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Comparative Response of Monoecious and Dioecious Hydrilla to Endothall

ANGELA G. POOVEY[1] AND K. D. GETSINGER

ABSTRACT

Hydrilla (*Hydrilla verticillata* [L.f.] Royle) is an aggressive submersed weed that has invaded many United States waterbodies. While both the monoecious and dioecious biotypes are present in the United States, monoecious populations have continued to spread along the eastern seaboard and in the Great Lakes Region. There is limited documentation of this biotype's response to herbicides; therefore, we conducted two laboratory studies to compare the efficacy of endothall against monoecious and dioecious hydrilla under various concentrations and exposure times. In the first experiment, plants were propagated from shoot fragments. In the second experiment, plants were propagated from subterranean turions (tubers). Results showed that endothall is efficacious against both monoecious and dioecious hydrilla, reducing biomass by >85% with concentrations of 2 mg ai L^{-1} coupled with exposure times of 48 h for dioecious and 72 h for monoecious plants grown from shoot fragments. Higher concentrations (4 mg ai L^{-1}) or extended exposure times (96 h) were required to control hydrilla grown from tubers. Treatment of newly sprouted monoecious tubers may be an effective application strategy because most monoecious tubers sprout during spring and summer. Endothall efficacy against monoecious and dioecious hydrilla grown from tubers requires further study.

Key words: aquatic herbicide, chemical control, shoot fragment, submersed aquatic plant, subterranean turion, tuber.

INTRODUCTION

Hydrilla (*Hydrilla verticillata* [L.f.] Royle) is an aggressive submersed weed that has invaded many United States waterbodies. Its photosynthetic capabilities, rapid growth rate, and vegetative reproduction strategies enable hydrilla to establish large monospecific stands that are difficult to manage (Langeland 1996). While both the monoecious and dioecious biotypes are present in the United States, the monoecious strain is more prevalent in the cooler regions of the country. The monoecious biotype of hydrilla has spread throughout the eastern seaboard, stretching from Georgia north toward Maine, with occurrences in the western states of Washington and California (Jacono et al. 2008). New infestations have been found in Indiana and Wisconsin (Maki and Galatowitsch 2008). Although the dioecious biotype is typically found in the southern tier of the United States, it was discovered in Connecticut (Les et al. 1997), Pennsylvania (Madeira et al. 2000), and more recently, near Boise, Idaho, where the water is warmed by geothermal wells (Jacono et al. 2008). Distribution overlaps of monoecious and dioecious populations have been reported in Lake Gaston, a reservoir on the Virginia-North Carolina border, (Ryan et al. 1995), and water bodies in California, South Carolina, and Georgia (Madeira et al. 2000).

Monoecious and dioecious plants are genetically and morphologically different (Verkleij et al. 1983, Madeira et al. 2000, 2004). Each is related to different Asian accessions. The monoecious biotype is of Korean origin, while the dioecious biotype is from the Indian subcontinent (Madeira et al. 2004, Coetzee 2009). Monoecious hydrilla has longer internode distances and shorter leaves that are lighter green in appearance than dioecious plants (Ryan et al. 1995). Shoot growth is generally spread at the sediment surface with numerous root crowns and high shoot densities (Van 1989). Once lateral spread slows, shoots grow upward toward the water surface, forming dense canopies. Conversely, dioecious shoots first elongate to the surface and then form profuse and dense branches; however, in subtropical springs where light is not limited, dioecious hydrilla remains close to the sediment surface, indicating that growth habit is contingent upon environmental conditions (Spencer and Bowes 1993). Monoecious hydrilla has been described as an annual that dies back in the winter (Harlan et al. 1985), while dioecious hydrilla is considered a perennial that grows throughout the year, albeit at a reduced rate during the winter (Ryan et al. 1995).

Differences in hydrilla vegetative reproduction and turion ecology have been summarized (Netherland 1997). Briefly, both hydrilla biotypes form subterranean turions (tubers) and axillary turions (turions). Tubers from dioecious hydrilla are larger in size, though smaller in number, compared to tubers produced by monoecious hydrilla. Both bioytpes are able to reproduce from stem fragments, roots, and rhizomes (Langeland and Sutton 1980). Although monoecious plants are capable of viable seed production (Langeland and Smith 1984, Steward 1993), data on seedling survival *in situ* are lacking.

