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Surveys for Pathogens of Monoecious Hydrilla

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PURPOSE: This technical note describes the results of surveys for pathogenic agents on monoecious hydrilla.

INTRODUCTION: There are two biotypes of *Hydrilla verticillata* (L.f.) Royle (hydrilla) in the United States. The pistillate dioecious hydrilla biotype was introduced from Sri Lanka into Florida in the 1950's (Schmitz et al. 1990). It has spread throughout the Southeast United States, as far west as Texas and into parts of California (Madeira et al. 2004). Monoecious hydrilla was first discovered in Delaware in 1976 and later in the Potomac River (Haller 1982, Steward et al. 1984). It has now expanded its distribution through the Atlantic States and northward to Maine (Madeira et al. 2004). Separate populations have been reported in Iowa, Ohio, Indiana, Wisconsin, Kansas, Missouri, California, and Washington State (Nonindigenous Aquatic Species (NAS) 2011). The Washington State population no longer exists due to an aggressive eradication program.[1] It is believed that populations in Iowa and Wisconsin have also been eradicated.[2] The most recent invasions have appeared in Lake Cuyuga and the Erie Canal at North Tonawonda, both in upstate New York (Cornell Cooperative Extension (CCC) 2011, Lansing Star 2012). Shortly after its discovery in 1982, Steward et al. (1984) predicted that monoecious hydrilla had the potential to invade all of the lower 48 states and southern and central Canada. Although not known to exist in Minnesota, Maki and Galatowitsch (2008) ran a CLIMEX model that indicated the state was at risk for invasion of monoecious hydrilla.

The growth forms of dioecious and monoecious hydrilla biotypes are very different. Compared to the monoecious biotype, dioecious plants tend to have more vigorous growth, extending vertically to the water surface and then spreading laterally and forming a mat (Van 1989). Madeira et al. (1997) hypothesized that this growth form was an adaptation to deep water generated from monsoons on the Indian subcontinent. The vegetative propagules (i.e. tubers of the dioecious biotype) are larger than those of the monoecious biotype and are formed under short-day conditions (Van 1989). In contrast, the tubers of the monoecious biotype are produced under long-day photoperiods and are smaller. When they germinate, the stems tend to grow laterally, generating new root crowns along the sediment surface, which results in high shoot densities (Van 1989). When the monoecious hydrilla mat declines in the fall, it breaks loose and fragments containing numerous axillary propagules, i.e. turions, drift in the water currents dispersing the plant (Steward and Van 1987). Madeira et al. (1997) hypothesized

[1]Personal Communication. 2012. J. Parsons, Aquatic Plant Specialist, State of Washington Department of Ecology, Olympia, WA.
[2] Personal Communication. 2013. Michael Netherland, Research Biologist, US Army Engineer Research and Development Center, Vicksburg, MS.

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that this growth habit suggested a temperate origin of the plant that was consistent with its probable Korean origin.

While dioecious hydrilla has been surveyed for pathogenic agents periodically over the past 25 years (Joye and Cofrancesco 1991, Shabana and Charudattan 1996, Shabana et al. 2003, Shearer 2012), monoecious hydrilla has received little attention. In part, this was due to its limited distribution in a few eastern states, but its expansion in recent years to widely different geographic regions of the United States has given it new status as an invasive species of note.

Monoecious hydrilla management is primarily through chemical control using endothall (Poovey and Getsinger 2010), fluridone, and a combination of copper and diquat.[1] Grass carp (*Ctenopharyngodon idella*) would be a potential biological control agent because it is a non-specific feeder of aquatic plants and in all likelihood would feed on monoecious hydrilla. At this time, it is unknown if carp have been specifically released at any sites infested by monoecious hydrilla. While the ephydrid fly *Hydrellia pakistanae*, a biocontrol insect, has successfully established populations on the dioecious hydrilla biotype, there are no records that document its establishment on the monoecious hydrilla biotype, even after concerted release efforts.

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; Netherland and Shearer 1996; Shearer 1998, 2009a, 2009b; Shearer and Nelson 2002; Shearer and Jackson 2006), *Fusarium culmorum* (Charudattan et al. 1984), and *Plectosporium tabacinum* (Smither-Kopperl et al. 1999). To date, no pathogens have been researched as potential biological control agents for management of monoecious hydrilla. The purpose of the study presented herein was to survey some known populations of monoecious hydrilla and isolate potential fungal pathogens.

