



# The Screening of Pathogens as Potential Biological Control Agents for Management of *Hydrilla verticillata*

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**PURPOSE:** The purpose of this study was to survey hydrilla infestations in the southeastern United States for new pathogens that might be used as biocontrol agents for the management of hydrilla.

**INTRODUCTION:** Since its introduction into Florida in the 1950s, *Hydrilla verticillata* (L. f.) Royle (hydrilla) has spread throughout the Southeast (Schmitz et al. 1991). In the 1970s, Charudattan et al. (1980) recommended an integrated approach to hydrilla management by employing herbicides, biological agents, and mechanical harvesters. Toward that end, a search was begun for microbial agents that would be compatible with other control strategies and that offered an environmentally safe alternative (Charudattan et al. 1980). An isolate of *Fusarium roseum* 'Culmorum' (Lk & Fr.) found on *Stratiotes aloides* L. (Hydrocharitaceae) from the Netherlands was evaluated in quarantine at the University of Florida, Gainesville, Florida. It was found to be efficacious on hydrilla, killing the plant in two to three weeks. However, it was never developed into a bioherbicide because it was a foreign isolate that was not host specific.

The U.S. Army Corps of Engineers began pathogen research on hydrilla in the 1980s. In the fall of 1987 and 1988, microbial isolates were obtained from hydrilla plants collected in 15 lakes and three rivers in the southern United States (Joye and Cofrancesco 1991). The material yielded 200 fungal and 27 bacterial isolates. Many of the isolates were determined to be members of the genera *Penicillium*, *Aspergillus*, *Trichoderma*, and *Rhizopus*, and were not considered further for biocontrol agents because they are saprophytic or secondary invaders. Two fungal isolates — FHy18 and FHy20 — both identified as *Macrophomina phaseolina* (later determined to be *Mycoleptodiscus terrestris* (Gerdeman) Ostazeski) (Shearer 1993) damaged hydrilla significantly and reduced hydrilla aboveground biomass between 95 and 99% in greenhouse bioassays. None of the bacterial isolates were efficacious on hydrilla (Joye and Cofrancesco 1991). Field tests using *M. terrestris* were conducted at Sheldon Reservoir northeast of Houston, Texas. Enclosures (1m x 1m x 2 m high) were each inoculated with 7 L of a *M. terrestris* fungal slurry in fall of 1988 and 1989 (Joye 1990). In both years, shoot dry weight of treated plants was significantly lower than that of untreated plants. Subsequent testing in the greenhouse and in the field produced similar results (Shearer 1996).

Since it had been over 20 years since the last surveys for pathogens of hydrilla had taken place, and since the invasive aquatic macrophyte has greatly expanded its range, new surveys were undertaken. The results of those surveys and laboratory evaluations are documented herein.

**MATERIALS AND METHODS:** During summer 2009 and 2010, surveys were conducted in the southeastern United States to collect hydrilla samples for the purpose of isolating potential pathogenic biological control agents. In total, 31 hydrilla samples were collected from sites in six states (Alabama,

Florida, Louisiana, Tennessee, Texas, and Virginia) and shipped overnight in refrigerated coolers to the biocontrol laboratory at the U.S. Army Engineer Research and Development Center (ERDC), Vicksburg, Mississippi. Upon arrival, the samples were thoroughly washed in tap water to remove any soil or debris attached to stems and leaves. The samples were wrapped in moist paper toweling, placed in plastic bags, and kept at 4 °C until they could be processed.

