Biocontrol of *Hydrilla verticillata* with the Endemic Fungus *Macrophomina phaseolina*

G. F. JOYE, Research Plant Pathologist, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS 39180-6199, and Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge 70803

ABSTRACT

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An isolate of *Macrophomina phaseolina* discovered in Lake Houston, TX, caused a disease of the submersed plant *Hydrilla verticillata*. In repeated greenhouse and field tests, this fungus greatly reduced the biomass of hydrilla within 3–4 wk after inoculation. Pathogenicity studies indicated that this fungus may be useful as a biocontrol agent.

Additional keywords: aquatic plants, biological control, microsclerotia

Hydrilla (*Hydrilla verticillata* (L. fil.) Royle) (Hydrocharitaceae) is a submersed aquatic plant that is one of the most invasive pests of waterways in tropical and subtropical regions of the world (15). It impedes navigation, clogs drainage and irrigation canals, interferes with recreational activities, and disrupts wildlife habitats (14). Hydrilla's "competitive edge" has been attributed to several factors, the most important being its ability to thrive under low light, which many native aquatic plants lack (3).

Hydrilla is controlled largely through the use of chemical herbicides or mechanical removal. The high cost of these control measures, as well as concern for the environment, has increased interest in the biological control of aquatic weeds. At present, biocontrol agents used to manage hydrilla include sterile hybrid grass carp (white amur) (4) and two insects, a tuber-feeding weevil and a leaf-mining fly (5).

The use of plant pathogens to control aquatic plants has been studied for nearly 20 yr, and several promising fungal pathogens have been identified. An endemic isolate of *Cercospora rodmanii* Conway was found to incite a severe leaf spot disease on water hyacinth (*Eichhornia crassipes* (Mart.) Solms.) (11), but under optimal growing conditions, the plant can reproduce at a rate that renders the disease epidemic ineffective (7). *Fusarium roseum* (Link:Fr.) var. *culmorum* Snyd. & Hans. (8,10), an exotic isolate from The Netherlands, has demonstrated the potential to control hydrilla but has not been cleared for general release in the United States.

The discovery of endemic plant pathogens with potential as biocontrol agents for aquatic weeds has been a major objective of research at the U.S. Army Engineer Waterways Experiment Station in Vicksburg, MS. In 1987, a pathogenic isolate of *Macrophomina phaseolina* (Tassi) Goid. was collected from hydrilla growing in Lake Houston, TX (12,13). After demonstrating that Koch's postulates were satisfied under laboratory conditions, we began to investigate this pathogen as a possible agent for biocontrol of hydrilla. This paper reports the results of greenhouse and field efficacy tests.

MATERIALS AND METHODS

Greenhouse studies. Clear plastic tubes 150 cm long and 13.75 cm in diameter were used for greenhouse studies. Unsterilized lake sediment (20 cm) was placed in the bottom of each tube and covered with 7.5 cm of fine, washed silica sand. The sand helped support the hydrilla sprigs and reduce turbidity. Aluminum foil was wrapped around the outside of the sediment-filled portion of the tubes to prevent light from penetrating to the root zone. Three 15-cm sprigs of fresh hydrilla were planted in the sediment in each tube, and 16 L of nutrient solution was added. The nutrient solution contained, per liter, 0.179 g of $Ca(NO_3)_2$, 0.092 g of $CaCl_2$, 0.033 g of $MgSO_4$, 0.015 g of $KHCO_3$, and 0.059 g of $NaHCO_3$ (16). The tubes were aerated and maintained under prevailing greenhouse conditions.

Two isolates of *M. phaseolina*, FHY18 and FHY20, were evaluated. Inoculum of each isolate was grown in modified sterile Richard's V-8 broth (10 g of glucose, 10 g of KNO_3, 3 g of $CaCO_3$, 200 ml of V-8 juice, and 800 ml of distilled water) (20). Cultures of the fungus were grown for 5–7 days in a 10-L fermenter (model M1085-2000; New Brunswick Scientific Co., Edison, NJ) at 27 C, then aerated at 6.9 kPa and agitated at 200 rpm. Excess liquid was drained off by filtering the fungal culture through three layers of cheesecloth. The recovered fungal material was comminuted for 15 sec in a Waring Blendor. The fungal culture was suspended in 350 ml of deionized water to ensure rapid dispersal of the inoculum and to provide a fungal propagule concentration of 4.7×10^7 cfu/ml (mycelia and microsclerotia) to yield a final concentration of 1×10^6 cfu/ml after dilution in the 16 L of water for each column. Dilutions of the fungal suspensions were plated on potato-dextrose agar to determine propagule density.

After the hydrilla plants had grown to the top of the water column (100 cm), in about 4 wk, they were inoculated with the test isolates. Control plants were treated with 350 ml of deionized water. Plants were observed daily for disease symptoms. Three weeks after inoculation, the remaining living biomass was collected, dried at 100 C, and weighed.