Differences in genetic variation, growth habit, phenology, and reproductive propagules probably represent distinct survival strategies between dioecious and monoecious hydrilla and may have consequences for control strategies (Verkleij et al. 1983, Spencer et al. 1987). Over the past two decades, operational practices to control submersed weeds, and research efforts to improve that control, have focused on utilizing herbicides in a species-selective manner, targeting

[1]U.S. Army Engineer Research and Development Center, 3909 Halls Ferry Rd., Vicksburg, MS 39180. Corresponding author, e-mail: Angela.G.Poovey@erdc.usace.army.mil. Received for publication 17 April 2009 and in revised form 14 September 2009.

control of weedy species and minimizing impacts on nontarget vegetation (Getsinger et al. 2008). Much of this work has focused on dioecious hydrilla, with limited information generated on the monoecious biotype.

In laboratory studies, monoecious and dioecious biotypes responded similarly to the herbicides diquat [6,7-dihydrodipyrido (1,2-1a:2',1'-c) pyrazinediium] and the dipotassium salt of endothall (7-oxabicyclo [2.2.1] heptane-2,3-dicarboxylic acid; Steward and Van 1987, Van and Conant 1988). Endothall has been used to control hydrilla (primarily dioecious) throughout the United States for more than 40 years. Currently, it is the most widely used chemical alternative for managing fluridone-resistant hydrilla in Florida (Netherland et al. 2005). Concentration-exposure time (CET) relationships developed for dioecious hydrilla (Netherland et al. 1991) show that it can be effectively controlled at high concentrations (4 to 5 mg ai L^{-1}; 5 mg ai L^{-1} is the maximum label rate) and relatively short exposure times (12 to 18 h). Lower application rates (2 to 3 mg ai L^{-1}) require longer exposure times of 48 to 72 h. Label rates of the Aquathol® K formulation (United Phosphorus, Inc., King of Prussia, PA) reflect the CET concept by recommending 2 to 3 mg ai L^{-1} endothall for entire lake or large treatment areas and 3 to 4 mg ai L^{-1} endothall for spot treatments or along the shoreline.

Continued spread of monoecious hydrilla along the eastern seaboard and in the Great Lakes Region, limited documentation of its response to herbicides, and the potential of both hydrilla biotypes to occur in the same water body has led to a renewed interest in developing chemical control strategies for selectively controlling this plant. To investigate the potential differences in hydrilla biotypes in response to an aquatic herbicide, we conducted two small-scale studies to evaluate the efficacy of endothall against monoecious hydrilla grown from different plant structures under various concentrations and exposure times and compared results to dioecious hydrilla. We used field collected shoots and tubers because each represents a different reproductive strategy. Shoot fragments are an important means of long distance dispersal (Steward 1992), while tubers are the primary source of annual hydrilla reinfestation in a localized area (Haller et al. 1976, Spencer et al. 1987).

MATERIALS AND METHODS

Plants Established from Apical Shoots

An experiment was conducted in a walk-in controlled environment growth chamber (48 m^2) at the U.S. Army Engineer Research and Development Center (ERDC) in Vicksburg, Mississippi. Ambient conditions were set to provide optimum conditions for submersed plant growth: air temperature of 21 ± 2 C, light intensity of 520 ± 50 µmol m^{-2} sec^{-1}, and photoperiod of 14:10 h light:dark cycle. Lighting was provided by a combination of 400 watt high-pressure sodium and metal halide bulbs.

Monoecious hydrilla shoots were collected from Lake Durant, Raleigh, North Carolina on 29 September 2004 and shipped overnight to the ERDC. Four healthy apical cuttings (15 cm) were planted to a depth of 2 cm in 300 ml beakers (diameter = 7 cm, depth = 12 cm) filled with natural lake sediment amended with 150 mg L^{-1} ammonium chloride. A 1-cm layer of silica sand was added to the sediment surface to prevent dispersion of nutrients and sediment into the water column. Four beakers were placed in each of 28 aquaria (volume = 53 L; 27 cm long by 27 cm wide by 73 cm high). All aquaria were then filled with culture solution (Smart and Barko 1985).

Dioecious hydrilla shoots were collected from ponds at the University of Florida, Gainesville, on 6 October 2004 and shipped overnight to the ERDC. These shoots were planted as described above. Beakers of the dioecious biotype were placed side by side with beakers of the monoecious biotype in each aquarium; therefore, each aquarium was considered a replicate containing a total of eight planted beakers, four of each biotype.