The samples were processed by dilution plating. A 10-g sub-sample of stem and leaf tissue was surface sterilized in a 3.5 percent sodium hypochlorite solution for one minute and rinsed in deionized water for one minute. The sub-sample was blotted dry with sterile paper towels, then added to 100 ml of sterile water and macerated in a blender for 30 sec, providing a dilution factor of 1/10. The resulting slurry was further diluted to concentrations of 1/100 and 1/50. All dilutions were plated in 1-ml aliquots onto Martin's agar (H<sub>2</sub>O, 1 L; agar, 20 g, KH<sub>2</sub>PO<sub>4</sub>, 0.5 g; MgSO<sub>4</sub>·7 H<sub>2</sub>O, 0.5 g; peptone, 0.5 g; dextrose, 10 g, yeast extract, 0.5 g; rose Bengal, 0.05 g; streptomycin sulfate 0.03 g) plates (3 plates per dilution concentration). The plates were incubated in the dark at 25 °C for 1 week. Small pieces (~ 1 mm x 1 mm) were cut from the leading edge of filamentous fungal colonies on the plates and transferred to Potato Dextrose Agar (PDA) (Difco Inc., Detroit, MI) slants. An agar slant is simply a test tube placed at an angle during cooling to give a large slanted surface for inoculation. After 7 to 10 days, the slants were sorted together and enumerated into morphospecies based on gross colony morphology and color. The cultures were stored at 4 °C until they could be plated for identification. Each morphological "species" was plated onto Potato Carrot Agar (PCA) (Dinghra and Sinclair 1995) and PDA and incubated at 25 °C under a grow light for 1 to 3 weeks to induce sporulation. Both agars are important for isolate identification because characteristic colors and growth patterns developed on PDA and on PCA colonies readily produce asexual and/or sexual spores. Those cultures that sporulated were identified to genus and species when possible. Those that did not sporulate were placed in categories of moniliaceous (hyaline or brightly colored hyphae) or dematiaceous (dark hyphae) Ascomycetes.

After the fungi were identified, they were selected for efficacy testing on hydrilla. Fungi were selected for screening if they were in a genus having species that had been documented as being pathogenic (e.g. *Fusarium*, *Phoma*, *Colletotrichum*, *Drechslera*, *Mycocleptodiscus*, *Myrothecium*). Those fungi recognized to be secondary invaders or saprophytes were not tested. Between 8 and 10 isolates were plated each week onto PDA and allowed to grow 8 to 10 days at 25 °C. Approximately one half of the culture on the plate was cut into small pieces (1 mm x 1 mm) and added to a 250 ml baffled flask containing 100 ml of Richards's V-8 juice broth (glucose, 10 g; KNO<sub>3</sub>, 10 g; CaCO<sub>3</sub>, 3 g; V-8 juice (Campbells, Camden NJ), 200 ml; H<sub>2</sub>O, 800 ml). The flasks were placed on a platform shaker (New Brunswick, Edison, NJ) set at 300 rpm. Flasks were swirled daily to prevent fungal buildup along the sides of the flasks. After 7 days, the flasks were examined for contamination and the culture medium characterized by color, amount of mycelium produced (sparse to abundant), presence or absence of microsclerotia (melanized survival structures), chlamydospores, and conidia (thin walled asexual spores). Contents of the flask were ground in a blender for 30 seconds to homogenize the culture. The resulting slurry was pipetted in 1 ml aliquots into 250 ml Erlenmeyer flasks containing 100 ml deionized water and a hydrilla apical shoot 15 cm in length. Each treatment was replicated 5 times. The flasks were incubated in a growth chamber (Conviron, Pembina, ND) at 25 °C under a 12/12 light dark photoperiod for 14 days. The hydrilla shoots were visibly rated for presence/absence of disease on a rating scale of 0-4 (0 = no disease, tissues green and healthy; 1 = slight chlorosis; 2 = general overall

chlorosis; 3 = tissues discolored and stems beginning to fragment; 4 = total discoloration and tissues collapsed, no possibility of regrowth).

