ERDC/TN APCRP-BC-37
April 2016

Screening of Pathogenic Agents for Efficacy on Monoecious Hydrilla 2015

by Judy F. Shearer

PURPOSE: This technical note describes the results of screening plant pathogenic fungi for use as potential biological control agents for management of monoecious hydrilla.

INTRODUCTION: In the United States, monoecious hydrilla (*Hydrilla verticillata* (L.f.) Royle) was first reported in Delaware in 1976 (Haller 1982, Steward et al. 1984). It was initially misidentified as *Elodea canadensis* Mich. (Steward et al. 1984). The plant was subsequently found in the reflecting pool on the National Mall in 1980 (Haller 1982; Fincham 2009). In 1982, additional surveying near Washington, DC uncovered three additional sites, including a cove on the west bank of the Potomac River near Alexandria, VA; an abandoned section of the Chesapeake and Ohio Canal; and an area in Kenilworth Aquatic Gardens (Steward et al. 1984). Collections from Delaware, Lilypons Gardens north of Frederick, MD, and collections from Kenilworth Aquatic Gardens were sent to Ft. Lauderdale, Florida United States Department of Agriculture (USDA) Aquatic Weed Lab for culture and examination, where they were positively determined to be monoecious hydrilla after male and female flowers developed on the same plant (Steward et al. 1984).

In the 1980s, monoecious hydrilla was also discovered in North Carolina (Langeland and Schiller 1983). It too was initially misidentified as an *Elodea* spp. By 1992, monoecious hydrilla was found in Lake Gaston on the North Carolina/Virginia border growing in association with the dioecious female biotype (Ryan et al. 1995). The two biotypes were also thought to coexist in Strom Thurmond Reservoir, SC, and in Lake Guntersville, AL. Recent collections that were analyzed using genetic markers indicated that the populations in Strom Thurmond were all monoecious and those found in Lake Guntersville were mixed populations of monoecious and dioecious hydrilla. Madeira et al. (2004) reported that monoecious infestations were distributed throughout the Atlantic states and northward to Maine. Populations have also been reported in the Midwest and the Northwest. Those in Iowa, Wisconsin, and Washington have since been eradicated.

In 2003, Peterson et al. used Genetic Algorithm for Rule Set Prediction (GARP) analysis to predict the potential distribution of four invasive plant species in North America. Hydrilla was predicted to extend from the Atlantic Coast west to California and north to Washington, Kansas, Missouri, Illinois, Indiana, West Virginia, New York and east to southern New England. Balciunas and Chen (1993), during surveys for biological control agents in China supplemented by herbarium specimens and literature citations plus climatic data, indicated that hydrilla had the potential to grow in aquatic habitats throughout North America, including in Canada and Alaska. These predictions are increasingly becoming a reality. The 2011 United States Geologic Survey (USGS) map (Figure 1) shows the widespread distribution of hydrilla across watersheds in the United States (USGS 2015). More recently, the New York Department of Environmental Conservation (DEC) has published

several announcements about monoecious hydrilla being discovered in Cayuga Lake, Erie Canal, at North Tonawonda in upstate New York, and in the Croton River near New York City (DEC 2015).