To achieve similar canopy formation in both biotypes before herbicide application, monoecious plants grew for 4 weeks, while dioecious plants grew for 3 weeks after planting. Four beakers of each biotype were randomly sampled for a pretreatment shoot biomass. Shoots were clipped at the sediment surface, dried at 70 C for 48 h and weighed. Pretreatment dry weights (mean ± 1 SE, n = 4) for monoecious and dioecious hydrilla were 0.48 ± 0.09 g $beaker^{1}$ and for 1.16 ± 0.13 g $beaker^{-1}$, respectively. The monoecious biotype in our experiment had shoot biomass comparable to early spring biomass in North Carolina (Harlan et al. 1985), while the dioecious biotype had shoot biomass comparable to early spring biomass in Florida (Bowes et al. 1979).

For herbicide application, a stock solution of endothall as the liquid formulation Aquathol® K was prepared as 5.07 g ai L^{-1} based on the dipotassium salt. Endothall rates of 1, 2, and 4 mg ai L^{-1} were used with exposure times ranging from 24 to 96 h to determine CET relationships (Table 1). All treatments hereafter will be referred to as the endothall rate followed by its respective exposure time (e.g., 1/48). Untreated reference aquaria were included to assess plant growth in the absence of herbicide exposure. Immediately following herbicide exposure times, all aquaria, including references, were drained and filled with fresh culture solution three times to remove all aqueous herbicide residues. Each treatment, including the reference, was replicated three times.

Water samples (60 ml) were collected 25 cm below the water surface from one treatment replicate for each CET combination at 24 h to ensure nominal herbicide concentrations were achieved. Samples were stored at 4 C until shipped for analysis, using the enzyme-linked immunosorbent assay (ELISA) technique (Toth 1999), which can detect endothall concentrations as low as 7 µg ai L^{-1}.

Water temperature was measured continuously with an Optic Stowaway® Temperature Probe (Onset Computer Corp., Bourne, MA) in reference aquaria. The pH was measured at the beginning and end of the experiment with a WTW pH 315i meter (WTW Measurement Systems, Ft. Meyers, FL).

For each biotype, herbicide efficacy was assessed 6 weeks after treatment (WAT) by harvesting shoot biomass from two beakers from each aquarium (replicate) at each time interval. Means for each replicate were calculated from post-treat-

TABLE 1. ENDOTHALL CONCENTRATION AND EXPOSURE TIME COMBINATIONS EVALUATED AGAINST MONOECIOUS (M) AND DIOECIOUS (D) HYDRILLA GROWN FROM SHOOT FRAGMENTS AND TUBERS.

Shoot Experiment			Tuber Experiment		
Concentration (mg ai L^{-1})	Exposure time (h)	Biotype	Concentration (mg ai L^{-1})	Exposure time (h)	Biotype
1	48	M, D	1	48	M, D
1	96	M, D	1	96	M, D
2	48	M, D	2	48	M, D
2	72	M, D	2	72	M, D
2	96	M, D	2	96	M
4	24	M, D	4	24	M, D
4	48	M, D	4	48	M, D
4	72	M, D			

ment harvest data (n = 2), then subjected to a one-way analysis of variance (ANOVA) to determine herbicide effects on percent biomass reduction based on shoot dry weights (n = 3). If the main effects were significant ($p \leq 0.05$), means were compared using the Student-Newman-Keuls method (S-N-K).

Plants Established from Tubers

An experiment was conducted under the same experimental conditions as described for the shoot experiment above. Tubers of monoecious hydrilla were collected from Lake Gaston, North Carolina-Virginaia on 7 October 2004 and shipped overnight to the ERDC. After receipt, tubers were refrigerated in the dark at 4 C for two days. Tubers were then placed in reverse osmotic (RO) water, aerated, and allowed to sprout and grow in the environmental growth chamber for 3 weeks. Mean tuber weight (±1 SE) was 0.14 ± 0.01 g fresh weight (FW; n = 24).

Three sprouted tubers (shoot length = 3.32 ± 0.12 cm) were planted to a depth of 2 cm in 300 ml beakers (dia = 7 cm, depth = 12 cm) filled with natural lake sediment amended with 150 mg L^{-1} ammonium chloride. A 1-cm layer of silica sand was added to the sediment surface to prevent dispersion of nutrients and sediment into the water column. Three beakers were placed in each aquarium (volume = 10 L; 13 cm long by 13 cm wide by 62 cm high) filled with growth solution (Smart and Barko 1985).