**RESULTS AND DISCUSSION:** A total of 92 strains of fungi were screened for efficacy on hydrilla sprigs in the flask study. Of these, 27% could not be identified because they did not sporulate on PDA or PCA; thus, they were designated as Dematiaceous Ascomycetes or Moniliaceous Ascomycetes (Table 1). Of the strains that were tested, 83% caused no damage (0 disease rating), 14% elicited a disease rating of less than 3, and 3% elicited a disease rating greater or equal to 3. In general, any strain eliciting a disease rating less than 3 would not be considered a good candidate for pathogen biocontrol because hydrilla damage was not severe enough to prevent rapid regrowth. Only three fungal strains, *Myrothecium verrucaria*, *Myrothecium roridum*, and *Mycoleptodiscus terrestris* had mean disease ratings of 4, 3.8, and 3, respectively, and have potential for further development as biocontrol agents. None of the unidentified strains of non-sporulating Ascomycetes induced disease.

<b>Table 1. Collection number and disease ratings of pathogens isolated from hydrilla in 2009/2010.</b>		
<b>Isolate #</b>	<b>Mean disease rating</b>	<b>Species</b>
000309	0	<i>Colletotrichum gloeosporioides</i>
000409	0	<i>Phoma</i> sp.
000509	0	<i>Colletotrichum gloeosporioides</i>
001009	0	<i>Phialophora</i> sp.
001109	0	<i>Phoma</i> sp.
001209	0	Dematiaceous Ascomycete
001309	0	Dematiaceous Ascomycete
011909	0	<i>Isaria</i> sp.
012009	0	<i>Microsphaeropsis olivacea</i>
012109	0	Moniliaceous Ascomycete
012209	0	<i>Nodulisporium</i> sp.
012309	0	<i>Myrothecium roridum</i>
012409	0	Moniliaceous Ascomycete
012509	0	<i>Pestalotiopsis guepinii</i>
018809	0	Hyphal <i>Mycoleptodiscus terrestris</i>
019009	0	<i>Hansfordia</i> sp.
019109	0	Dematiaceous Ascomycete
019209	0	<i>Virgaria nigra</i>
019409	0	Moniliaceous Ascomycete
019509	0	<i>Pestalotiopsis guepinii</i>
019609	0	<i>Scopulariopsis koningii</i>
019809	0	<i>Khuskia oryzae</i>
019909	0	<i>Hansfordia</i> sp.
020009	0	<i>Isaria</i> sp.
020109	0	<i>Macrophoma</i> sp.
020209	0	<i>Plectosphaerella cucumerina</i>
044809	0	<i>Pestalotiopsis guepinii</i>
044909	0.4	<i>Calcarisporium</i> sp.
045109	0	<i>Isaria</i> sp.
045309	0	Dematiaceous Ascomycete

045409	0	<i>Calcarisporium</i> sp.
045509	0.8	<i>Colletotrichum lindemuthianum</i>
045609	0.8	<i>Khuskia oryzae</i>
045709	0	<i>Phoma</i> sp.
045809	1.2	Dematiaceous Ascomycete
045909	0	<i>Acremonium</i> sp.
046109	1	<i>Fusarium stillboides</i>
046209	0.4	<i>Acremonium</i> sp.
046309	0	Dematiaceous Ascomycete
046409	0	<i>Alternaria alternata</i>
002310	0.2	<i>Coniella petrackii</i>
004410	0.2	<i>Microsphaeropsis olivacea</i>
004810	0	<i>Scopulariopsis</i> sp.
004910	0	<i>Pseudeurotium</i> sp.
005110	0	<i>Isaria</i> sp.
005210	0.2	<i>Sclerotium rolfsii</i>
005310	0	<i>Hansfordia ovulispora</i>
005410	0	<i>Phoma</i> sp.
005510	0	<i>Pestalotiopsis guepinii</i>
005610	0	<i>Emericellopsis minima</i>
005810	4	<i>Myrothecium verrucaria</i>
005910	0	Dematiaceous Ascomycete
006010	0	Dematiaceous Ascomycete
006210	0	Dematiaceous Ascomycete
006510	0	<i>Phoma</i> sp.
006610	0	Dematiaceous Ascomycete
006910	0	<i>Plectosphaerella cucumerina</i>
013810	0	Dematiaceous Ascomycete
013910	0	Dematiaceous Ascomycete
014110	0	<i>Melanospora</i> sp.
014210	0	Moniliaceous Ascomycete
014610	0	Moniliaceous Ascomycete
014910	0	Dematiaceous Ascomycete
015110	0	Dematiaceous Ascomycete
015910	0	<i>Myrothecium roridum</i>
016010	0	Dematiaceous Ascomycete
016110	0	<i>Phoma</i> sp.
016210	0	<i>Fusarium equiseti</i>
016610	0	Moniliaceous Ascomycete
019010	0	<i>Isaria</i> sp.
019110	0	<i>Chrysosporium</i> sp.
019210	0	Dematiaceous Ascomycete
019510	0	<i>Pestalotiopsis guepinii</i>
019610	0	<i>Isaria</i> sp.
019710	0	Moniliaceous Ascomycete
019810	2	<i>Mycocleptodiscus terrestris</i>
020010	3	<i>Mycocleptodiscus terrestris</i>
020110	0	Moniliaceous Ascomycete

