

NOTES

Combinations of diquat and carfentrazone-ethyl for control of floating aquatic plants

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INTRODUCTION

Water hyacinth (*Eichhornia crassipes* Mart. Solms) is a common problem in waterways throughout the southern United States where it impedes the recreational use of rivers and lakes (fishing, swimming and boat traffic) and the generation of hydroelectric power. Water hyacinth increases the potential for flooding, reduces phytoplankton production, and alters ecosystem properties (Toft et al. 2003). Common duckweed (*Lemna minor* L.) infestations often reduce the use and aesthetics of small water bodies, may impact native submersed plant growth, and may be responsible for oxygen depletion in the water column (Hillman 1961, Parr et al. 2002). Nuisance populations of these plant species are often associated with influxes of nutrients; and in recent years, the amount of nutrients finding their way into waterbodies is increasing (Vitousek et al. 1997, Bedford et al. 1999). The influx of nutrients often results in increased growth rates, greater plant densities, and a source for new infestations. As environmental conditions for plant growth become more favorable, coupled with the ease at which floating species can disperse, new management recommendations need to be developed that result in rapid effective control.

The use of contact herbicides such as diquat (6,7-dihydrodiprido (1,2-a:2',1'-c) pyrazinedium dibromide) have been effective for control of both water hyacinth and common duckweed (Langeland et al. 2002, Wersal and Madsen 2009). Diquat typically offers rapid results, although high applications rates are often used making the treatment of large areas cost prohibitive. Therefore, the use of low dose combinations of herbicides may offer an effective alternative than maximum rates of one product alone. Herbicide combinations have been used extensively in terrestrial situations, evident by the multitude of commercially available herbicide mixes; however, this approach is much less utilized and understudied in aquatic plant management.

Previous work has demonstrated that combinations of carfentrazone-ethyl (a,2-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzenepropanoic acid, ethyl ester) and 2,4-D results in faster Eurasian watermilfoil (*Myriophyllum spicatum* L.) and parrot-

feather (*Myriophyllum aquaticum* Vell. Verdc.) control than 2,4-D alone (Gray et al. 2007). The combination of endothall (dipotassium salt of 7-oxabicyclo [2,2,1] heptane-2,3-dicarboxylic acid) and 2,4-D or triclopyr (triethylamine (TEA) salt of [(3,5,6-trichloro-2-pyridinyl) oxy]acetic acid) reduces the exposure time needed for Eurasian watermilfoil control as opposed to applying 2,4-D or triclopyr alone (Madsen et al. 2010). However, combinations of diquat and penoxsulam (2-(2,2-difluoroethoxy)-N-(5,8 dimethoxy [1,2,4] triazolo [1,5-c] pyrimidin-2-yl)-6 (trifluoromethyl) benzenesulfonamide) resulted in an antagonistic response between the herbicides when applied to water hyacinth and resulted in reduced efficacy than when applying penoxsulam alone (Wersal and Madsen 2010).

The antagonistic response is likely due to the rapid cell destruction by diquat that limits the translocation and efficacy of the slower acting enzyme inhibiting herbicides. Therefore, we evaluated the efficacy of low rates of diquat alone and in combination with low rates of another fast acting contact herbicide, carfentrazone-ethyl. The objective of this study was to determine if combinations of low rates of diquat and carfentrazone-ethyl would enhance control of water hyacinth and common duckweed.

MATERIALS AND METHODS

The study was conducted once, in mesocosms, at the R. R. Foil Plant Science Research Center, Mississippi State University, from July through August 2008. Water hyacinth and common duckweed were planted into mesocosms from greenhouse stock held at Mississippi State University. Water hyacinth was placed into forty-five 378 L mesocosms to cover the water surface; and common duckweed was placed into forty-five 151 L mesocosms in a similar fashion. Plants were allowed to acclimate to growing conditions for approximately 2 weeks. Mesocosms were amended with 30 mg L⁻¹ of Miracle Gro®¹ fertilizer (24-8-16) weekly to maintain growth (Wersal and Madsen 2009).

