Stratioides aloides* L. in The Netherlands (Charudattan and McKinney 1977, 1978; Charudattan et al. 1984).

Although promising results have been obtained from this pathogen, its use for biocontrol in the United States has not been resolved (Joye, in press). Several other fungi have been tested against hydrilla, including species of *Rhizoctonia*, *Pythium*, *Phytophthora*, and *Sclerotium* (Charudattan 1973).

9. The discovery of endemic plant pathogens with potential for use in biocontrol has been a major objective in our research. In 1987, a pathogenic isolate of *Macrophomina phaseolina* was collected from hydrilla growing in Lake Houston, Texas (Joye 1988, 1989). The present report focuses on the studies that led to the discovery of this potential biocontrol agent of hydrilla and describes the results of greenhouse and field efficacy tests and host range studies.

Materials and Methods

Microbial isolation and culture

10. In the fall of 1987 and 1988, fungal and bacterial isolates were collected from hydrilla plants obtained from natural populations growing in 15 lakes and three rivers of the southeastern United States (Table 1). Three to five samples of hydrilla tissue of at least 100 g each were taken from each location. The samples were placed in plastic bags and packed in ice for transport to the laboratory and stored at 4° C until ready for use.

11. The samples of hydrilla tissue were further divided into four equal parts of 25 g each, washed for several minutes in tap water to remove soil and debris, surfaced sterilized for 20 sec in 10-percent sodium hypochlorite, and washed in sterile deionized distilled water for 1 min. A puree of the tissue was formed by blending in a Waring blender (Waring Products Division, New Hartford, CT) for 30 sec. The puree was serially diluted from 10^0 to 10^5 .

12. A 0.1-ml aliquot was taken from each dilution with a 1-ml pipette, placed on potato dextrose agar (PDA) + 3 $\mu\text{g/ml}$ of streptomycin and nutrient agar (NA) + 3 $\mu\text{g/ml}$ of benomyl, and evenly spread over the media with a glass rod formed into the shape of a hockey stick. Two to five days after plating, individual isolates of fungi (on PDA) and bacteria (on NA) were taken from the dilution plates and recultured on fresh PDA and NA. Pure cultures of each

isolate were stored at -80°C in a 1:1 solution of 10-percent skim milk/40-percent glycerol (Dhingra and Sinclair 1985).

Test tube bioassays

13. The fungal and bacterial isolates collected from hydrilla tissue were screened in a test tube bioassay using a 7-cm hydrilla sprig grown in 20- by 2.5-cm glass tubes filled with 60 ml of a sterile nutrient solution and covered with a metal cap. The nutrient solution consisted of $\text{Ca}(\text{NO}_3)_2$ (0.179), CaCO_2 (0.092), MgSO_4 (0.033), KHCO (<0.015), and NAHCO_3 (0.059 g/l) (Smart and Barko 1985).

14. The test fungi were grown in 250-ml Erlenmeyer flasks with 100 ml of V8 juice broth (glucose, 10 g; KNO_3 , 10 g; CaCO_3 , 3 g; V8 juice, 200 ml; distilled water, 800 ml) and were agitated at 160 rpm for 3 to 5 days. Bacteria were grown on nutrient agar and suspended in sterile distilled water. Hydrilla plants in the tubes were inoculated with a concentration of 1×10^6 colony forming units (cfu)/ml of each fungal or bacterial isolate. Dilutions of the fungal suspensions were plated on PDA to determine propagule density. Bacterial counts were made using a hemacytometer.

15. The hydrilla tubes were placed in a growth chamber (adjusted to 25°C with 12/12 hr light/dark periods (Figure 2). After 6 weeks, any remaining green and apparently healthy tissue was harvested, dried at 100°C for 48 hr, and weighed. Control plants were treated with 2 ml of V8 broth. Each fungal, bacterial, and control treatment was replicated 10 times. Biomass values were compared using Tukey's Test, following analysis of variance (Steel and Torrie 1980).

Greenhouse bioassays

16. Clear acrylic tubes (150 by 13.75 cm) were used for these studies. Twenty centimeters of unsterilized lake sediment was placed in the bottom of each tube and overlain with 7.5 cm of fine washed silica sand. The sand helped support the planted hydrilla sprigs and reduce turbidity. Aluminum foil was wrapped around the exterior of the tubes near the sediment-filled portion to prevent light penetration to the sediment of each tube. Three 15-cm sprigs of hydrilla were planted in the sediment of each tube after which 16 l of nutrient solution was added. The nutrient solution was the same as previously described except that $\text{Ca}(\text{NO}_3)_2$ was omitted in order to reduce algal blooms (Smart and Barko 1985). The tubes of hydrilla were aerated and maintained at 25°C in a 1,200-l waterbath with an attached cooling system (Figure 3).

17. The inoculum for each test fungal isolate was grown in a sterile V8 broth as previously described. Excess supernatant was drained off by filtering the fungal culture through three layers of cheesecloth. Sufficient inoculum of each fungus was produced to yield a concentration of 1×10^6 cfu/ml after dilution in the 16 ℓ of nutrient solution for each column. To ensure rapid dispersal of the inoculum in the tubes, the fungi were suspended in 350 ml of deionized water. Control hydrilla plants were treated with 350 ml of deionized water.

18. When the hydrilla plants had grown to the top of the water column (100 cm) in the tubes, they were inoculated with the test fungi. The plants were observed daily for disease symptoms. Three weeks after inoculation, the remaining living tissue was collected, dried at 100° C for 3 days, and weighed. Treatments were replicated five times. Mean comparisons were made using Tukey's Test, following analysis of variance (Steel and Torrie 1980).