020410	2.8	<i>Pestalotiopsis guepinii</i>
020510	0	<i>Cladosporium cladosporioides</i>
020610	0	<i>Pestalotiopsis guepinii</i>
020710	0	<i>Pestalotiopsis guepinii</i>
020810	0	Moniliaceous Ascomycete
020910	0	<i>Acremonium</i> sp.
021010	0	<i>Pestalotiopsis guepinii</i>
021110	0	<i>Colletotrichum gloeosporioides</i>
021210	0	<i>Phoma levelii</i>
021310	3.8	<i>Myrothecium roridum</i>
021410	0	<i>Phialophora</i> sp.
021510	0	<i>Trichoderma pseudokoningii</i>
021610	2.6	<i>Drechslera erythrospila</i>
021710	2	<i>Mycocleptodiscus terrestris</i>

*Mycocleptodiscus terrestris* has been studied since the late 1980s as a candidate for inundative biocontrol of hydrilla (Joye 1990, Joye and Cofrancesco 1991, Shearer 1993, 1996, 1997, 1998, 2009a, 2009b, Shearer and Jackson 2006). The fungus has also been used with herbicides in an integrated approach to hydrilla management (Nelson and Shearer 2009, Nelson et al. 1998, Netherland and Shearer 1996, Shearer and Nelson 2002, 2009). Applied together in low doses, the combinations were more efficacious than either herbicide or fungus applied alone. In the present study, the four strains of *M. terrestris* varied in pathogenicity. The strictly hyphal strain did not cause any damage to hydrilla; whereas the other strains all produced microsclerotia in broth culture and caused chlorosis or fragmentation of stems, but no total collapse of shoot tissues. None of the strains outperformed the strain currently in use for hydrilla management.

Both *Myrothecium* species are cosmopolitan and have been reported on a variety of hosts (Farr et al. 1980). *Myrothecium verrucaria* has been researched as a biocontrol agent for management of kudzu (*Pueraria montana* (Lour.) var *lobata* (Willd.) Maeson & Sl. Almeida) (Boyette et al. 2002, 2008a 2008b, Hoagland et al. 2007), redvine (*Brunnichia ovata* (Walter) Shinnery), and trumpetcreeper (*Campsis radicans* (L.) Seem. ex Bureau) in the United States (Boyette et al. 2008b). *Myrothecium roridum* has been researched as a potential biocontrol agent for water hyacinth (*Eichhornia crassipes* (Mart.) Solms) in Africa (Okunowo et al. 2010). Both *M. verrucaria* and *M. roridum* caused severe damage to hydrilla in this study, and both will potentially make good pathogen biocontrol agents. Neither isolate produced spores in the Richard's V-8 juice broth medium. Enhancing their performance as biocontrol agents may require the development of a liquid medium that induces sporulation in broth culture. Disease of hydrilla caused by the two *Myrothecium*s in this study could very likely result from the release of trichothecenes, mycotoxins that are produced as secondary metabolites in the two strains (Agrios 2005).