MATERIALS AND METHODS: During the summer of 2012, monoecious hydrilla was collected in California, Texas, Mississippi, Virginia, and South Carolina. With the exception of Mississippi, samples were shipped overnight to the biomanagement laboratory located at the U.S. Army Corps of Engineers Research and Development Center (USAERDC) in Vicksburg, Mississippi. Upon arrival, the samples were washed in running 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 subsample of stem and leaf tissue from each collection was surface sterilized in a 3.5% sodium hypochlorite solution for 1 min, placed in a sieve, and rinsed in deionized water for 1 min. Excess moisture was drained off the subsample and it was added to a blender containing 100 ml of sterile water. The subsample was macerated in the blender for 30 s, providing a dilution factor of 1/10. The resulting slurry was further diluted to concentrations of 1/50 and 1/100. All dilutions were plated onto Martin's agar plates (three plates per dilution concentration). Martin's agar consisted of 1 L H_2O; 20 g agar, 0.5 g KH_2PO_4; 0.5 g $MgSO_4 \cdot 7\ H_2O$; 0.5 g peptone; 10 g dextrose, 0.5 g yeast extract; 0.05 g rose Bengal; 0.03 g

[1] Personal Communication. 2013. Michael Netherland, Research Biologist, US Army Engineer Research and Development Center, Vicksburg, MS.

streptomycin sulfate). The Martin's agar plates were incubated in the dark at 25° C for 1 week. Small pieces (~1 by 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, Michigan) slants (test tubes placed at an angle during cooling to give a large slanted surface for inoculation). After 7-10 days, the slants from each of the geographic regions 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 1985) 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 develop on PDA, and colonies readily produce asexual and/or sexual spores on PCA. Those cultures that sporulated were identified to genus and species when possible. Those that did not sporulate were placed in categories of moniliaceous (hyaline hyphae) or dematiaceous (dark hyphae) Ascomycetes.

RESULTS AND DISCUSSION: The number of monoecious hydrilla samples processed from different geographical regions of the United States in 2012 were as follows: California (2), Texas (2), South Carolina (3), Virginia (3), and Mississippi (1). The samples were collected in the northern Sierra foothills Oregon House area of Yuba County, California; a culture pond located on the grounds of the Corps of Engineers Lewisville Aquatic Ecosystem Research Facility, Lewisville, Texas; Strom Thurmond Reservoir, South Carolina; Lake Gaston, Virginia; and a culture tank at the USAERDC Aquatic and Wetland Ecosystems Research and Development Facility located in Vicksburg, Mississippi. A total of 173 isolates were obtained and following sorting, they were separated into 72 morphospecies (Table 1). Together singletons (44) and doubletons (14) accounted for 58 species or 81% of the total.

Table 1. Fungal isolates from monoecious hydrilla collected in CA (California), VA (Virginia), MS (Mississippi), TX (Texas), and SC (South Carolina).

Species	CA	VA	MS	TX	SC
Candida	1				
Dematiaceous Ascomycete 1	12				
Dematiaceous Ascomycete 2	6				
Stachybotrys charticola	1				
Cladosporium sphaerospermum	2				2
Cladosporium cladosporioides	1				2
Trichoderma harzianum	1	1			3
Scopulariopsis koningii	1				
Moniliaceous Ascomycete 1	1				
Penicillium sp.	1				
Penicillium sp.	1				
Mucor spinosus	1				
Dematiaceous Ascomycete 3	1				
Rhinocladiella sp.	1				
Acremonium charticola	2				
Geotrichum sp.	1				
Hansfordia ovalispora	1				1
Pythium sp.	6		2	1	6

Table 1. Continued.					
Species	**CA**	**VA**	**MS**	**TX**	**SC**
Acremonium curvulum	1				
Anguillospora longissima	1				
Monodictys castanae	2				
Gliomastix murorum	2				
Monodictys levis	1				
Phoma sp.	1				
Myrothecium roridum		1			
Aspergillus niger		1			1
Phycomyete		1			
Talaromyces helicus		1			4
Gliocadium viride		1			
Dimorphospora foliicola		1			
Plectosphaerella cucumerina		1			
Isaria sp.		1			
Moniliaceous Ascomycete 2		1			
Moniliaceous Ascomycete 3		1			
Alternaria alternata				8	
Mortierella sp.				2	
Sporobolomyces				1	
Cladosporium tenuissimum				1	
Acremonium furcatum				2	
Dinemosporium strigosum				2	
Khuskia oryzae				1	
Phoma herbarum				3	1
Moniliaceous Ascomycete 4				1	
Ulocladium alternariae				1	
Moniliaceous Ascomyete 5					2
Dematiaceous Ascomycete 3					28
Moniliaceous Ascomycete 6					2
Hansfordia pulvinata					1
Penicillium sp.					1
Dematiaceous Ascomycete 4					2
Acremonium psammosporum					2
Phoma nebulosa					1
Moniliaceous Ascomyete 7					1
Penicillium sp.					1
Penicillium sp.					1
Microsphaeropsis olivacea					1
Dematiaceous Ascomycete 5					1
Penicillium sp.					3
Moniliaceous Ascomycete 8					4
Emericellopsis minima					2
Moniliaceous Ascomycete 9					1