Host specificity

19. Plants used for this study included submersed and floating aquatic plants, wetland terrestrial plants, and crop plants (Table 2). A humidity chamber (100 by 100 by 240 cm) was constructed of wood and clear polyethylene plastic. Styrofoam cups (150 ml) were filled with potting mix (Sunshine No. 4, Fisons Western Corporation, Vancouver, BC, Canada). Seeds or plant sprigs of each species were planted in each cup and watered as needed. Relative humidity was maintained between 90 and 99 percent using a cool-water vaporizer (Hanksraft model 240, Gerber Products Corporation, Reedsburg, WI). Temperature could not be controlled and ranged between 25° and 35° C. Ten replicates were prepared for each treated and nontreated plant species. Inoculum was grown in a V8 broth as prescribed previously.

20. One month after planting, the wetland terrestrial plants and crop plants were submersed in an inoculum of *Macrophomina phaseolina* (mycelium and microsclerotia) with a concentration of 1×10^8 cfu/ml plus surfactant (Tween 20 at 0.5 ml/ ℓ). Control plants were submersed in water and surfactant only. Aquatic plants were inoculated with 150 ml of concentrated inoculum of 1×10^8 cfu/ml. Control aquatic plants were treated with 150 ml of water plus surfactant. The plants were maintained in the humidity chamber for 14 days after inoculation and observed daily for any disease symptoms. Data were reported as plants being resistant if no reaction occurred or if only hypersensitive flecking was observed. Plants were considered susceptible if the

plants showed significant symptoms of disease. All plant species were tested at least twice.

Field tests

21. In 1988 and 1989, an isolate of *Macrophomina phaseolina* was tested under field conditions at the Sheldon Reservoir, located 20 miles (32 km) northeast of Houston, TX. The test site was a dense, monospecific hydrilla stand growing in a lentic environment with a water depth of 1 m. Enclosures (1 by 1 by 2 m) were constructed of polyvinyl chloride tubing frame (2.54 cm, Schedule 40) covered with a clear polyethylene (6 mil), and secured in the sediment. The enclosures were erected 1 month prior to inoculation to allow the plants to naturalize (Figure 4).

22. On 29 September 1988 and 10 October 1989, hydrilla plants growing within the enclosures were treated with an inoculum of 1×10^9 cfu/ml of *M. phaseolina*, isolate FHyl8, to produce a final concentration of 1×10^4 cfu/ml. After 4 weeks, the remaining living biomass was collected, dried at 100° C, and weighed. Treated and nontreated plots were replicated five times. Mean comparisons were made using the t-test (Steel and Torrie 1980).

Results and Discussion

Microbial isolation and culture

23. Nearly 200 fungal isolates and 27 bacterial isolates were collected from hydrilla foliage. The most frequent fungal species collected were members of *Penicillium*, *Trichoderma*, *Rhizopus*, and *Aspergillus*, which were isolated from plants growing at every sample site. Isolates within these genera were not tested for pathogenicity because of their typically wide host range and production of nonspecific enzymes or toxins. Except for *Rhizopus*, Charudattan has previously reported isolates of these genera to have some pathogenicity toward hydrilla (Charudattan 1973, Charudattan and Lin 1974). Other less frequently isolated fungi included species of *Fusarium*, *Rhizoctonia*, and numerous other unidentified nonsporulating Hypomycetes. An isolate of *M. phaseolina* was isolated from hydrilla growing only in Lake Houston (Figure 5).

24. Bacterial identifications were not made conclusively; however, genera that were represented included *Pseudomonas* and *Bacillus*.

Test tube bioassays

25. Of the fungal isolates collected from our survey, two isolates identified as *M. phaseolina* (FHyl8 and FHy20) damaged hydrilla significantly more than other test isolates ($P > F = 0.05$) (Figures 6 and 7). Twenty-two of 27 bacterial isolates were tested. No bacterial isolate significantly damaged hydrilla ($P > F = 0.33$) (Figure 8).

26. Since hydrilla is not native to the Western Hemisphere and its presence in the United States has been noted for only about 30 years, we hypothesized that microorganisms strongly pathogenic to hydrilla would rarely be found. The latest published checklist of fungi on plants in the United States (Farr et al. 1989) lists for hydrilla only *Sclerotium rofsii*, which is a plant pathogen with an extremely wide host range. However, several fungi have been reported to cause various levels of disease of hydrilla (Charudattan 1973, 1975; Charudattan and Lin 1974; Charudattan and McKinney 1977, 1978; Charudattan et al. 1984).

Greenhouse bioassays

27. Although only one fungus, *M. phaseolina* (isolates FHyl8 and FHy20), was found to be effective in the test tube bioassay, three isolates of fungi previously reported on hydrilla (Charudattan et al. 1984, Pennington 1985) were included in the greenhouse experiments for comparative purposes. Isolates FHyl8 and FHy20 reduced the biomass between 95 and 99 percent (Figure 9). The final biomass of hydrilla treated with these two isolates was significantly ($P < 0.05$) lower than that of the controls or plants treated with other isolates (Table 3).

28. A similar effect was observed in a repeated experiment. Plants treated with isolate 621P (*Fusarium roseum* var. *culmorum*) had significantly lower biomass than those treated with isolates 224 (*Fusarium moniliforme* var. *subglutinans*) and 236 (*Cladosporium cladosporioides*).

29. Disease symptoms appeared on treated plants 7 days after inoculation. Initially, the disease was expressed as interveinal chlorosis, leaving only the midvein with green color. This progressed into a complete loss of color in all leaf tissue. The stem gradually became transparent. After 10 days, plants began to deteriorate. By 14 days after inoculation, plants appeared dead. By 21 days after inoculation, the time plants were harvested, no living tissue was evident (Figure 5).

30. Prior to this study, isolate 621P, an isolate of *F. roseum* var. *culmorum*, was thought to be the most virulent pathogen to hydrilla. Our

studies have shown that an isolate of *M. phaseolina* was significantly more pathogenic than was isolate 621P. Isolate 621P was originally collected from diseased tissue of *Stratioides aloides* L. growing in The Netherlands (Charudattan and McKinney 1977). However, before this fungus can be used as a biocontrol agent in the United States, further experimental testing is required (Joye 1989).