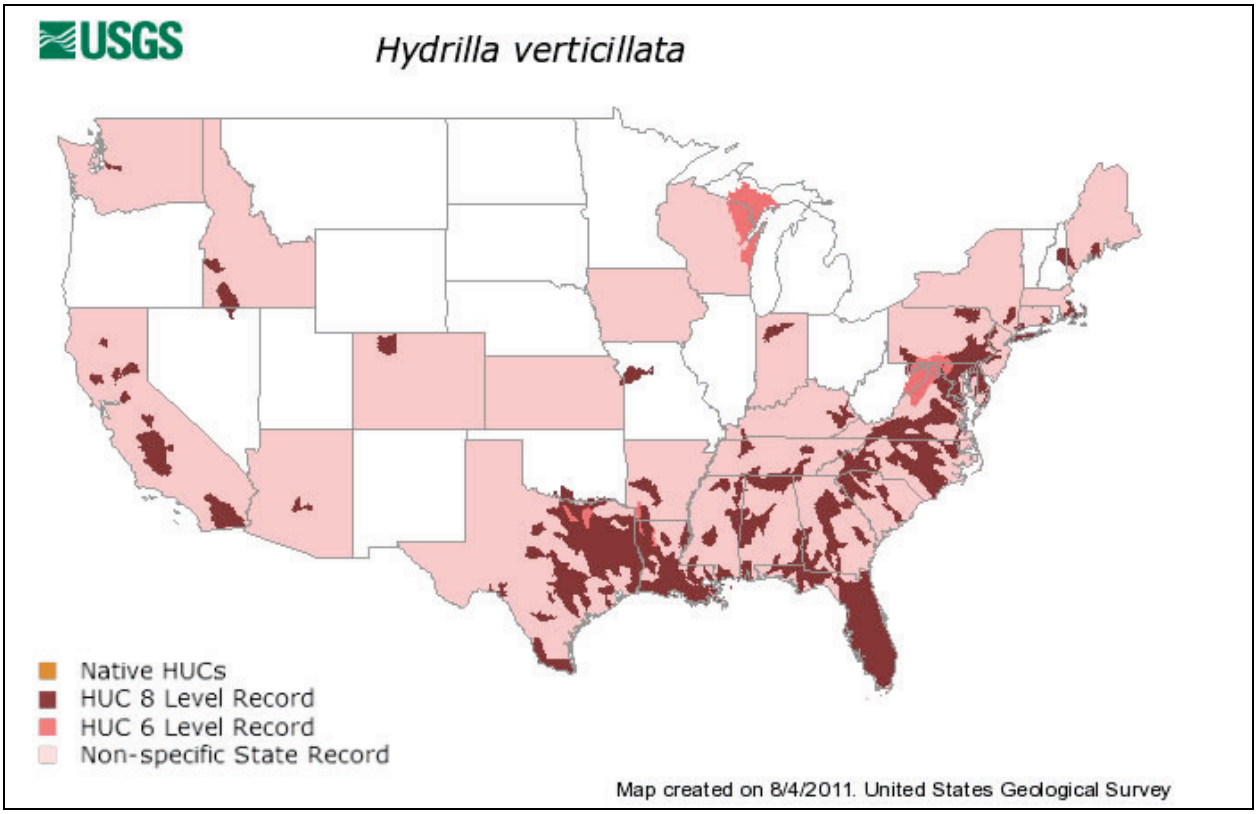

Figure 1. Distribution of hydrilla in watersheds in the lower 48 states.

Monoecious hydrilla is reported to be an extremely tolerant and competitive plant. It is almost impossible to eradicate because it produces both axillary turions and subterranean turions (tubers) in great numbers. Nawrocki (2011) found densities of over 3000 tubers per m^2 in North Carolina. They can remain viable in undisturbed soil up to four years (Van and Steward 1990) and sometimes longer. In laboratory trials, tubers had a very high germination rate, often over 90% (Harlan et al. 1985, Van and Steward 1990). Once sprouted, monoecious hydrilla tubers send out shoots laterally rather than vertically, as observed with dioecious tubers (Van 1989). The plant has sometimes been called an herbaceous perennial (Richardson 2013), but its phenology is much more like an annual in that maximum growth occurs in summer; in winter, the soft aboveground biomass dies back to the sediment. Regrowth in the spring is entirely dependent on turion and tuber germination (Harlan et al. 1985, Sutton et al. 1992).