Following the acclimation period, foliar applications of diquat as Reward®² and carfentrazone-ethyl as Stingray®³ were applied alone and in combination at ratios of 15:1, 7.4:1, 3.7:1, and 1.85:1 (diquat:carfentrazone-ethyl). An untreated reference was included for statistical comparisons to treated plants. A 0.25% v:v non-ionic surfactant (Cygnet Plus®⁴) was added to the spray solution and applied at 468 L ha⁻¹ (50 gal

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acre⁻¹) using a CO₂ pressurized single-nozzle spray apparatus. Each treatment was replicated in three mesocosms. At 4 WAT, one biomass sample was harvested in all water hyacinth mesocosms using a 0.10 m² quadrat and two samples harvested in all common duckweed mesocosms using a 0.002 m² sampling device (Wersal and Madsen 2009). Dead above ground water hyacinth leaves and petioles were removed, although all root biomass was included in all samples. All common duckweed fronds were included in the harvesting and subsequent analysis. Plants were washed dried at 70 C for 72 h and weighed to determine biomass.

Statistical analyses

A general linear model was used in SAS® to determine differences in biomass and herbicide treatments within plant species. If a significant difference was detected, treatment means were separated using the Fisher's Protected LSD test. All analyses were conducted at a p < 0.05 level of significance.

RESULTS AND DISCUSSION

Water hyacinth

Water hyacinth treated with the combination of diquat and carfentrazone-ethyl at 280 and 152 g ai ha⁻¹ resulted in a 99% reduction in biomass 4 WAT; although control was similar to diquat applied at 560 g ai ha⁻¹, carfentrazone-ethyl applied at 76 and 152 g ai ha⁻¹, diquat+carfentrazone-ethyl at 140+76 g ai ha⁻¹, and all other combinations containing 280 g ai ha⁻¹ of diquat (Figure 1). Diquat applied alone at 560 g ai ha⁻¹

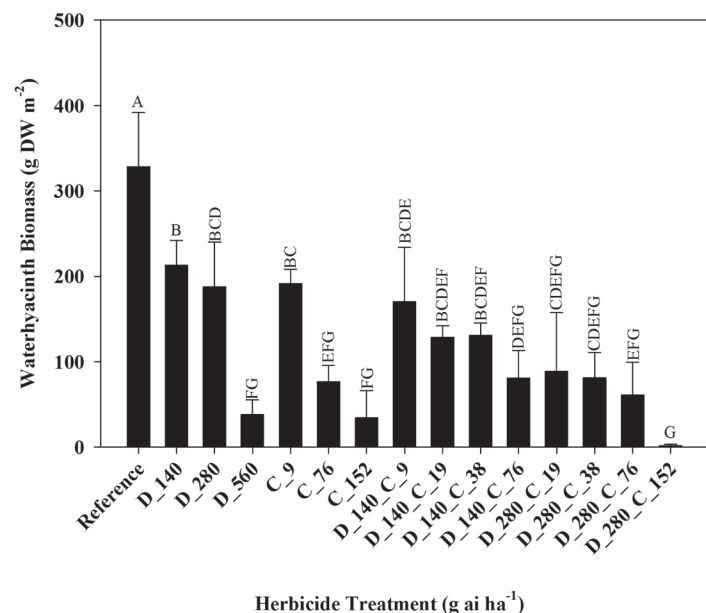


Figure 1. Mean biomass (± 1 SE) of water hyacinth 4 weeks after treatment with low rates of diquat and carfentrazone-ethyl alone and in combination. Bars sharing the same letter are not significantly different according to a Fisher's Protected LSD analysis at a p < 0.05. On the x-axis, a D indicates a treatment of diquat and a C indicates a treatment of carfentrazone-ethyl, with the number that follows signifying the rate of the active ingredient in g ai ha⁻¹.

resulted in an 88% reduction in water hyacinth biomass with respect to untreated reference plants. Carfentrazone-ethyl applied at 152 g ai ha⁻¹ resulted in 89% biomass reduction 4 WAT. Diquat applied at 4600 g ai ha⁻¹ resulted in greater than 95% control of water hyacinth 14 days after treatment (DAT; Langeland et al. 2002). Data from this study suggest that comparable water hyacinth control can be achieved using reduced rates of diquat and carfentrazone-ethyl out to 4 WAT. Additionally, the combination of herbicides did not offer increased efficacy when compared to diquat applied at 560 g ai ha⁻¹ or carfentrazone-ethyl applied at 76 and 152 g ai ha⁻¹.