Host specificity

31. *Macrophomina phaseolina*, isolate FHyl8, was nonpathogenic to 44 of 46 species and subspecific taxa within 22 families (Table 2). Only hydrilla and duck lettuce (*Ottelia alismoides* (L.) Pers.) were susceptible. Duck lettuce is an introduced Afro-Asian species, populations of which are known to exist in southern Louisiana (Correll and Johnston 1970).

32. The host range of *M. phaseolina* is generally considered broad, with at least 284 plant species reported to be susceptible to isolates of this pathogen (Farr et al. 1989). Results of our studies suggest that different isolates of this fungus may vary in their pathogenicity. Variation in the pathogenicity of other isolates of this fungus has been reported in relation to chlorate sensitivity (Pearson, Leslie, and Schwenk 1986, 1987). With this in mind, additional work is needed on the host range of this isolate of *M. phaseolina*.

Field tests

33. Disease symptoms of hydrilla were observed on plants in treated plots 2 weeks after inoculation. Symptoms were similar to those observed in the greenhouse studies. Initially, chlorotic interveinal areas of the foliage appeared, which later progressed into widespread tissue disintegration. Control plants were healthy and vigorous. In 1988 and 1989, there was a significant ($P > t = 0.03$ and 0.02 , $df = 4$) reduction in dry weights of treated plots (Table 4) with a 60.3- and 58-percent difference in dry weights between treated and untreated plots.

Conclusions

34. The effectiveness of this *M. phaseolina* on hydrilla in a field test has not been observed with other pathogenic fungal isolates on hydrilla. However, Charudattan et al. (1984) reported similar results under greenhouse conditions with the Dutch isolate of *F. roseum* var. *culmorum*. The isolate of *M. phaseolina* used in these studies has shown outstanding performance as a

biocontrol agent of hydrilla under controlled and field conditions; however, additional research is needed before this plant pathogen is considered for commercial use. Aspects of this biocontrol agent which must be determined include its fate, epidemiology, host range, toxicity, and effectiveness as a formulated product.

35. Two years of laboratory, greenhouse, and field data have shown that biological control of hydrilla with the plant pathogen *M. phaseolina* is possible. This pathogen apparently satisfies requirements for a biocontrol agent. It can be produced in abundance on artificial media, it has shown a high degree of host specificity, and the prepared inoculum of the pathogen was able to rapidly destroy hydrilla within 4 weeks after inoculation.

36. The effects of application of a biocontrol agent such as that described to an aquatic environment are not well understood. However, in general, biological control agents have little negative effect on the environment. Application of this pathogen may result in an effect similar to that observed with more traditional chemical control agents as a result of the rapid killing of the target weed; i.e., the rapid decay of organic matter will likely promote eutrophication and decrease oxygen levels. Nevertheless, long-term effects on the environment would be practically nonexistent. No persistence of harmful residues in the water, sediment, or within the tissues of plants and animals would occur.

References

- Bennett, F. D. 1977. Insects as Agents for Biological Control of Aquatic Weeds. Aquatic Botany 3:165-174.
- Blanchard, J. L. 1967. Economic Aspects of Weed Control in the Lakes of Winter Park, Florida. Hyacinth Control Journal 6:21-22.
- Bowes, G., Van, T. K., Garrard, L. A., and Haller, W. T. 1977. Adaptation to Low Light Levels of Hydrilla. Journal of Aquatic Plant Management 15:32-35.
- Burkhalter, A. P. 1975. The White Amur Controversy. Weeds, Trees, and Turf 14:26-35.
- Cassani, J. R. 1981. Biological Control of Aquatic Weeds in Southwest Florida. Journal of Aquatic Plant Management 19:49-50.
- Center, T. D. 1989. Release and Establishment of Insect Biocontrol on Hydrilla. In Proceedings, 23rd Annual Meeting, Aquatic Plant Control Research Program, 34-40. Miscellaneous Paper A-89-1. US Army Engineer Waterways Experiment Station, Vicksburg, MS.
- Charudattan, R. 1973. Pathogenicity of Fungi and Bacteria from India to Hydrilla and Waterhyacinth. Hyacinth Control Journal 11:44-48.

- Charudattan, R. 1975. Use of Plant Pathogens for Control of Aquatic Weeds. Ecological Research Series 660-3-75-301. US Environmental Protection Agency, pp 127-153.
- Charudattan, R., and Lin, C. Y. 1974. Isolates of *Penicillium*, *Aspergillus*, and *Trichoderma* Toxic to Aquatic Plants. Hyacinth Control Journal 12:70-73.
- Charudattan, R., and McKinney, D. E. 1977. A *Fusarium* Disease of the Submerged Aquatic Weed *Hydrilla verticillata*. Proceedings, American Phytopathology Society 4:222.
- Charudattan, R., and McKinney, D. E. 1978. A Dutch Isolate of *Fusarium roseum* 'Culmorum' May Control *Hydrilla verticillata* in Florida. In Proceedings, 5th EWRS International Symposium on Aquatic Weeds, Amsterdam, pp 219-224.
- Charudattan, R., Freeman, T. E., Cullen, R. E., and Hofmeister, F. M. 1984. Evaluation of *Fusarium roseum* 'Culmorum' as a Biological Control Agent of *Hydrilla verticillata*. Technical Report A-85-5. US Army Engineer Waterways Experiment Station, Vicksburg, MS.
- Charudattan, R., Linda, S. B., Kluefel, M., and Osman, Y. A. 1985. Biocontrol Efficacy of *Cercospora rodmanii* on Waterhyacinth. Phytopathology 75:1263-1269.
- Correll, D. S., and Johnston, M. C. 1970. Manual of the Vascular Plants of Texas. Texas Research Foundation, Renner, TX.
- Dhingra, O. D., and Sinclair, J. B. 1985. Basic Plant Pathology Methods. CRC Press, Boca Raton, FL.
- Environmental Laboratory. 1985. Monoecious Hydrilla in the Potomac River. Miscellaneous Paper A-85-5. US Army Engineer Waterways Experiment Station, Vicksburg, MS.
- Farr, D. F., Bills, G. F., Chamuris, G. P., and Rossman, A. Y. 1989. Fungi on Plants and Plant Products in the United States. APS Press, St. Paul, MN.
- Joye, G. F. 1988. Biological Control of *Hydrilla verticillata* Using an Endemic Fungal Plant Pathogen. Phytopathology 78:1593 (A642).
- Joye, G. F. 1989. Biological Control of *Hydrilla verticillata* (L.f.) Royle Using an Endemic Plant Pathogen. In Proceedings, 23rd Annual Meeting, Aquatic Plant Control Research Program. Miscellaneous Paper A-89-1. US Army Engineer Waterways Experiment Station, Vicksburg, MS, pp 41-50.
- Joye, G. F. Biological Control of Aquatic Plants with Plant Pathogens. In Symposium on Biological Control of Weeds with Microbes and Microbial Products (in press). American Chemists Society.
- Langeland, K. A., and Sutton, D. L. 1980. Regrowth of Hydrilla from Axillary Buds. Journal of Aquatic Plant Management 18:27-29.
- May, B. E. 1973. Comparative Effects of Diquat Plus Copper Sulphate on Aquatic Organisms. Weed Science 21:249-253.
- Miller, J. L., Garrard, L. A., and Haller, W. T. 1976. Some Characteristics of Hydrilla Tubers Taken from Lake Ocklawaka During Drawdown. Journal of Aquatic Plant Management 14:29-32.
- Pearson, C. A. S., Leslie, J. F., and Schwenk, F. W. 1986. Variable Chlorate Resistance in *Macrophomia phaseolina* from Corn, Soybean, and Soil. Phytopathology 76:646-647.