Tubers of dioecious hydrilla were collected from ponds at the University of Florida, Gainesville, on 8 November 2004 and shipped overnight to the ERDC. Upon arrival, tubers were weighed (mean = 0.47 ± 0.05 g FW, n = 24), placed in aerated RO water, and left in the growth chamber to sprout for 3 days. Three sprouted tubers were planted in beakers as described above (shoot length = 15.0 ± 2.20 cm). Three beakers of the dioecious biotype were placed in each 10-L aquarium filled with growth solution.

Monoecious plants grew for 7 weeks and dioecious for 5 weeks to achieve the initial formation of a surface canopy prior to herbicide application. The day before herbicide application, one beaker was removed from each aquarium for a pretreatment biomass estimate of each biotype. Pretreatment dry weights (mean ± 1 SE) were 0.43 ± 0.07g $beaker^{-1}$ (n = 24) and 0.32 ± 0.03 g $beaker^{-1}$ (n = 21) for monoecious and dioecious hydrilla, respectively. The monoecious biotype had shoot biomass comparable to spring hydrilla biomass in North Carolina (Harlan et al. 1985), while the dioecious biotype had shoot biomass comparable to early spring hydrilla biomass in Florida (Bowes et al. 1979).

For herbicide application, a stock solution of endothall of the liquid formulation Aquathol® K was prepared as 5.07 g ai L^{-1} based on the dipotassium salt. Herbicide rates of 1, 2, and 4 mg ai L^{-1} were used with exposure times ranging from 24 to 96 h to determine CET relationships (Table 1). Application technique, water sample collection, and biomass harvest protocols were all identical to the shoot experiment described earlier. There were 45 total aquaria (replicates): 24 aquaria planted with monoecious hydrilla and 21 aquaria planted with dioecious hydrilla. There were three replicates for each treatment for each biotype.

Herbicide efficacy was assessed at 6 WAT by harvesting shoot biomass from one beaker of each aquarium. A one-way ANOVA was conducted to determine herbicide effects on percent biomass reduction based on shoot dry weights (n = 3). If the main effects were significant ($p \leq 0.05$), means were compared using the S-N-K method.

RESULTS

Shoot Experiment

During the experiment, water temperatures in aquaria ranged from 20.4 to 23.2 C, while the pH ranged from 8.6 to 9.1. Aqueous endothall residues (mean ± 1 SE) were 1.03 ± 0.03 mg ai L^{-1} (n = 6), 2.23 ± 0.03 mg ai L^{-1} (n = 9), and 4.28 ± 0.13 mg ai L^{-1} (n = 9) for the nominal concentrations of 1, 2, and 4 mg ai L^{-1}, respectively.

Control of dioecious hydrilla shoot biomass was similar (≥90%) for all endothall treatments (Figure 1A), except for the 1 mg ai L^{-1} concentration with a 48-h exposure time, (1/48) in which percent biomass reduction was only 37 ± 13%. Monoecious plants dosed with 1 mg ai L^{-1} for a 48-h exposure time were reduced by 32 ± 11% (Figure 1B). For both biotypes, viable green shoots with new growth were present 6 WAT, indicating that plants from the 1/48 treatment might recover if left to grow for a longer period of time. Doubling the exposure time to 96 h increased efficacy of this low endothall concentration. Biomass reduction was >85% for dioecious hydrilla, while biomass reduction was >95% for