*Plectosporium tabacinum* the anamorph of *Plectosphaerella cucumerina* has been studied as a potential biological control agent for hydrilla (Smither-Kopperl et al. 1999). It can colonize its host as a saprophyte and under certain environmental conditions (particularly those having to do with water quality) can become pathogenic and cause disease. However, the two strains that were isolated in this study caused no damage to hydrilla and would not be considered as potential agents. The strain that was isolated in Florida that showed some promise as a biocontrol agent (Smither-Kopperl et al. 1999) was never developed and sold commercially.

**FUTURE WORK:** The fungal strains that caused minimal damage to hydrilla in the flask study will not undergo further testing as biocontrol agents. The strains that induced a disease rating of 3 or greater will be re-evaluated on hydrilla springs in a flask test and evaluated on a larger scale in aquariums with planted hydrilla.

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## REFERENCES

- Agrios, G. N. 2005. *Plant Pathology* 5<sup>th</sup> ed. Burlington, MA: Elsevier Academic Press.
- Boyette C. D., H. L. Walker, and H. K. Abbas. 2002. Biological control of kudzu (*Pueraria lobata*) with an isolate of *Myrothecium verrucaria*. *Biocontrol Science and Technology* 12:75-82.
- Boyette, C. D., M. A. Weaver, R. E. Hoagland, and K. C. Stetina. 2008a. Submerged culture of a mycelial formulation of a bioherbicidal strain of *Myrothecium verrucaria* with mitigated mycotoxin production. *World J. Microbiol Biotechnol.* 24:2721-2726.
- Boyette, C. D., R. E. Hoagland, M. A. Weaver, and K. N. Reddy. 2008b. Redvine (*Brunnichia ovata*) and trumpetcreeper (*Campsis radicans*) controlled under field conditions by a synergistic interaction of the bioherbicide, *Myrothecium verrucaria*, with glyphosate. *Weed Biology and Management* 8:39-45
- Charudattan, R., T. E. Freeman, R. E. Cullen, and F. M. Hofmeister. 1980. Evaluation of *Fusarium roseum* 'Culmorum' as a biological control for *Hydrilla verticillata*: Safety. In *Proceedings of the Vth International Symposium on Biological Control of Weeds, July 1980, Brisbane, Australia*, ed. E. S. Delfosse., 3307-323, Melbourne, Australia: Commonwealth Scientific and Industrial Research Organisation.
- Dhingra O. E., and J. F. Sinclair. 1995. *Basic plant pathology methods*. CRC Press, Inc., Boca Raton, FL.
- Farr, D. F., G. F. Bills, G. P. Chamuris, and A. Y. Rossman. 1980. *Fungi on plants and plant products in the United States*. St. Paul, MN: APS Press.
- Hoagland, R. E., C. D. Boyette, and H. K. Abbas. 2007. *Myrothecium verrucaria* isolates and formulations as bioherbicide agents for kudzu. *Biocontrol Science and Technology* 17:721-731.
- Joye, G. F. 1990. Biocontrol of hydrilla with the endemic fungus *Macrophomina phaseolina*. *Plant Dis.* 74:1035-1036.
- Joye, G. F., and A. F. Cofrancesco. 1991. *Studies on the use of fungal plant pathogens for control of Hydrilla verticillata (L.f.) Royle*. Technical Report A-91-4. Vicksburg, MS: U.S. Army Engineer Research and Development Center.
- Netherland, M. D., and J. F. Shearer. 1996. Integrated use of fluridone and a fungal pathogen for control of hydrilla. *J. Aquat. Plant Manage.* 33-4-8.