- Pearson, C. A. S., Leslie, J. F., and Schwenk, F. W. 1987. Host Preference Correlated with Chlorate Resistance in *Macrophomina phaseolina*. Phytopathology 71:827-831.
- Pennington, J. C. 1985. Biological Control of *Hydrilla verticillata* (L.f.) Royle with Lytic Enzyme-Producing Organisms. Technical Report A-85-3. US Army Engineer Waterways Experiment Station, Vicksburg, MS.
- Pieterse, A. H. 1981. Hydrilla - A Review. Abstracts on Tropical Agriculture 7:9-34.
- Robson, T. O. 1976. A Review of the Distribution of Aquatic Weeds in the Tropics and Sub-tropics. In Aquatic Weeds in South East Asia, Proceedings, Regional Seminar on Noxious Aquatic Vegetation, New Delhi. W. Junk, The Hague, pp 25-30.
- Sculthorpe, C. D. 1967. The Biology of Aquatic Vascular Plants. Edward Arnold, London.
- Smart, M., and Barko, J. W. 1985. Laboratory Culture of Submersed Freshwater Macrophytes on Natural Sediment. Aquatic Botany 21:251-263.
- Sobal, B. M. 1987. Environmental Effects of Aquatic Disposal of Chopped Hydrilla. Journal of Aquatic Plant Management 25:19-23.
- Stanley, J. G. 1976. A Review of Methods for Obtaining Monosex Fish and Progress Report on Production of Monosex White Amur. Journal of Aquatic Plant Management 14:68-70.
- Steel, R. G. D., and Torrie, J. H. 1980. Principles and Procedures of Statistics. McGraw-Hill, New York.
- Sutton, D. L., Weldon, L. W., and Blackburn, R. D. 1970. Effect of Copper on Uptake of Diquat 14C by Hydrilla. Weed Science 18:703-707.
- Van, T. K., Haller, W. T., and Garrard, L. A. 1978. The Effect of Daylength and Temperature on Hydrilla Growth and Tuber Production. Journal of Aquatic Plant Management 16:57-59.
- Vandiver, V. V. 1978. Herbicides for Control of Hydrilla in Florida. Agronomy Facts 77:1-2.
- Yeo, R. R., Falk, R. H., and Thurston, J. R. 1984. The Morphology of Hydrilla (*Hydrilla verticillata* (L.f.) Royle). Journal of Aquatic Plant Management 22:1-17.

Table 1
Survey Sample Sites for Collection of Endemic
Microorganisms from Hydrilla

<u>State</u>	<u>Sample Site</u>
Texas	Lake Lewis
	Lake Houston
	Sheldon Reservoir
	Lake Somerville
	Lake Limestone
	Fairfield Lake
	Lake Athens
	Lake Palestine
	Lake Nacogdoches
	Sam Rayburn
	Toledo Bend
	Lake Conroe
Louisiana	Bayou Lafourche
	Lake Theriot
Florida	Oklawaha Lake
	Lake Seminole
	St. Johns River
	Chasakowhitzka River

Table 2

Reaction of Various Plant Species to FHyl8 Isolated from Hydrilla*

<u>Family/Species</u>	<u>Disease Reaction**</u>
Aceraceae	
Drummond's red maple (<i>Acer rubrum</i> L. var. <i>drummondii</i> (Hook & Arn.) Sarg.)	R
Alismataceae	
Arrowhead (<i>Sagittaria latifolia</i> Willd.)	R
Amaranthaceae	
Alligatorweed (<i>Alternanthera philoxeroides</i> (Mart.) Griseb.)	R
Araceae	
Waterlettuce (<i>Pistia stratiotes</i> L.)	R
Ceratophyllaceae	
Coontail (<i>Ceratophyllum demersum</i> L.)	R
Commelinaceae	
Dayflower (<i>Commelina</i> sp.)	R
Cucurbitaceae	
Cantaloupe (<i>Cucumis melo</i> L.) 'Halesbest'	R
Meloncito (<i>Melothria pendula</i> L.)	R
Squash (<i>Cucurbita pepo</i> var. <i>meloepo</i> (L.) Alef.)	
'Yellow summer crookneck'	R
Watermelon (<i>Citrullus vulgaris</i> Schrad.)	R
Cyperaceae	
Spikerush (<i>Eleocharis</i> sp.)	R
Fabaceae	
Alfalfa (<i>Medicago sativa</i> L.) 'Pioneer'	R
Red clover (<i>Trifolium pratense</i> L.)	R
Soybean (<i>Glycine max</i> (L.) Merr.)	
'Bedford'	R
'Braxton'	R
'Coker 368'	R
'Davis'	R
'Forrest'	R
'Hartz 8112'	R
'H6385'	R
'H7110'	R
Haloragaceae	
Parrotfeather (<i>Myriophyllum brasiliense</i> Camb.)	R
Eurasian watermilfoil (<i>Myriophyllum spicatum</i> L.)	R