Common duckweed

The herbicide rates and combinations evaluated in this study did not result in control of common duckweed 4 WAT (p = 0.57), and no treatment resulted in >8% reduction in biomass (data not shown). There were early visual injury symptoms observed on plants treated with 560 g ai ha⁻¹ of diquat, but these symptoms were minimal at the conclusion of the study. Previous studies have reported >95% control of common duckweed when using 4600 g ai ha⁻¹ of diquat (Langeland et al. 2002, Wersal and Madsen 2009). The use of carfentrazone-ethyl alone or in combination with diquat, at the rates tested in this study, also did not offer control of common duckweed. The closely related landoltia (*Landoltia punctata* [G. Mey.]) was reported to have an EC₉₀ value of 772.7 g ai ha⁻¹ and was the most tolerant of floating plants to carfentrazone-ethyl (Koschnick et al. 2004). Our results, and those previously published, suggest that higher rates of diquat and carfentrazone-ethyl are needed to control common duckweed.

Herbicide combinations have been well documented in agricultural settings to improve efficacy, reduce the costs associated with weed control, and identify antagonistic combinations (Green 1989). As additional chemistries become available for use in aquatic habitats and as plant community compositions change due to nonnative species or environmental factors, studies that assess the compatibility and efficacy of herbicide combinations will be of greater importance. In aquatic plant management this could lead to reduced costs through lower use rates, greater herbicide efficacy, meeting contact and exposure requirements, increasing management efficiency by targeting more than one plant species with a single application, and could complement herbicide stewardship programs. Future research needs to assess herbicide interactions for aquatic plant management, identify additional beneficial herbicide combinations, and effective rates for herbicide combinations.

ACKNOWLEDGMENTS

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SOURCES OF MATERIAL

¹Miracle-Gro® Water Soluble All Purpose Plant Food, The Scotts Company, PO Box 606 Marysville, OH 43040

²Reward® Landscape and Aquatic Herbicide, Syngenta Professional Products, PO Box 18300 Greensboro, NC 27419

³Stingray®, FMC Corporation, 1735 Market Street, Philadelphia, PA 19103.

⁴CygnnetPlus®, Brewer International, PO Box 690037, Vero Beach, FL 32969.

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Evaluations of contact aquatic herbicides for controlling two populations of submersed flowering rush

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ABSTRACT

Flowering rush (*Butomus umbellatus* L.) is a rapidly spreading invasive aquatic plant in the northern United States. Introduced from Eurasia, it grows as an emergent plant along shorelines and as a submersed plant in deeper water of lakes and rivers. Because submersed flowering rush grows in fluctuating water levels, management of this plant has been inconsistent and unpredictable. Two small-scale experiments were conducted to evaluate contact herbicide efficacy on the submersed form of flowering rush from two triploid populations, one from Minnesota and one from Idaho. In the first experiment, various concentrations and exposure times of diquat, endothall, and flumioxazin were applied to Minnesota flowering rush. In the second experiment, concentration-exposure time relationships were investigated for flumioxazin against Idaho flowering rush.

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One treatment of endothall was used to compare flumioxazin, a newly registered compound, with an older chemistry. Results of both experiments showed that contact herbicides are effective against flowering rush. Although flumioxazin (200 µg ai L⁻¹) did not significantly reduce shoot biomass for exposure periods of 12 or 24 h, concentrations of diquat (370 µg ai L⁻¹) for exposure times of 6 and 12 h, and endothall (1500 and 3000 µg ai L⁻¹) for exposure times of 12 and 24 h reduced shoot biomass of Minnesota submersed flowering rush by >70%; however, these treatments did not significantly impact root biomass. Lateral rhizome buds, which serve as a source of annual reinfestation, were found in all treatments. Concentrations of flumioxazin (400 µg ai L⁻¹) and endothall (3000 µg ai L⁻¹) for exposure times of 24 h controlled Idaho submersed flowering rush by successfully reducing shoot and root biomass by >70%. Application strategies for complete control of triploid flowering rush shoots and roots with contact herbicides may require repeat applications and/or combinations with each other and systemic herbicides. Further evaluation of herbicides for controlling triploid as well as diploid flowering rush is warranted.

Key Words: *Butomus umbellatus*, diquat, endothall, flumioxazin, triploid.

INTRODUCTION

Flowering rush (*Butomus umbellatus* L.), a monocot introduced from Eurasia, has become invasive in the northern United States. It grows as an emergent plant along shorelines and as a submersed plant in deeper water of lakes and rivers (>3 m). Flowering rush can form monospecific stands, interfering with intended water uses and crowding out native plants (Boutwell 1990, Les and Mehrhoff 1999). The reproductive biology of flowering rush is quite complex and varies among populations (Kliber and Eckert 2005). The triploid or sterile phenotype spreads clonally by rhizomes and lateral rhizome buds. The diploid or fertile phenotype spreads through seed dispersal and reproductive structures (bulbils) that form on the root and inflorescence and break off to generate new plants. Once established, however, both phenotypes of flowering rush are difficult to control.