Table 1. Continued.					
Species	**CA**	**VA**	**MS**	**TX**	**SC**
Penicillium sp.					1
Hansfordia biophila					2
Penicillium purpurogenum					1
Pseudeurotium ovalis					1
Penicillium sp.					1
Dematiaceous Ascomycete 6					1
Aureobasidium pullulans					2
Moniliaceous Ascomyete 10					1
Venturia sp.					2
Dematiaceous Ascomycete 7					1
Mycoleptodiscus terrestris					1

The most frequently isolated species was Dematiaceous Ascomycete 3 with 28 total isolates followed by *Pythium* with 12, Dematiaceous Ascomycete 1 with 12, and *Alternaria alternata* with eight. The monoecious hydrilla samples yielded 17 non-sporulating species herein noted as dematiaceous (dark mycelium) or moniliaceous (hyaline mycelium) Ascomycetes (Table 1).

The majority of the species isolated during the study could be described as cosmopolitan saprobes, or secondary weak pathogens. As such, they would not make good candidates for biological control of monoecious hydrilla. Potential exceptions could be *Acremonium curvulum*, *Myrothecium roridum*, *Plectosphaerella cucumerina*, *Venturia* sp., and *Mycoleptodiscus terrestris*.

Andrews et al. (1981) identified *A. curvulum* as a potential biocontrol pathogen of *Myriophyllum spicatum* (Eurasian watermilfoil). At times, the fungus also occurred benignly in some watermilfoil populations as an endophyte. The researchers found that when *A. curvulum* was inoculated onto watermilfoil plants that were endophyte-free it was only mildly pathogenic, but when it was inoculated onto plants that were endophyte-infected, the stressed plants usually died. These findings resulted in curtailment of further development of the agent due to inconsistent efficacy in the laboratory. It is unknown at the present time if pathogen performance would be similar on monoecious hydrilla.

Myrothecium roridum has been suggested as a possible mycoherbicidal agent for control of waterhyacinth (Okunowo et al. 2010b). The published paper focused on optimum growth parameters of the fungus, but efficacy testing on waterhyacinth was not included. Because species of *Myrothecium* can produce cellulolytic enzymes, *M. roridum* might have potential as a pathogenic agent (Moreira et al. 2005, Okunowo et al. 2010a) both for waterhyacinth and monoecious hydrilla.

In the late 1990s, Smither-Kopperl et al. (1999) isolated *Plectosporium tabacinum* (anamorph *Plectosphaerella cucumerina*) from asymptomatic dioecious hydrilla. In an aquarium study, the fungus could spread to other plants from a single infected shoot. However, it was considered weakly pathogenic and the authors recommended it be used with herbicides in an integrated approach for hydrilla management. This approach might also be applied when using *P. cucumerina* as a biocontrol fungus for monoecious hydrilla management.

Members of the genus *Venturia* are leaf, stem, and fruit pathogens of a variety of plants (Farr et al. 1989). *Venturia inaequalis*, for example, causes a serious disease of apples in areas where environmental conditions are cool and moist during the early growing season when trees are beginning to set fruit. The disease causes scab, which severely reduces the quality of infected fruit (Agrios 2005). Similar diseases affect pears (*V. pyrina*) and hawthorns (*V. inaequalis* sp.f. *pyracanthae*) (Agrios 2005). The fact that moist conditions predispose apples, pears, and hawthorns to disease might be a good indication that the *Venturia* isolate from monoecious hydrilla is well adapted to aquatic conditions and thus the fungus could prove to be a good biocontrol agent.

Mycoleptodiscus terrestris has been found to be efficacious on dioecious hydrilla used alone (Joye 1990, Shearer and Jackson 2006, Shearer 1998, Shearer 2009a, Shearer 2012) and in combination with herbicides (Netherland and Shearer 1996, Shearer and Nelson 2002, Nelson and Shearer 2009). The isolation of the fungus from monoecious hydrilla indicates it can invade host tissues. Only future testing will determine if the isolate is efficacious on the monoecious hydrilla biotype.

FUTURE WORK: The above-mentioned potential pathogens will be screened for pathogenicity on monoecious hydrilla in a flask study. Testing will also include all the unknown dematiaceous and moniliaceous Ascomycetes and those known as weak pathogens. Those isolates identified as cosmopolitan saprobic species will not be tested.

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