(Continued)

* Ten plants of each variety were dipped in inoculum containing 1×10^8 cfu/ml and surfactant (Tween 20, 0.5 ml/l). Controls were dipped in water with surfactant only. Plants were evaluated daily for 14 days.

** R = resistant; S = susceptible.

Table 2 (Concluded)

Family/Species	Disease Reaction
Hydrocharitaceae	
Hydrilla (<i>Hydrilla verticillata</i> (L.f.) Royle)	S
Duck lettuce (<i>Ottelia alismoides</i> (L.) Pers.)	S
Eelweed (<i>Vallisneria americana</i> Michx.)	R
Limnaceae	
Duckweed (<i>Lemna minor</i> L.)	R
Malvaceae	
Okra (<i>Abelemoschus esculentus</i> (L.) Moench.) 'Clemson spineless'	R
Najadaceae	
Southern naiad (<i>Najas guadalupensis</i> (Spreng.) Magnus)	R
Nyssaceae	
Tupelo gum (<i>Nyssa aquatica</i> L.)	R
Onagraceae	
Water primrose (<i>Ludwigia peploides</i> (H.B.K.) Raven.)	R
Water primrose (<i>Ludwigia</i> sp.)	R
Poaceae	
Wheat (<i>Triticum aestivum</i> L.) 'Coker 762' 'FL302' 'McNair 1003' 'Rosen' 'Terra 1812'	R R R R R
Rice (<i>Oryza sativa</i> L.) 'Lemont' 'Mars' 'Mercury' 'TeBonnet'	R R R R
Polygonaceae	
Smartweed (<i>Polygonum</i> sp.)	R
Rubiaceae	
Buttonweed (<i>Diodia virginica</i> L.)	R
Saururaceae	
Lizardtail (<i>Saururus cernuus</i> L.)	R
Solanaceae	
Tomato (<i>Lycopersicon esculentum</i> Mill.) 'Sweet million 5352'	R
Taxodiaceae	
Bald cypress (<i>Taxodium distichum</i> (L.) Rich.)	R

Table 3

Effect of Applications of *Macrophomina phaseolina* Mycelia and
Microsclerotia to Hydrilla in Greenhouse Tests*

<u>Treatment**</u>	<u>Mean Dry Weight, g†</u>	
	<u>Experiment 1</u>	<u>Experiment 2</u>
Isolate		
FHy18	0.08 a	0.39 a
FHy20	0.2 a	0.13 a
Untreated	6.56 b	7.6 b

* Plants grown in clear plastic tubes (150-cm length × 13.75-cm diameter) filled with 20 cm of lake sediment and 16 l of nutrient solution were evaluated. Each plot (tube) was planted with three 15-cm hydrilla sprigs.

** Plants were inoculated with a concentration of 1×10^6 cfu/ml. Colony forming units were mycelia and microsclerotia. Untreated plants were treated with water only.

† Values represent the average living plant material of five replicates, 21 days after treatment. Means followed by the same letter are not significantly different (Tukey's Test, $P = 0.05$).

Table 4

Effects of Applications of *Macrophomina phaseolina* Mycelia and
Microsclerotia to Hydrilla in Field Tests*

<u>Treatment**</u>	<u>Mean Dry Weight, g†</u>	
	<u>1988</u>	<u>1989</u>
Inoculated	137.18	106.9
Control	354.16	258.8

* Plots (1 m²) were established within a natural dense monospecific stand of hydrilla growing in 1 m of lentic water.

** Treated plants were inoculated with a concentration of 1×10^4 cfu/ml. Colony forming units were mycelia and microsclerotia. Control plants received no amendments.

† Values are the average living plant material of five replicates, 4 weeks after inoculation. The mean values of the inoculated plants were significantly different from the controls for both years as determined by the t-test ($P > t = 0.03$ and 0.02 , and $df = 4$).