Aquatic herbicides may be an effective option to control flowering rush, yet systematic research of herbicide efficacy is lacking. Because both emergent and submersed forms create nuisance conditions, each morphology requires an independent control strategy. Focusing on the submersed plants, success or failure of herbicide treatment depends on the aqueous herbicide concentration that comes in contact with the target plant concomitant with the length of time the target plant is exposed to the dissipating herbicide concentration. Understanding this concentration/exposure time (CET) relationship is critical to achieve desirable control of nuisance submersed plants (Getsinger and Netherland 1997).

Because submersed flowering rush grows in waterbodies with fluctuating water levels, management of this plant has been inconsistent and unpredictable. Herbicide applications in these systems are typically subject to more extreme environmental variables than applications made to lakes with limited water flow. Most notably, lakes or a chain of lakes connected by a river have variable water exchange patterns that will impact aqueous distribution of herbicides resulting in reduced chemical exposure times against target plants and reduced efficacy. One option for control of flowering rush in these environments is the use of contact herbicides.

Contact herbicides are products that are fast-acting, usually requiring short exposure times that range from 6 to 24 h, depending on the active ingredient. They have a broad spectrum of activity and can be used to control most submersed plant species. The disadvantage of these products is that they rarely kill the entire plant, with the exception of annual plants and very young perennial plants (with poorly developed rootstock or root crown tissue). Robust perennial species treated with contact herbicides usually have the ability to recover from the herbicide exposure, and typically regrow from rootstock or root crown tissue located at or below the surface of the sediment.

In two separate experiments, we evaluated endothall (dipotassium salt) [7-oxabicyclo (2.2.1) heptane-2,3-dicarboxylic acid], diquat [6,7-dihydro-dipyrido (1,2-a:2',1'-c) pyrazinediium dibromide], and flumioxazin (2-[7-fluoro-3,4-dihydro-3-oxo-4-(2-propynyl)-2H-1,4-benzoxazin-6-yl]-4,5,6,7-tetrahydro-1H-isoindole-1,3(2H)-dione) for the control of submersed flowering rush under relatively short aqueous exposure time scenarios. In one experiment, all three herbicides were used against the flowering rush field-collected

from Minnesota. In another experiment, we used flowering rush that was field-collected from Idaho for a CET study of flumioxazin, which was recently registered by the US Environmental Protection Agency (EPA) for aquatic use. We incorporated one endothall treatment as a comparison using an older chemistry. Results of these evaluations will be used to provide guidance for management of submersed flowering rush in water bodies with short contact times.

MATERIALS AND METHODS

Minnesota flowering rush experiment

Flowering rush rhizomes were field-collected on 19 July 2010 from Big Detroit Lake, Pelican River Watershed, Minnesota. The Pelican River Watershed is composed of five natural lakes and two reservoirs connected by short segments of the Pelican River. These lakes are considered "ice-block" lakes, and are located in an outwash plain. Big Detroit Lake is one of the natural lakes in the watershed. It has an 840 ha surface area with 12.4 km of shoreline. The average depth is 5.6 m, and the maximum depth is 25 m. The littoral zone encompasses 40% of the lake. The shore of Big Detroit Lake is heavily populated and lined with residential development. Flowering rush occupies 25 ha of the lake.

After collection, rhizomes were shipped overnight to the US Army Engineer Research and Development Center (ERDC) in Vicksburg, Mississippi. Rhizomes surrounded by sediment were subjected to a cold treatment (4 C) for at least 3 weeks before sprouting. Rhizomes (4 to 5 cm in length) were then washed to remove sediment, placed in culture solution (Smart and Barko 1985) that was aerated, and allowed to sprout in an environmental growth chamber (48 m²) for 3 weeks.

Ambient conditions were set to provide optimum conditions for submersed plant growth: air temperature of 21 ± 2 C, light intensity ranging from 353 to 517 μmol m⁻² sec⁻¹, and photoperiod of 14 h:10 h light:dark cycle. On 31 August 2010, one sprouted rhizome (1 to 3 shoots; shoot length = 27 ± 1.6 cm) was planted to a depth of 4 cm in 1 L high-density polyethylene (HDPE) beakers filled with fertilized (150 mg L⁻¹ ammonium chloride) potting soil¹ and kitty litter². A thin layer of masonry sand (2 cm) 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 = 48 L) filled with culture solution (Smart and Barko 1985) amended with chelated iron (0.1 mg L⁻¹). Plants grew for 3 weeks to achieve the initial formation of a surface canopy (~60 cm) prior to herbicide application.