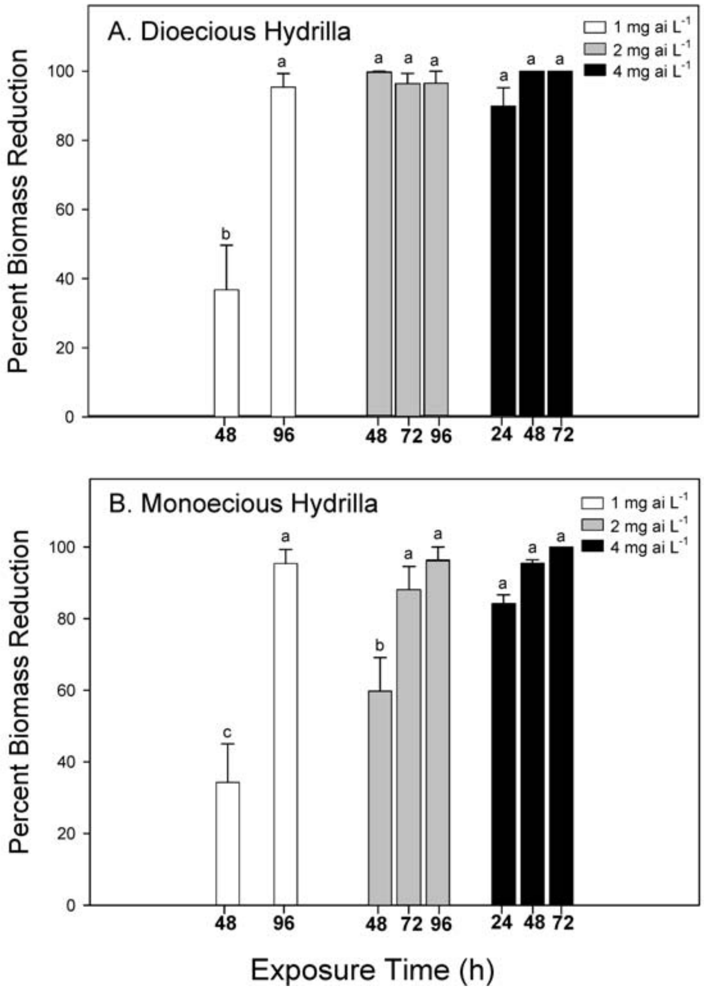

Figure 1. Percent biomass reduction of (A) dioecious and (B) monoecious hydrilla grown from shoot clippings 6 weeks following endothall applications of 1, 2, and 4 mg ai L^{-1} under various exposure periods (24, 48, 72, and 96 h). Means are ±1 SE (n = 3). Treatments with different letters are significantly different for each biotype (S-N-K; $p \leq 0.05$).

monoecious hydrilla in the 1/96 treatment. Remaining plant shoots from these treatments were brown, decayed, and essentially dead, with little recovery potential.

Endothall treatments of 2 and 4 mg ai L^{-1} had little remaining biomass with no visual evidence of shoot recovery potential for either biotype. Percent biomass reduction with these CETs ranged from 90 to 100% for dioecious (Figure 1A) and monoecious hydrilla, with exception of the 2/48 treatment of monoecious (Figure 1B). Endothall applications of 2 mg ai L^{-1} for a 48-h exposure time reduced monoecious hydrilla shoot biomass by 60 ± 9%. Plants were brown with decaying shoots and few viable stems; it is uncertain whether the plants would survive and resprout via root crowns.

Tuber Experiment

Water temperatures in aquaria were 22.4 ± 0.04 C, while pH was 9.0 ± 0.1 throughout the experiment. Aqueous endothall residues (mean ± 1 SE) were 0.97 ± 0.03 mg ai L^{-1} (n = 4), 2.07 ± 0.03 mg ai L^{-1} (n = 6), and 4.29 ± 0.02 mg ai L^{-1} (n = 3) for the nominal concentrations of 1, 2, and 4 mg ai L^{-1}, respectively.

Dioecious shoot biomass reduction was similar for 2 and 4 mg ai L^{-1} endothall treatments, ranging from 74 to 99% (Figure 2A). Disintegration of plant canopy with decaying stems was evident in these treatments as soon as 1 WAT, with plant death occurring by 3 WAT. Endothall treatments of 1 mg ai L^{-1} were ineffective regardless of exposure time. Percent biomass reduction for a 1/48 exposure time was 13 ± 11%, while biomass reduction for a 1/96 exposure time was 41 ± 15%. Shoots were still green and healthy with new growth present for both treatments indicating eventual recovery.

Reduction of monoecious hydrilla shoot biomass ranged from 29 to 88% for all concentrations and exposure times evaluated (Figure 2B). As with dioecious hydrilla, endothall applied at 1 mg ai L^{-1} was ineffective against monoecious hydrilla regardless of exposure time. Plants in the 2/48, 2/72, and 4/24 treatments provided partial control (45 to 65%),