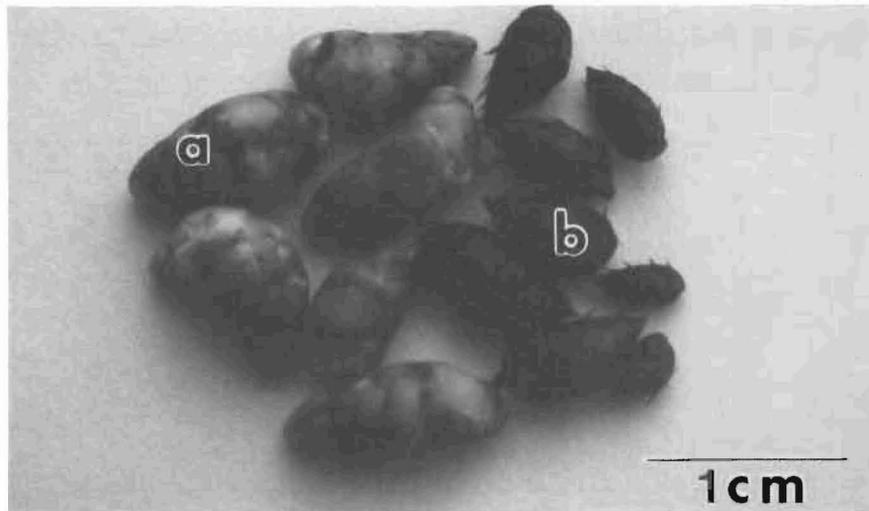


Figure 1. Tubers (a) and turions (b) produced by *Hydrilla verticillata*

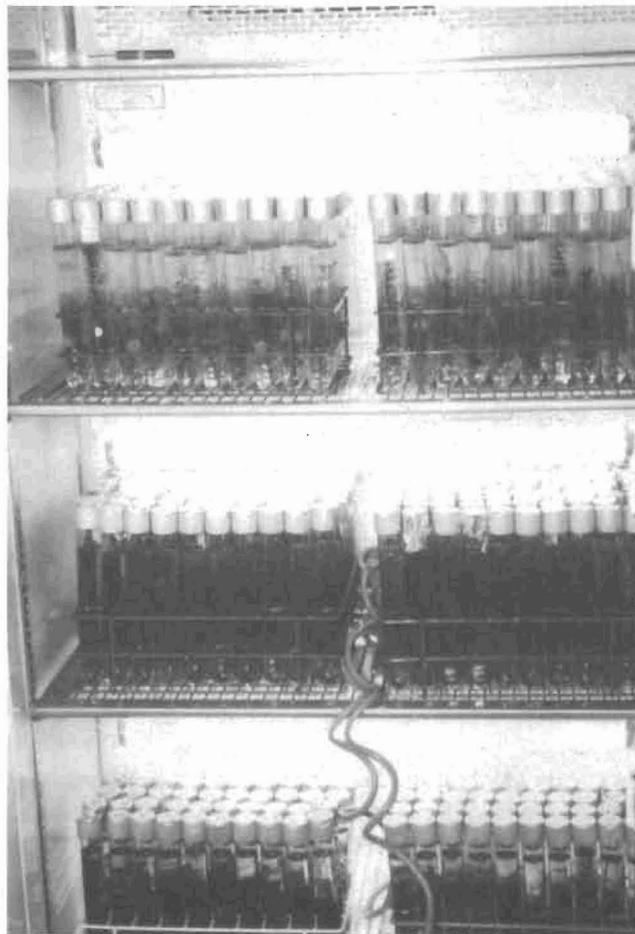


Figure 2. Test tube bioassays (a growth chamber with test tubes placed on racks)

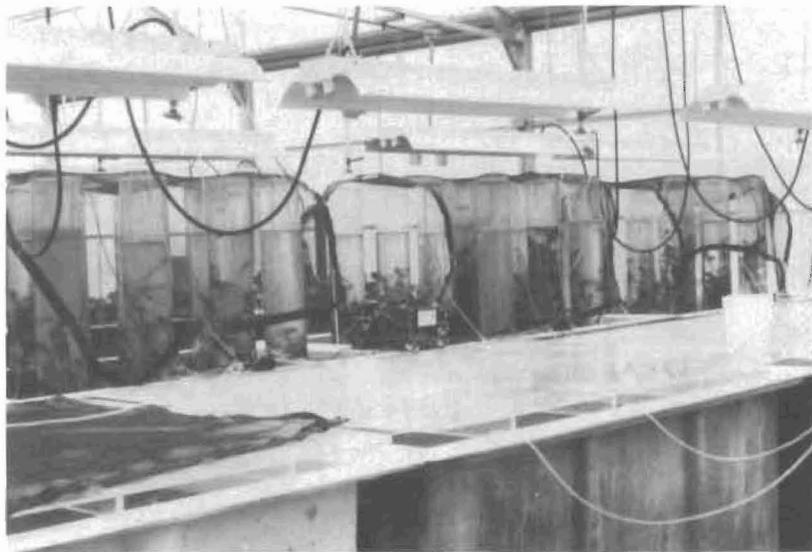
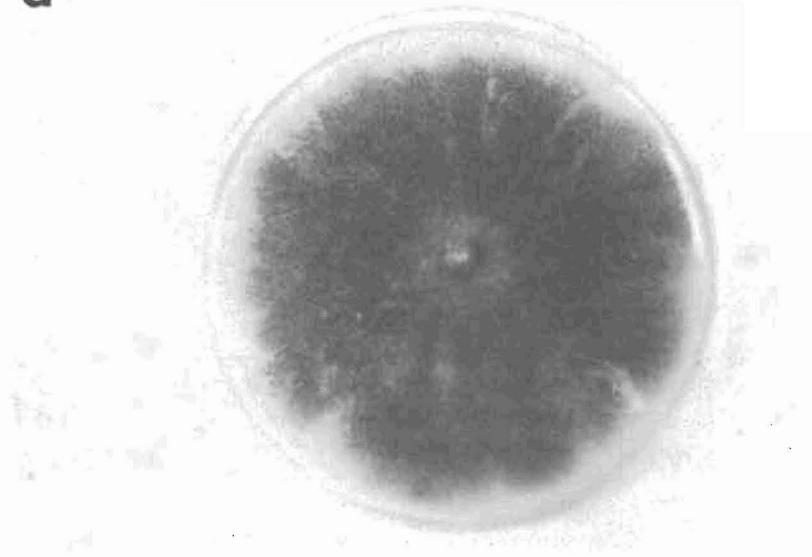


Figure 3. Greenhouse bioassay for testing fungal plant pathogens for control of hydrilla