Herbicide application rates (Table 1) were selected based on several factors. Medium to high concentrations of each product were used because aqueous exposure times can be short where flowering rush grows. For example, exposure times in Detroit Lakes ranged from 4 to 78 h in field trials using 3000 μg active ingredient (ai) L⁻¹ endothall (Skogerboe 2010).

For herbicide application, stock solutions of endothall (Aquathol® K)³, diquat (Reward®)⁴ and flumioxazin (Clipper®)⁵ were prepared by diluting formulation concentrates in distilled water. From the stock, each herbicide was applied subsurface using a pipette to provide nominal concentrations in the treatment aquaria for the appropriate exposure

TABLE 1. CONCENTRATIONS AND EXPOSURE TIMES OF ENDOTHALL, DIQUAT, AND FLUMIOXAZIN EVALUATED AGAINST FIELD-COLLECTED MINNESOTA FLOWERING RUSH.

Herbicide Treatment	Concentration ($\mu\text{g ai L}^{-1}$)	Exposure (h)
Endothall	1500, 3000	12, 24
Diquat	370	6, 12
Flumioxazin	200	12, 24
Reference	0	24

time (Table 1). 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. The experiment was concluded on 19 October 2010, 4 weeks after treatment (WAT).

Water samples were collected 25 cm below the water surface from the diquat and endothall treatments at the end of each exposure time to ensure nominal herbicide concentrations were achieved. Samples were stored at 4 C until shipped for analysis. Endothall residues were analyzed in-house at ERDC using the enzyme-linked immunosorbent assay (ELISA) technique (Toth 1999), which can detect endothall concentrations as low as $7 \mu\text{g ai L}^{-1}$. Diquat residues were analyzed using HPLC-UV method following EPA protocol 549.1 by Pacific Agricultural Labs, Portland, Oregon. Water samples were not collected or analyzed for flumioxazin due to its rapid degradation at pH levels observed in this experiment (9.4 ± 0.1). Investigations have shown that flumioxazin degrades rapidly in pH >8.5 under laboratory and mesocosm conditions, with an aqueous half-life ranging from 17.5 to 102 min (Katagi 2003, Mudge 2007, Mudge et al. 2010).

Water temperature was measured continuously with an Optic Stowaway® Temperature Probe⁶ in reference aquaria. Water temperatures in aquaria were 21 ± 0.02 C during the experiment. The pH was measured with a handheld multi-parameter probe⁷.

Treatments were randomly assigned to individual aquaria and replicated three times, including the reference ($n = 3$). Herbicide efficacy was assessed by harvesting shoot and root biomass at 4 WAT. Biomass from all beakers in each aquarium were harvested, dried, and weighed for a dry weight measurement (g DW). Shoot and root biomass were analyzed using one-way analysis of variance (ANOVA) to determine herbicide effects. If effects were significant ($p \leq 0.05$), means were compared using the Tukey test.

Idaho flowering rush experiment

Flowering rush shoots, roots, and associated rhizomes were field-collected on 10 August 2010 from Lake Pend Oreille, a large natural lake in northern Idaho that stretches for 105 km beyond Albeni Falls Dam, which sits on the Pend Oreille River. The lake is fed by the Clark Fork and Pack Rivers and has a surface area of 380 km². The lake basin is deep and steep-sided with a maximum depth of 377 m and average depth of 164 m. The lake is located in a valley surrounded by mountains, national forests, and few small towns. Flowering rush coverage ranges from 20 to 81 ha.

After collection, plants were shipped overnight to ERDC. The next day, plants were washed and then floated in culture solution (Smart and Barko 1985) for one day before planting. The Idaho flowering rush experiment was also conducted in a walk-in controlled environment growth chamber (52 m²) at ERDC with ambient conditions similar to the Minnesota flowering rush experiment described above.