- Nelson, L. S., and J. F. Shearer. 2009. *Integrated weed management strategies for control of hydrilla*. APCRP Technical Notes Collection. ERDC/TN APCRP-CC-09/ Vicksburg, MS: U.S. Army Engineer Research and Development Center.
- Nelson, L. S., J. F. Shearer, and M. D. Netherland. 1998. Mesocosm evaluation of integrated fluridone-fungal pathogen treatment of four submersed plants. *J. Aquat. Plant Manage.* 36:73-77.
- Okunowo, W. O., G. O. Gbenle, A. A. Osuntoki, and A. A. Adekunle. 2010. Media studies on *Myrothecium roridum* Tode: A potential biocontrol agent for water hyacinth. *Journal of Yeast and Fungal Research* 1:55-61.
- Schmitz, D. C., B. V. Nelson, L. E. Nall, and J. D. Schardt. 1991. Exotic aquatic plants in Florida: A historical perspective and review of the present aquatic plant regulation program. In *Proceedings of the symposium of exotic pest plants: November 2-4, 1988. University of Miami, Rosentiel School of Marine and Atmospheric Science, Miami, Florida*, ed. T. D. Center et al., 303-323, Washington DC: U.S. Department of Interior, National Park Service Document.
- Shearer, J. F. 1993. Biocontrol of hydrilla and milfoil using plant pathogens. In *Proceedings of the 27<sup>th</sup> Annual Meeting of the Aquatic Plant Control Research Program*. Misc Pap. A-93-2. Vicksburg, MS: U.S. Army Environmental Laboratory.
- Shearer, J. F. 1996. Field and laboratory studies of the fungus *Mycocleptodiscus terrestris* as a potential agent for management of the submersed aquatic macrophyte *Hydrilla verticillata*. Technical Report A-96-3. Vicksburg MS: U.S. Army Engineer Research and Development Center.
- Shearer, J. F. 1997. *Endemic pathogen biocontrol research on submersed macrophytes: Status report 1996*. APCRP Technical Report A-97-3. Vicksburg, MS: US Army Engineer Waterways Experiment Station.
- Shearer, J. F. 1998. Biological control of hydrilla using an endemic fungal pathogen. *J. Aquat. Plant Manage.* 36:54-56.
- Shearer, J. F. 2009a. *Preliminary testing of Mycoleptodiscus terrestris formulations*. APCRP Technical Notes Collection. ERDC/TNAPCRP-BC-10. Vicksburg MS: U.S. Army Engineer Research and Development Center.
- Shearer, J. F. 2009b. *Storage stability of dried microsclerotia of the biological pathogen Mycoleptodiscus terrestris*. APCRP Technical Notes Collection. ERDC/TNAPCRP-BC-13. Vicksburg MS: U.S. Army Engineer Research and Development Center.
- Shearer, J. F., and M. A. Jackson. 2006. Liquid culturing of microsclerotia of *Mycocleptodiscus terrestris*, a potential biological control agent for the management of hydrilla. *Biol. Control* 38:298-306.
- Shearer, J. F., and L. S. Nelson. 2002. Integrated use of endothall and a fungal pathogen for management of the submersed macrophyte *Hydrilla verticillata* (L.f.) Royle. *Weed Technol.* 16:224-230.
- Shearer, J. F., and L. S. Nelson. 2009. *Combining ALS-inhibiting herbicides with the fungal pathogen Mycoleptodiscus terrestris for control of hydrilla*. APCRP Technical Notes Collection. ERDC/TN APCRP-CC-11. Vicksburg, MS: U.S. Army Engineer Research and Development Center.
- Smither-Kopperl, M. L., R. Charudattan, and R. D. Berger. 1999. *Plectosporium tabacinum*, a pathogen of the invasive aquatic weed *Hydrilla verticillata* in Florida. *Plant Dis.* 83:24-28.

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