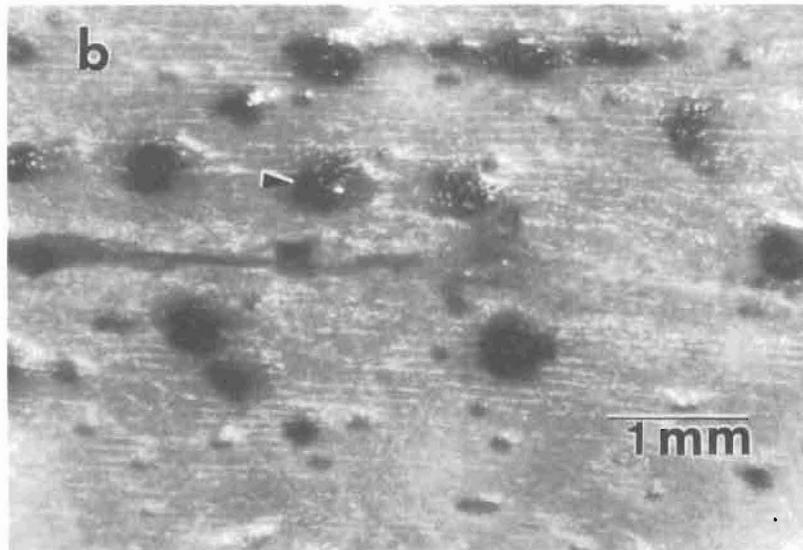
Figure 4. Enclosures for the 1988 biocontrol field test using *Macrophomina phaseolina* on hydrilla established at the Sheldon Reservoir, Texas

a



a. Growth in pure culture on V8 juice agar

b



b. Microsclerotia embedded in a leaf of hydrilla

Figure 5. *Macrophomina phaseolina*

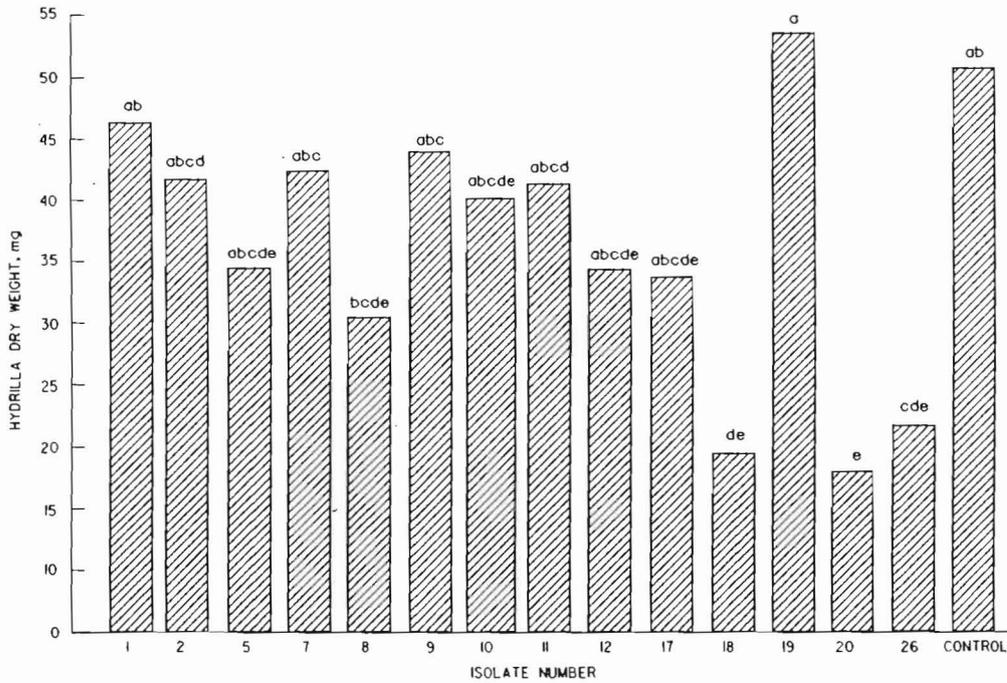


Figure 6. Fungal test tube bioassay, showing effects of fungal isolates on hydrilla. Bars with the same letter are not significantly different, Tukey's Test ($P = 0.05$, $n = 10$)

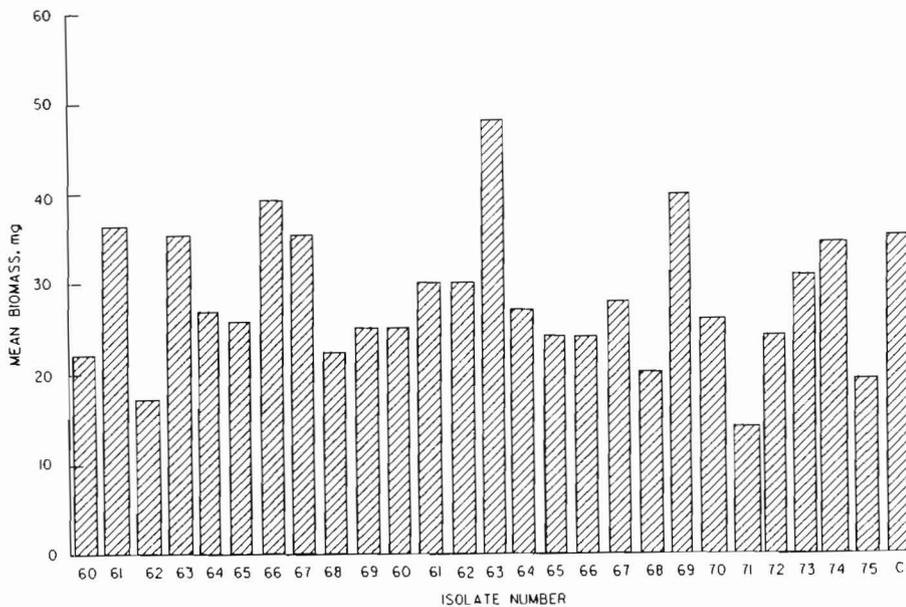


Figure 7. Fungal test tube bioassay, showing effects of fungal isolates on hydrilla. Based on the analysis of variance procedure, no fungal isolate was significantly different from the control in this bioassay; thus, no mean separation procedure was performed