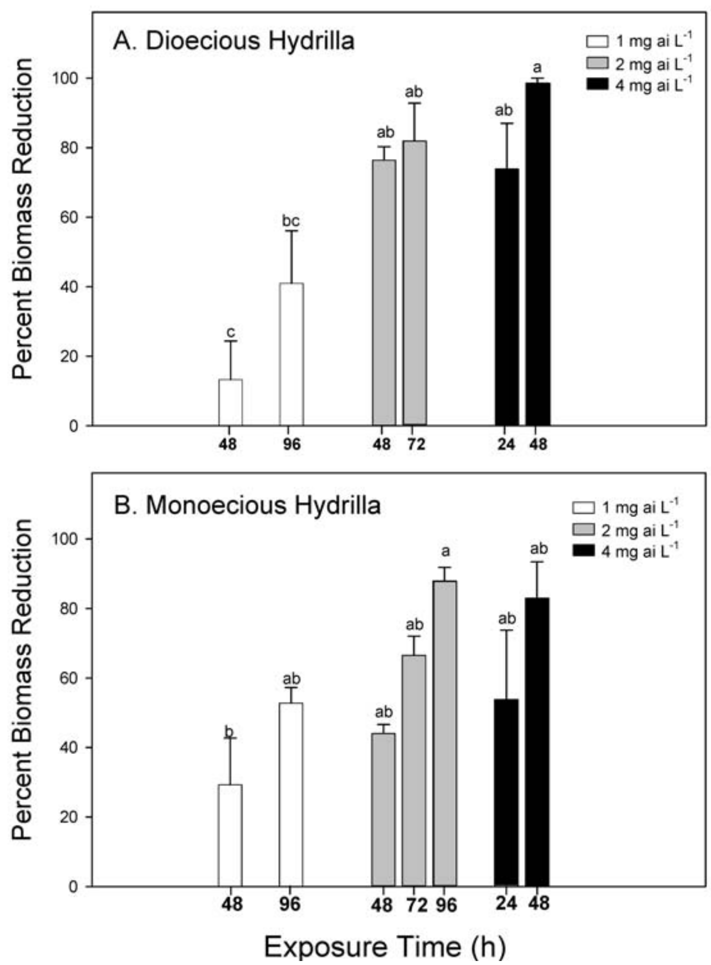

Figure 2. Percent biomass reduction of (A) dioecious and (B) monoecious hydrilla propagated from tubers 6 weeks following endothall applications of 1, 2, and 4 mg ai L^{-1} under various exposure periods (24, 48, 72, and 96 h). Means are ±1 SE (n = 3). Treatments different letters are significantly different for each biotype (S-N-K; $p \leq 0.05$).

and showed symptoms of herbicide injury (bleaching and broken stems) at 1 WAT. By 3 WAT, many of these plants had collapsed canopies and decaying stems; however, by 6 WAT, plants were regaining biomass with green and healthy stems, suggesting recovery. Only two endothall treatments would be considered successful, reducing monoecious shoot biomass by >80% (Figure 2B). These are the 2/96 and 4/48 treatments with percent biomass reduction of 83 ± 11 and 88 ± 4%, respectively.

DISCUSSION

Control of dioecious hydrilla grown from shoot fragments in this experiment was similar to other studies (Van and Conant 1988, Skogerboe and Getsinger 2001, Shearer and Nelson 2002), including the CET evaluation conducted in the same laboratory system by Netherland et al. (1991). Our results also showed that endothall is efficacious against monoecious hydrilla, providing excellent control with low concentrations (≥1 mg ai L^{-1}) coupled with adequate exposure times (≥96 h). If these exposures are not maintained, control will be limited. Langeland and Pesacreta (1985) attributed poor control of monoecious hydrilla by endothall to inadequate contact time in North Carolina stream impoundments, which had high flushing rates due to high drainage to volume ratios.

In our experiment, endothall concentrations of 1 mg ai L^{-1} were effective in reducing both monoecious and dioecious hydrilla grown from shoot fragments when exposure times were at least 96 h; however, these concentrations were ineffective against both biotypes grown from tubers, regardless of exposure time. Although greenhouse and field studies have demonstrated that endothall combined with diquat, copper, or the mono (N,N-dimethlyalkalamine) salt of endothall can enhance control (Pennington et al. 2001, Skogerboe et al. 2004), adequate exposure times are imperative for significant shoot biomass reduction. For example, 1 mg ai L^{-1} endothall combined with 0.5 mg ai L^{-1} copper was effective in reducing dioecious hydrilla shoot biomass up to one year posttreatment because the nominal concentrations were maintained in the treatment area for at least 65 h (65-h half-life; Skogerboe et al. 2004). When 3 mg ai L^{-1} endothall was combined with 0.5 mg ai L^{-1} copper using a 27-h half-life, shoot biomass was reduced for 3 months but had returned to pretreatment levels 1 year posttreatment (Skogerboe et al. 2004).

Shoot biomass reduction was less for both dioecious and monoecious hydrilla grown from tubers (Figure 2) than grown from shoot fragments (Figure 1). Depending on plant source, hydrilla shoot fragments may have fewer stored reserves, and therefore respond differently to stress than plants grown from sprouted tubers (Spencer et al. 1994). Unlike the response of sago pondweed (*Stukenia pectinata* L.) to fluridone (1-methyl-3-phenyl-5-[3-trifluoromethyl)phenyl]-4(1*H*)-pyridinone; Spencer et al. 1989), endothall efficacy against hydrilla was not determined by tuber size. Although dioecious tubers were larger than monoecious tubers, shoot biomass reduction for many CET combinations was greater in dioecious than monoecious hydrilla.