A variety of methods are available for management of monoecious hydrilla, with registered aquatic herbicides being the most widely used. Grass carp (*Ctenopharyngodon idella*) are a potential biological control agent as a non-specific feeder of aquatic plants and have been observed feeding on

monoecious hydrilla.[1] Grass carp have been released in Lake Gaston along the North Carolina/Virginia border where they have tried to maintain target population densities of 3.2 to 20.5 fish per hydrilla ha^{-1}; however, there has been little evidence that grass carp have contributed to hydrilla control in the lake. While the ephydrid fly *Hydrellia pakistanae*, a biocontrol insect, has successfully established populations on the dioecious hydrilla biotype, there are no published records of establishment on the monoecious hydrilla biotype even after concerted release efforts. Both greenhouse and outdoor-pond studies have documented that *Hydrellia* flies have reduced survival rates and longer developmental times on monoecious than dioecious hydrilla (Grodowitz et al. 2010). In north Texas, *Hydrellia* flies survive and overwinter as larval stages in stems of dioecious hydrilla (Harms and Grodowitz 2011). This overwintering survival mechanism may preclude fly establishment on monoecious hydrilla populations because the plant survives as subterranean tubers and turions (Steward and Van 1987) and not as aboveground biomass.

Several pathogens have been researched as potential biological control agents for management of dioecious hydrilla, including *Mycoleptodiscus terrestris* (Joye and Cofrancesco 1991; Joye 1990; Joye and Paul 1991; Nelson et al. 1998; Nelson and Shearer 2009; Netherland and Shearer 1996; Shearer 1998; Shearer 2009a, 2009b; Shearer and Nelson 2002; Shearer and Jackson 2006), *Fusarium culmorum* (Charudattan et al. 1984), and *Plectosporium tabacinum* (Smither-Kopperl et al. 1999). Isolations from monoecious hydrilla in 2009 and 2010 have yielded at least three strains of *Myrothecium roridum*, which have proved to be efficacious on monoecious hydrilla in small flask studies. Additional isolates would increase the number of potential agents that would be available for management purposes against hydrilla and other aquatic and riparian nuisance plant species.

MATERIALS AND METHODS: During the summer/fall 2014, monoecious hydrilla was collected from Cayuga Lake and Erie Canal in New York, Strom Thurmond Reservoir in Georgia/South Carolina, Lake Guntersville in Alabama, the Potomac River in Virginia, and from several culture tanks in Gainesville, Florida. Isolations were made for pathogenic fungi that might be efficacious on the target host. The isolates were identified and stored on Potato Dextrose Agar (PDA) and ½ strength Corn Meal Agar (CM) (Difco Inc., Detroit, MI) slants at 4˚ C in a refrigeration unit. A total of 94 potential pathogenic agents were selected for screening against monoecious hydrilla.

Each week, 10 isolates were retrieved from storage and plated onto PDA petri plates. The cultures were allowed to grow at 25˚ C on a lab bench for one to three weeks until they approximately filled the 15 cm diameter plate. About ½ the culture on each plate was chopped into $1mm^2$ pieces with a sterile scalpel and added to a 250 ml baffled flask containing 100 ml Richard's V-8 juice broth (10 g dextrose, 10 g KNO_3, 3 g $CaCO_3$, 200 ml V-8 juice (Campbell's, Camden, NJ) 800 ml H_2O per L). The cultures were placed on a platform rotary shaker (Innova Eppendorf, Inc. Hauppaige. NY) set to 200 rpms. The cultures were hand shaken daily to prevent fungal build-up on the walls of the flasks. After one week, the fungal slurry in each flask was examined microscopically to ensure the cultures had not become contaminated. The entire contents of the flask were macerated in a sterile blender for 30 sec. Two hundred fifty-milliliter Erlenmeyer flasks containing 100 ml of H_2O and a 15 cm apical shoot of monoecious hydrilla that had been rinsed with deionized (DI) water were inoculated with 1 ml of fungal slurry. Each treatment was replicated five times.