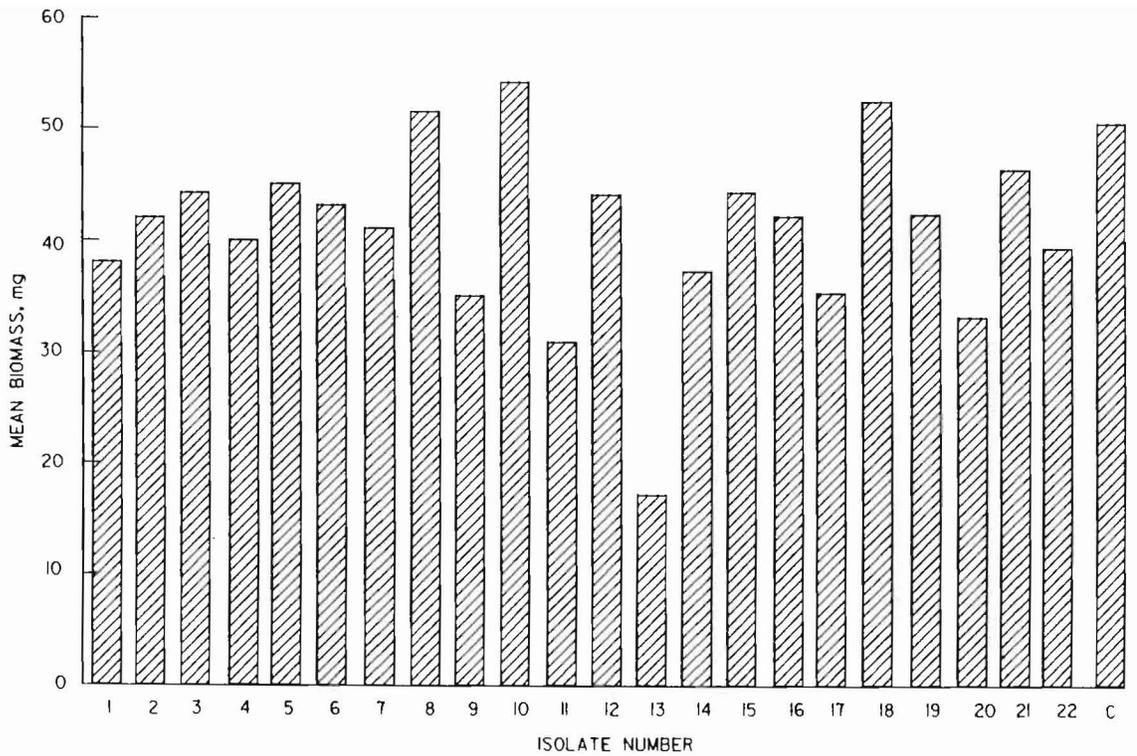
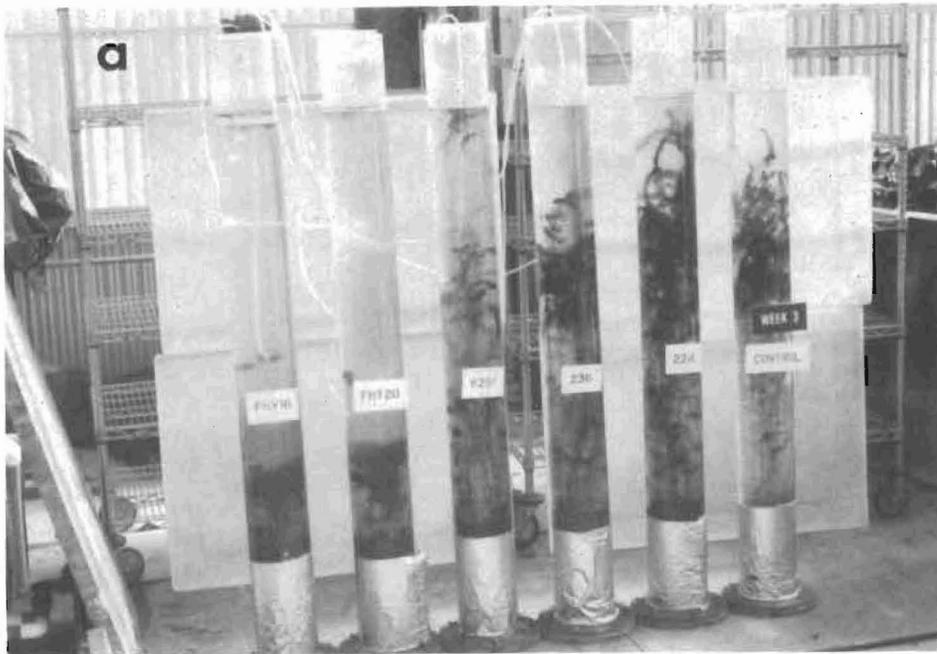
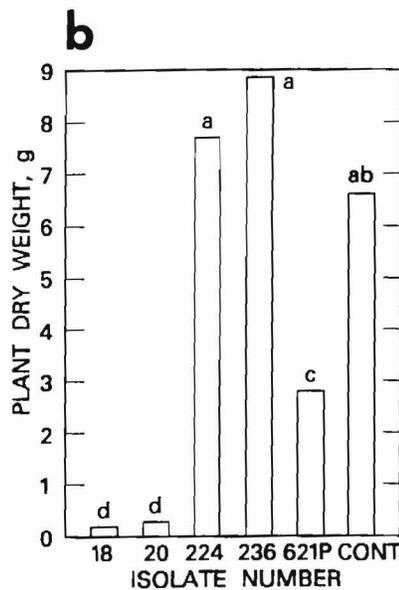


Figure 8. Bacterial test tube bioassay, showing effects of bacterial isolates on hydrilla. Based on the analysis of variance procedure, no bacterial isolate was significantly different from the control in this bioassay; thus, no mean separation procedure was performed



a. Effects of selected fungal isolates on hydrilla. Isolates 18 and 20 are *Macrophomina phaseolina*; isolates 224, 236, and 621P are *Cladosporium cladosporioides*, *Fusarium moniliforme* var. *subglutinans*, and *Fusarium roseum* var. *culmorum*, respectively



b. Tukey's Test ($P = 0.05$, $n = 5$). Bars with the same letter are not significantly different

Figure 9. Greenhouse column bioassay