On 13 August, whole plants (shoots, roots, and rhizomes) were planted to a depth of 4 cm in 1 L HDPE beakers filled with fertilized (300 mg L^{-1} ammonium chloride) potting soil¹. A thin layer of silica sand (2 cm) was added to the sediment surface to prevent dispersion of nutrients and sediment into the water column. Two beakers were placed in each aquarium (volume = 48 L) filled with culture solution amended with chelated iron (0.1 mg L^{-1}). After 10 days, new shoots were emerging from the sediment surface, while older shoots were senescing. Plants grew for another week before herbicide application to allow the newly emerged shoots to reach the water surface (50 cm); senesced shoots were removed from the water column. Plants were young and actively growing at the time of herbicide application on 31 August. A handheld multi-parameter probe⁷ was used to measure water temperature and pH before herbicide application. Average water temperature was 22 C and pH was 9.0.

Flumioxazin application rates (Table 2) were selected to further refine CETs for this product against submersed flowering rush, and the endothall rate was used as a comparison with a product that was used in field trials (Skogerboe 2010). For herbicide application, stock solutions of each product were made and applied as in the previous experiment. The experiment continued for 6 WAT and was completed on 12 October 2010.

Treatments were randomly assigned to individual aquaria and replicated four times, including the reference ($n = 4$). Herbicide efficacy was assessed by harvesting shoot and root biomass at 6 WAT. Biomass from all beakers in each aquarium were harvested, dried, and weighed for a dry weight measurement (g DW). Shoot biomass and root biomass were analyzed using one-way ANOVA to determine herbicide effects. If effects were significant ($p \leq 0.05$), means were compared using the Tukey test.

Ploidy and genetic variation of flowering rush populations

Ploidy of the Minnesota flowering rush population has been documented to be triploid by Lui et al. (2005). Ploidy of Idaho flowering rush was determined using guard cell length following the methods of Kliber and Eckert (2005).

TABLE 2. CONCENTRATIONS AND EXPOSURE TIMES OF FLUMIOXAZIN AND ENDOTHALL EVALUATED AGAINST FIELD-COLLECTED IDAHO FLOWERING RUSH.

Herbicide Treatment	Concentration ($\mu\text{g ai L}^{-1}$)	Exposure Time (h)
Flumioxazin	50	24
	100	24
	200	12, 24
	400	12, 24
Endothall	3000	24
Reference	0	24

Plant populations with unknown ploidy can be classified using this method because triploid populations have had significantly larger mean guard cell length than diploids. Plant populations could be classified as triploid if mean guard cell length was $>42.63 \mu\text{m}$. We prepared slides for guard cell measurement by preserving the plant material in 3:1 ethanol and glacial acetic acid. This solution showed no cellular swelling compared to deionized water or saline. The plant material was mounted on slides in 3:1 ethanol and glacial acetic acid and sealed with acrylic. Measurements of a minimum of five guard cells per plant were obtained from four different plants within the Idaho population. The mean guard cell length was then used to infer ploidy.

We also examined genetic variation in the Minnesota ($n = 6$ individuals) and Idaho ($n = 10$ individuals) populations using amplified fragment length polymorphisms (AFLPs). Genomic DNA was extracted from fresh emergent vegetation using DNeasy Plant Mini Kits.⁸ AFLPs were prepared as described in Thum et al. (2011). We examined two selective primer pairs (EcoRI-ACA/ MseI-CAT and EcoRI-AGG/ MseI-CAT). Selective amplification products were run on an automated DNA sequencer⁹ using the internal size standard MapMarker1000 ROX¹⁰. We scored the AFLP data with GeneMapper v4.0,¹¹ limiting our analysis to fragments between 50 and 500 bp in length. Allele bins were determined using a peak height threshold (PHT) of 300 relative fluorescence units (RFU) and a bin width of 0.75 bp to ensure that only strong bands were included in the binset. We then automatically scored the data with the bin set using a PHT of 30 RFU, but visually checked and edited all allele calls.

RESULTS AND DISCUSSION

Minnesota flowering rush experiment

Aqueous diquat residues (mean ± 1 SE) were measured at $390 \pm 7 \mu\text{g ai L}^{-1}$ ($n = 6$) compared to the nominal concentration of $370 \mu\text{g ai L}^{-1}$. Aqueous endothall residues (mean ± 1 SE) were measured at $1822 \pm 61 \mu\text{g ai L}^{-1}$ ($n = 6$) and $3363 \pm 53 \mu\text{g ai L}^{-1}$ ($n = 6$) compared to the nominal concentrations of 1500 and 3000 $\mu\text{g ai L}^{-1}$, respectively. Because the recovery range was 100 to 110% of endothall in the ELISA analyses, the increase of 10% in actual concentrations compared to nominal concentrations is typical for aqueous residues of aquatic herbicides.