[1] Personal Communication. 2015. Gary O. Dick, Research Biologist, U.S. Army Engineer Research and Development Center, Lewisville, TX

The flasks were placed in a growth chamber (Conviron, Pembina, ND) set to 25˚ C and a 12-hour light/dark photoperiod for 2 weeks. Fungal efficacy was determined visually by rating plant disease on a scale of 0 to 4 (0 = no disease, tissues green and healthy; 1 = slight chlorosis; 2 = overall general chlorosis; 3 = tissues flaccid and fragmenting; 4 = tissues collapsed with no chance of recovery) (Shearer 1999, Shearer 2012, Shearer 2013).

RESULTS AND DISCUSSION: Eighty-six out of the 94 isolates tested for efficacy on monoecious hydrilla were non-pathogenic and were rated zero on the disease rating scale. Only one isolate, *Myrothecium roridum*, was given a disease rating of four (Table 1). Two weeks after inoculation with the pathogen, the monoecious hydrilla sprig tissues had lost all integrity and had collapsed into a decomposing mass in the bottom of the flask with no possibility of regrowth. The other seven species did not have disease ratings high enough to be considered biological control agents for monoecious hydrilla. A minimum rating of three would be necessary to be considered a potential agent (Shearer 2012).

Table 1. Monoecious hydrilla disease response to inoculation with eight potential pathogenic agents.

Species	Rating
Phoma sp.	2.4
Phialophora sp.	1.0
Sporobolmyces sp.	0.8
Pestalotiopsis guepinii	0.6
Mucor sp.	1.8
Myrothecium verrucaria	2.0
Phoma sp. 2	0.4
Myrothecium roridum	4.0

Myrothecium roridum has been suggested as a possible mycoherbicidal agent for control of several weedy species. Used alone, the fungus has been documented to impact *Eichhornia crassipes* (Mart.) Solms. (waterhyacinth) in India (Ponnappa 1970) and in Sri Lanka (Hettiarachchi et al. 1983) by causing a leaf spot disease. The isolate from India was highly pathogenic, producing symptoms within 18 to 48 hours, and under highly humid conditions it could kill the host (Ponnappa 1970). In studies in Africa, Okunowo et al. (2008) identified *M. roridum* as a pathogen having the level of virulence to make it a potential mycoherbicide for waterhyacinth management. Liyanage and Gunasekera (1989) combined *M. roridum* with 2,4-D for waterhyacinth management. They found that integrating the treatments using low doses of the fungus and the herbicide were significantly better than either treatment used alone.

Walker and Tilley (1997) suggested that some *Myrothecium* species might be developed as excellent broad-spectrum bioherbicides for a number of weedy species. Lee et al. (2008) tested *M. roridum* broth culture against seven weedy host species. The results indicated that the fungus could be used as a broad-spectrum mycoherbicide either as a seed germination inhibitor or to control weedy species in field crops in Korea.

FUTURE WORK: Strains of *Myrothecium roridum* will be evaluated against monoecious hydrilla in a larger scale aquarium study. They will also be tested on selected nuisance riparian and aquatic species to determine the utility of *M. roridum* as a broad-spectrum mycoherbicidal agent.

ACKNOWLEDGEMENTS: Support for this project was provided by the Aquatic Plant Control Research Program (APCRP).

POINTS OF CONTACT: For additional information contact the author, Dr. Judy F. Shearer, (601) 634-2516, *Judy.F.Shearer@usace. army.mil* or the APCRP Program Manager, Dr. Linda S. Nelson, (601) 634-2656, *Linda.S.Nelson@usace.army.mil*. This technical note should be cited as follows.

> Shearer, J. F. 2016. *Surveys for pathogens of monoecious hydrilla in 2015*. APCRP Technical Notes Collection. ERDC/TN APCRP-BC-37. Vicksburg, MS: U.S. Army Engineer Research and Development Center. *http://ed.eerdc.usace.army.mil/aqua/*

REFERENCES

Balciunas, J. K., and P. P. Chen. 1993. Distribution of hydrilla in Northern China: Implications on future spread in North America. *J. Aquat. Plant Manage*. 31:105-109.