Treatment of newly sprouted monoecious tubers may be an effective application strategy that should be tested in field sites. Van and Conant (1988) reported excellent control of monoecious hydrilla when endothall was applied to newly sprouted tubers (10 to 12 cm shoot length, 4 to 5 d of growth). Newly sprouted monoecious tubers were also found to be susceptible to chelated copper and fluridone (Steward and Van 1987, Van and Conant 1988). Sprouting of monoecious hydrilla tubers occurs in spring and summer (Harlan et al. 1985, Spencer and Ksander 2001), and there is speculation that most monoecious tubers sprout during this 3-month window (Spencer and Ksander 2001); however, it is uncertain exactly how many tubers sprout and how many remain quiescent in the sediment during a growing season (Netherland 1997). Treatment of newly sprouted dioecious tubers may prove more difficult because sprouting is nonseasonal and random, with a consistent percentage of tubers sprouting over time (Sutton and Portier 1985, Netherland and Haller 2006).

We demonstrated that CET developed for dioecious hydrilla by Netherland et al. (1991) were valid for dioecious hydrilla grown from either shoot fragments or tubers and for monoecious hydrilla grown from shoot fragments. Refinement of endothall efficacy against monoecious hydrilla grown from tubers is warranted, as stable populations of both biotypes occur near one another (Ryan et al. 1995). Research on herbicide efficacy on plants grown from axillary turions also would be valuable because these propagules have been implicated in the northward spread of monoecious hydrilla (Spencer et al. 1987, Maki and Galatowitsch 2008). Early herbicide application to newly sprouted tubers and turions may be a successful management strategy for monoecious hydrilla.

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LITERATURE CITED

Bowes, G. A., A. S. Holiday and W. T. Haller. 1979. Seasonal variation in the biomass, tuber density, and photosynthetic metabolism of hydrilla in three Florida lakes. J. Aquat. Plant Manage. 17:61-65.

Coetzee, J. A., M. P. Hill and D. Schlange. 2009. Potential spread of the invasive plant *Hydrilla verticillata* in South Africa based on anthropengic spread and climate suitability. Biol. Inv. 11:801-812.

Getsinger, K. D., M. D. Netherland, C. E. Grue and T. J. Koschnick. 2008. Improvements in the use of aquatic herbicides and establishment of future research directions. J. Aquat. Plant Manage. 46:32-41.

Haller, W. T., J. L. Miller and L. A. Garrard. 1976. Seasonal production and germination of hydrilla vegetative propagules. J. Aquat. Plant Manage. 14:26-29.

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

Jacono, C. C., M. M. Richerson and V. Howard Morgan. 2008. *Hydrilla verticillata*. USGS Nonindigenous Aquatic Species Database, Gainesville, FL. http://nas.er.usgs.gov/queries/FactSheet.asp?speciesID=6. Revision Date: 16 Dec 2008. Accessed 13 Mar 2009.

Langeland, K. A. 1996. *Hydrilla verticillata* (L.F.) Royle (Hydrocharitaceae), "the perfect weed." Castanea 61:293-304.

Langeland, K. A. and G. J. Pesacreta. 1985. Management of a monoecious strain of hydrilla in North Carolina. Aquatics 7:15-20.

Langeland, K. A. and C. B. Smith. 1984. Hydrilla produces viable seed in North Carolina lakes. Aquatics 6:20-21.

Langeland, K. A. and D. L. Sutton. 1980. Regrowth of hydrilla from axillary buds. J. Aquat. Plant Manage. 18:27-29.

Les, D. H., L. J. Mehrhoff, M. A. Cleland and J. D. Gabel. 1997. *Hydrilla verticllata* (Hydocharitaceae) in Connecticut. J. Aquat. Plant Manage. 35:11-15.

Madeira, P. T., C. C. Jacono and T. K. Van. 2000. Monitoring hydrilla using two RAPD procedures and the nonindigenous aquatic species database. J. Aquat. Plant Manage. 38:33-40.

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.

Maki, K. C. and S. M. Galatowitsch. 2008. Cold tolerance of the axillary turions of two biotypes of hydrilla and northern watermilfoil. J. Aquat. Plant Manage. 46:42-50.