Significant reduction of shoot biomass ranged from 74 to 95% for the endothall and diquat CETs evaluated (Figure 1A). Plants in the diquat treatments showed symptoms of herbicide injury (chlorotic and disintegrating stems) at 4 days after treatment. By 1 WAT, endothall treatments were exhibiting chlorosis and browning. By 4 WAT, plants dosed with diquat and endothall were necrotic; however, new healthy shoots were emerging from the sediment. Endothall treatments also had healthy green tissue at the lower levels of older shoots at the conclusion of the experiment. Neither the concentration nor the exposure time made a difference in the level of control achieved with endothall because all treatments were statistically similar; therefore, a concentration of 1500 $\mu\text{g ai L}^{-1}$ for a 12 h exposure was as effective as a concentration of 3000 $\mu\text{g ai L}^{-1}$ for a 24 h exposure. Flumioxazin applied at 200 $\mu\text{g ai L}^{-1}$

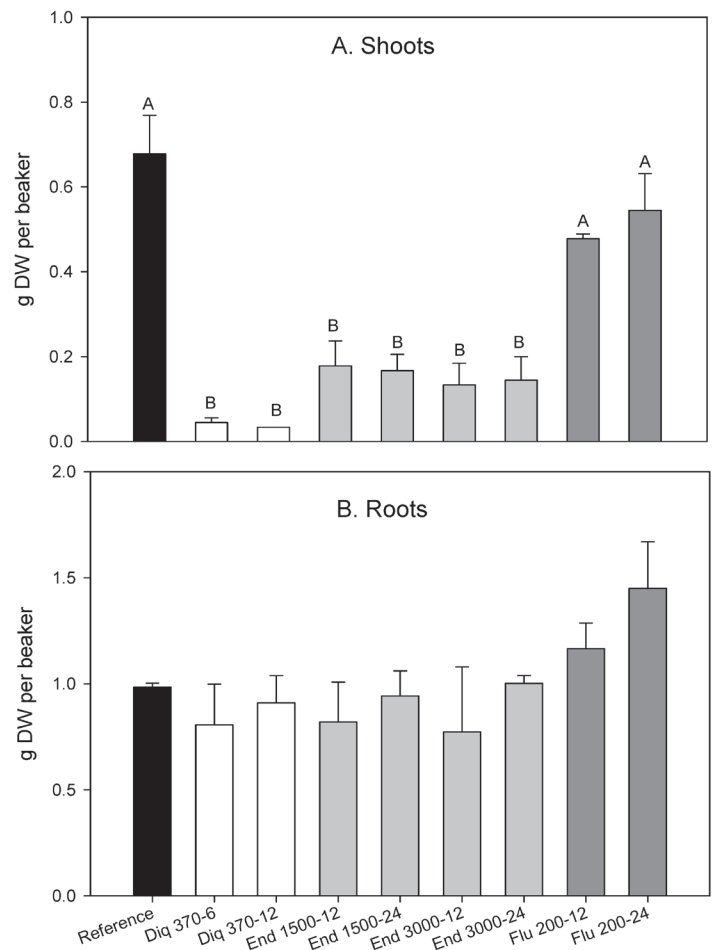


Figure 1. Shoot (A) and root (B) biomass (g DW) of Minnesota flowering rush (mean ± 1 SE, $n = 4$) at 4 weeks after treatment with diquat (Diq), endothall (End), and flumioxazin (Flu). Numbers behind herbicide abbreviations represent herbicide concentrations ($\mu\text{g ai L}^{-1}$) with exposure time (h). Treatments with the same letter are not significantly different (Tukey test, $p \leq 0.05$).

ai L^{-1} (50% maximum label rate) for 12 or 24 h exposure was not effective because shoot biomass was not significantly different from the untreated reference. By 1 WAT, plant stems in the flumioxazin treatments were chlorotic and red, but these early injury symptoms did not lead to major loss of mass at 4 WAT, with some shoots remaining green and viable. The lack of efficacy may be related to the high aqueous pH at time of treatment (9.4), thereby increasing herbicide concentration and/or exposure time required for plant death.

None of the contact herbicide treatments evaluated significantly reduced root biomass compared to the untreated reference (Figure 1B). All treatments had lateral rhizome buds present at the end of the experiment. Moreover, root biomass increased by almost 100% compared to pretreatment levels (0.44 g DW per beaker).

It is uncertain if the treatment of newly sprouted rhizomes would provide better control of flowering rush root biomass. Although application of contact herbicides to newly sprouted vegetative propagules (tubers) was successful in controlling hydrilla in small-scale studies (Van and Conant 1988), her-

bicide treatments in operational applications would be difficult if sprouting of flowering rush rhizomes is nonseasonal or random.