Charudattan, R., T. E. Freeman, R. E. Cullen, and F. M. Hofmeister. 1984. *Evaluation of* Fusarium roseum 'Culmorum*' as a biological control agent for* Hydrilla verticillata. Technical Report A-84-5. Vicksburg, MS: U.S. Army Engineers Waterways Experiment Station.

Fincham. M. W. 2009. Travels with hydrilla. The unnatural history of an accidental invader. *Chesapeake Quarterly* 8:1-5.

Grodowitz, M., J. Nachtrieb, N. Harms, and J. Freedman. 2010. *Suitability of using introduced* Hydrellia *spp. for management of monoecious* Hydrilla verticillata *(L.f.) Royle*. APCRP Technical Notes Collection. ERDC/TN APCRP-BC-17. Vicksburg MS: U.S. Army Engineer Research and Development Center.

Haller, W. T. 1982. Hydrilla goes to Washington. *Aquatics* 4:6-7.

Harlan, S. M., G. J. Davis, and G. J. Pesacreta. 1985. Hydrilla in three North Carolina lakes. *J. Aquat. Plant Manage*. 23:68-71.

Harms, N. E. and M. J. Grodowitz. 2011. Overwintering biology of *Hydrellia pakistanae*, a biological control agent of hydrilla. *J. Aquat. Plant Manage*. 49:114-117.

Hettiarachchi, S., S. A. Gunasekera, and I. Balasooriya. 1983. Leaf sport diseases of waterhyacinth in Sri Lanka. *J. Aquat. Plant Manage*. 21:62-63.

Joye, G. F. 1990. Biocontrol of *Hydrilla verticillata* 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.

Joye, G. F., and R. N. Paul. 1991. Histology of infection of *Hydrilla verticillata* by *Macrophomina phaseolina*. *Weed Sci.* 40:288-295.

Langeland, K. A., and D. Schiller. 1983. Hydrilla in North Carolina. A successful program begins. *Aquatics* 5:8-14.

Lee, H. B., C. J. Kim, K. S. Hong, and J. C. Kim. 2008. Evaluation of a fungal Strain, Myrothecium roridum F0252, as a bioherbicide agent. *Plant Pathol. J*. 24:453-460.

Liyanage, N. P., and S. A. Gunasekera. 1989. Integration of *Myrothecium roridum* and 2,4-D in waterhyacinth management. *J. Aquat. Plant Manage*. 27:15-20.

Madeira, P. T., T. K. Van, and T. D. Center. 2004. An improved molecular tool for distinguishing monoecious and dioecious hydrilla. *J. Aquat. Plant Manage*. 42:28-32.

Nawrocki, J. J. 2011. Environmental and physiological factors affecting submersed aquatic weed management (Masters Thesis). North Carolina State University. Available online *http://repository .lib.ncsu.edu/ir/handle/1840.16/7126.Nel*.

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.

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

New York Department of Environmental Conservation (DEC). 2015. Hydrilla publications. http://search.usa.gov/search?affiliate=nysdec&query=hydrilla&Search.x=0&Search.y=0 Accessed 11/17/2015.

Okunowo, W. O., G. O. Gbenle, A. A. Osuntoki, and A. A. Adekunie. 2008. Survey evaluation and molecular characterization of Nigerian native fungus for potential biocontrol of water hyacinth. *Phytopathology* 98: S115.

Ponnappa. K. M. 1970. On the pathogenicity of Myrothecium roridum – Eichhornia crassipes isolate. *Hyacinth Control Journal/J. Aquat. Plant Manage*. 8B:18-20.

Peterson, A. T., M. Papes, and D. A. Kluza. 2003. Predicting the potential invasive distributions of four alien plant species in North America. *Weed Science* 51:863-868.