Treatments were arranged in a randomized block design with five replications. The experiment was conducted twice. Data were subjected to analysis of variance, and Tukey's test (19) was used to compare means.

Field studies. In 1988 and 1989, the pathogen was tested under field conditions at the Sheldon Reservoir 32 km northeast of Houston, TX. The test site was a dense, monospecific hydrilla stand, growing in a lentic environment with an average water depth of 1 m. Enclosures (1 m × 1 m × 2 m high) were constructed of a polyvinyl chloride tubing frame (2.54 cm, schedule 40) covered with clear, 6-mil polyethylene. The enclosures were secured in the sediment 1 mo before inoculation to allow the plants to naturalize.

On 29 September 1988 and 10 October 1989, hydrilla growing within the enclosures was inoculated with 7 L of

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Table 1. Mean dry weight (g) of hydrilla treated with mycelia and microsclerotia of *Macrophomina phaseolina* in greenhouse tests[y]

Isolate	Experiment[z] 1	2
FHY18	0.1 a	0.4 a
FHY20	0.2 a	0.1 a
Untreated	6.6 b	7.6 b

[y] Plants were grown in clear plastic tubes (150 cm long and 13.75 cm in diameter) filled with 20 cm of lake sediment and 16 L of nutrient solution. Each plot (tube) was planted with three 15-cm sprigs of hydrilla.

[z] Values are average living plant material of five replicates 21 days after treatment. Means in a column followed by the same letter do not differ significantly (Tukey's test, $P > 0.05$).

Table 2. Mean dry weight (g) of hydrilla treated with mycelia and microsclerotia of *Macrophomina phaseolina* in the field[y]

Treatment	Year[z] 1988	1989
Inoculated	137.2	106.9
Control	354.2	258.8

[y] Plots (1 m^2) were established within a natural, dense, monospecific stand of hydrilla growing in 1 m of lentic water.

[z] Values are average living plant material of five replicates 4 wk after inoculation. The mean values of the inoculated plots were significantly different from those of the control plots for both years, as determined by the t test ($P > 0.03$ and 0.02 for 1988 and 1989, respectively).

a suspension of *M. phaseolina* containing 8.7×10^5 cfu/ml (6.1×10^9 cfu per 7 L) to produce a final concentration of 1×10^4 cfu/ml within the enclosures. At the time of inoculation, the water depth was 61 cm. In 1988, the depth increased to 100 cm before the experiment was completed; thus, assuming constant numbers of propagules, the corrected dilution concentration would have been 6.1×10^3 cfu/ml.

After 4 wk, the remaining biomass was collected, dried at 100 C, and weighed. Treated and untreated control plots were replicated five times. Means were compared using a t test (19).

RESULTS

Greenhouse studies. *M. phaseolina* (isolates FHY18 and FHY20) significantly reduced the biomass of hydrilla (95–99%) 21 days after inoculation compared with untreated plants ($P > 0.05$) (Table 1). Disease symptoms appeared 7 days after inoculation as interveinal chlorosis that progressed into a complete loss of color. After 10 days, plants treated with *M. phaseolina* began to disintegrate, apparently from loss of structural integrity. At 21 days, only small amounts of plant material could be recovered. Untreated plants were healthy and vigorous.

Field studies. In both 1988 and 1989, dry weight of field plots treated with *M. phaseolina* was significantly ($P > 0.03$ and 0.02, respectively) lower than that of untreated plots (Table 2). The reduction was 61.3 and 58.0% for 1988 and 1989, respectively. Disease symptoms on hydrilla in treated plots 2 wk after inoculation were similar to those observed in the greenhouse studies. Untreated plants were healthy and vigorous.

DISCUSSION

This is the first report of any isolate of *Macrophomina* causing disease of an aquatic plant. The isolates of *M. phaseolina* used in this study (mycelia and microsclerotia) can be produced in abundance on artificial media. Prepared inoculum of the pathogen was able to destroy hydrilla within a relatively short period after inoculation. *M. phaseolina* is the most effective of the potential mycoherbicides that have been tested to date on submersed plants (1,2,6,8–10, 14,17,18).

The application of large quantities of organic matter to the water in the field test was a concern at the outset of these experiments. However, in other studies using potential pathogens, none of which had any effect on hydrilla, the organic matter added to water columns did not adversely affect the growth of hydrilla (13). In addition, the pathogen was reisolated from diseased hydrilla tissue in these experiments.

Because this organism will undergo further scrutiny before any large-scale testing is conducted, we will continue to investigate host specificity, minimum titers for efficacy, toxicity, and propagule fate. Studies on the histopathology of colonization of hydrilla by this fungus are under way.

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