Netherland, M. D. 1997. Turion ecology of hydrilla. J. Aquat. Plant Manage. 35:1-10.

Netherland, M. D., K. D. Getsinger and D. R. Stubbs. 2005. Aquatic plant management: Invasive species and chemical control. Outlooks on Pest Manage. 16:100-104.

Netherland, M. D., W. R. Green and K. D. Getsinger. 1991. Endothall concentration and exposure time relationships for the control of Eurasian watermilfoil and hydrilla. J. Aquat. Plant Manage. 29:61-67.

Netherland, M. D. and W. T. Haller. 2006. Impact of management on the sprouting of dioecious hydrilla tubers. J. Aquat. Plant Manage. 44:32-36.

Pennington, T. G., J. G. Skogerboe and K. D. Getsinger. 2001. Herbicide/copper combinations for improved control of *Hydrilla verticillata*. J. Aquat. Plant Manage. 39:56-58.

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. and L. S. Nelson. 2002. Integrated use of endothall and a fungal pathogen for management of the submersed aquatic macrophyte *Hydrilla verticillata*. Weed Tech. 16:224-230.

Skogerboe, J. G. and K. D. Getsinger. 2001. Endothall species selectivity evaluation: Southern latitude aquatic plant community. J. Aquat. Plant Manage. 39:129-135.

Skogerboe, J. G., T. G. Pennington, J. M. Hyde and C. Aguillard. 2004. Use of endothall in combination with other herbicides for improved control of hydrilla - a field demonstration, APCRP Tech. Notes Coll. (TN APCRP-CC-04), US Army Eng. Res. and Dev. Center, Vicksburg, MS. 7 pp.

Smart, R. M. and J. W. Barko. 1985. Laboratory culture of submersed freshwater macrophytes on natural sediments. Aquat. Bot. 21:251-263.

Spencer, D. F., L. W. J. Anderson, M. D. Ames and F. J. Ryan. 1987. Variation in *Hydrilla verticillata* (L.f.) Royle propagule weight. J. Aquat. Plant Manage. 25:11-14

Spencer, D., L. Anderson, G. Ksander, S. Klaine and F. Bailey. 1994. Vegetative propagule production and allocation of carbon and nitrogen by monoecious *Hydrilla verticllata* (L.f.) Royle grown at two photoperiods. Aquat. Bot. 48:121-132.

Spencer, D. F. and G. G. Ksander. 2001. Field evaluation of degree-day based equations for predicting sprouting of hydrilla (*Hydrilla verticillata*) turions and tubers. J. Freshwater Ecol. 16:479-486.

Spencer, D. F., G. G. Ksander and L. C. Whiteland. 1989. Sago pondweed (*Potamogeton pectinatus*) tuber size influences its response to fluridone treatment. Weed Sci. 37:250-253.

Spencer, W. and G. Bowes. 1993. Ecophysiology of the world's most troublesome weeds, pp. 39-73. *In*: A. H. Pieterse and K. J. Murphy (eds.). Aquatic Weeds: The ecology and management of nuisance aquatic vegetation. Oxford University Press, Oxford.

Steward, K. K. 1992. Survival and growth of stem fragments from various hydrilla races. Florida Sci. 55:130-135.

Steward, K. K. 1993. Seed production in monoecious and dioecious populations of *Hydrilla*. Aquat. Bot. 46:169-183.

Steward, K. K. and T. K. Van. 1987. Comparative studies of monoecious and dioecious hydrilla (*Hydrilla verticillata*) biotypes. Weed Sci. 35:204-210.

Sutton, D. L. and K. M. Portier. 1985. Density of tubers and turions of hydrilla in south Florida. J. Aquat. Plant Manage. 23:64-67.

Toth, J. C. 1999. Method validation of immunochemical method for residues of endothall in water. Internal Report # KP-023-00, ELF Atochem North America, Inc.

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

Van, T. K. and R. D. Conant, Jr. 1988. Chemical control of hydrilla in flowing water: Herbicide uptake characteristics and concentrations versus exposure, APCRP Miscellaneous. Paper (A-88-2), US Army Eng. Waterways Exp. Sta., Vicksburg, MS. 33 pp.

Verkleij, J. A. C., A. H. Pieterse, G. J. T. Horneman and M. Torenbeek. 1983. A comparative study of the morphology and isoenzyme patterns of *Hydrilla verticillata* (L.f.) Royle. Aquat. Bot. 17:43-59.