Richardson, R. J. 2013. Monoecious hydrilla – a review of the literature. *www.nyis.info/user_uploads/files/Monoecious%20Hydrilla%20Lit%20Review%20-%20Final.pdf*. Accessed 11/11/15.

Ryan, F. J, C. R. Coley, and S. H. Kay. 1995. Coexistence of monoecious and dioecious hydrilla in Lake Gaston, North Carolina and Virginia. *J. Aquat Plant Manage*. 33:8-12.

Shearer, J. F. 1998. Biological control of hydrilla using an endemic fungal pathogen. *J. Aquat. Plant Manage*. 36:54-56.

Shearer, J. F. 1999. *Controlling hydrilla and Eurasian watermilfoil with fungal pathogens from the People's Republic of China*. APCRP Techical Notes Collection. ERDC/TN APCRP-BC-01. Vicksburg, MS: U.S. Army Engineer Research and Development Center.

Shearer, J. F. 2009a. *Preliminary testing of* Mycoleptodiscus terrestris *formulations*. APCRP Technical Notes Collection. ERDC/TN APCRP-BC-10. Vicksburg MS: U.S. Army Engineer Research and Development Center.

Shearer, J. F. 2009b. *Storage stability of dried microsclerotia of the biological control pathogen* Mycoleptodiscus terrestris. APCRP Technical Notes Collection. ERDC/TN APCRP-BC-13. Vicksburg MS: U.S. Army Engineer Research and Development Center.

Shearer, J. F. 2012. *Screening of pathogens as potential biological control agents for management of* Hydrilla verticillata. APCRP Technical Notes Collection. ERDC/TN APCRP-BC-27. Vicksburg, MS: U.S. Army Engineer Research and Development Center. *http://el.erdc.usace.army.mil/elpubs/pdf/tnel12-1.pdf*

Shearer, J. F. 2013. *Evaluation of a new biological control pathogen for management of Eurasian watermilfoil*. APCRP Technical Notes Collection. ERDC/TN APCRP-BC-30. Vicksburg MS: U.S. Army Engineer Research and Development Center.

Shearer, J. F., and M. A. Jackson. 2006. Liquid culturing of microsclerotia of *Mycoleptodiscus 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 aquatic macrophyte *Hydrilla verticillata*. *Weed Technol*. 16:224-230.

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.

Steward, K. K., T. K. Van, V. Carter, and A. H. Pieterse. 1984. Hydrilla invades Washington, D.C. and the Potomac. *Amer. J. Bot*. 71:162-163.

Steward, K. K., and T. K. Van. 1987. Comparative studies of monoecious and dioecious hydrilla (*Hydrilla verticillata*) biotypes. *Weed Sci*. 35:201-210.

Sutton, D. L., T. K. Van, and K. M. Portier. 1992. Growth of dioecious and monoecious hydrilla from single tubers. *J. Aquat Plant Manage*. 30:15-20.

United States Geologic Service (USGS). 2015. Hydrilla distribution Huc map. *http://www.bing.com/images/search?q=hydrilla+distribution+map&view=detailv2&&id=7C3498E768399B4EABD71AC8831F674D983D044F&selectedIndex=0&ccid=VTQSRLwC&simid=607991297723862779&thid=OIP.M55341244bc022f10677e2b6c71fd601co0&ajaxhist=0* Accessed 11/17/2015

Van, T. K., 1989. Differential responses to photoperiods in monoecious and dioecious *Hydrilla verticillata*. *Weed Science* 37:552-556.

Van, T. K., and K. K. Steward. 1990. Longevity of monoecious hydrilla propagules. *J. Aquat Plant Manage*. 28:74-76.

Walker, H. L., and A. M. Tilley. 1997. Evaluation of an isolate of *Myrothecium verrucaria* from sicklepos (*Senna obtusifolia*) as a potential mycoherbicide agent. *Biol. Control* 10:104-112.