Idaho flowering rush experiment

Flumioxazin concentrations of 400 $\mu\text{g ai L}^{-1}$ for a 24 h exposure time reduced shoot biomass by 82% and root biomass by 72% (Figure 2A). Visually, these plants had a collapsed canopy with dead shoots floating in the water column. Healthy green shoots were present growing from the sediment with a few shoots at the water surface.

When reducing the concentration by 50% (200 $\mu\text{g ai L}^{-1}$ for 24 h) or exposure time by 50% (400 $\mu\text{g ai L}^{-1}$ for 12 h), the efficacy of flumioxazin was reduced by 30 to 40%. These plants had intact canopies with healthy green shoots as well as brown dead ones. Although some reddening of stems and necrosis occurred in plants dosed with 50 and 100 $\mu\text{g ai L}^{-1}$ for 24 h and 200 $\mu\text{g ai L}^{-1}$ for 12 h, shoot biomass in these treatments was comparable to the reference. Shoot biomass in plants dosed with 3000 $\mu\text{g ai L}^{-1}$ endothall for 24 h was reduced by >90%.

Despite a trend of decreased root biomass with increasing herbicide concentration, root biomass for all flumioxazin treatments was statistically similar to the untreated reference due to treatment variation between replicates (Figure 2B). Plants dosed with 400 $\mu\text{g ai L}^{-1}$ for a 24 h exposure time were similar in root biomass to both the untreated reference and the endothall treatment. Reference plants and those that were dosed with 50 and 100 $\mu\text{g ai L}^{-1}$ had new rhizome growth. Few rhizomes were harvested in the 200 and 400 $\mu\text{g ai L}^{-1}$ flumioxazin treatments, and no rhizomes were recovered in the endothall treatments.

Flumioxazin has the potential to control submersed flowering rush at the maximum label rate (400 $\mu\text{g ai L}^{-1}$), even with a water column pH >9. Because flumioxazin potentially hydrolyzes in minutes at the high pH levels evaluated in this study (Katagi 2003), flowering rush and other aquatic plants are able to rapidly uptake flumioxazin. In a mesocosm study, the half-life of flumioxazin was 1.7 h at pH >8.5 in the water column, yet hydrilla was able to rapidly uptake flumioxazin with significant phytotoxic effects at 400 $\mu\text{g ai L}^{-1}$ (Mudge et al. 2010); however, for successful control of hydrilla (>70% biomass reduction) either higher concentrations or lower pH levels are required (Mudge and Haller 2010). Increased efficacy of flowering rush would likely be achieved in waterways with neutral pH ranges (pH = 7) because hydrolysis of flumioxazin is 16 to 18 h at lower pH levels (Katagi 2003, Mudge et al. 2010). Further testing of flumioxazin efficacy in a neutral pH would be beneficial in determining whether flowering rush is inherently tolerant or susceptible to this product.

Ploidy and genetic variation of flowering rush populations

Like the Minnesota population (Lui et al. 2005), the Idaho flowering rush population was determined to be triploid (mean ± 1 SE guard cell length = $58.9 \pm 1.6 \mu\text{m}$). Triploid plants produce more shoot, root, and rhizome biomass, as well as more numerous lateral rhizome buds than diploid plants; therefore, limited seed production in triploids is compensated for by more intensive vegetative reproduction (Hroudová and Zákřavský 1993). In these experiments, the root:shoot of reference Minnesota plants was 1.4 and the root:shoot for Idaho reference plants was 1.8. These ratios are similar to findings from a greenhouse experiment using emergent flowering rush that was predominately field-collected from locations in Midwestern North America (Lui et al. 2005).

The two selective primer pairs resulted in 136 bands in our AFLP analysis. Surprisingly, all of the individuals from both of our lakes were genetically identical for their AFLP genotypes. In addition, we analyzed several plants from each of four other populations (Silver and Long Lakes in WA and Noxon and Thompson Reservoirs in MT; data not shown), and these individuals also had identical AFLP profiles to the Minnesota and Idaho populations examined here. This suggests that these individuals are either all truly genetically identical (i.e., are different ramets of the same genet), or that genetic diversity for AFLPs is extremely low for flowering rush. Our results stand in contrast to earlier studies examining genetic variation in flowering rush that employed random amplified polymorphic DNA (RAPD) markers (Eckert

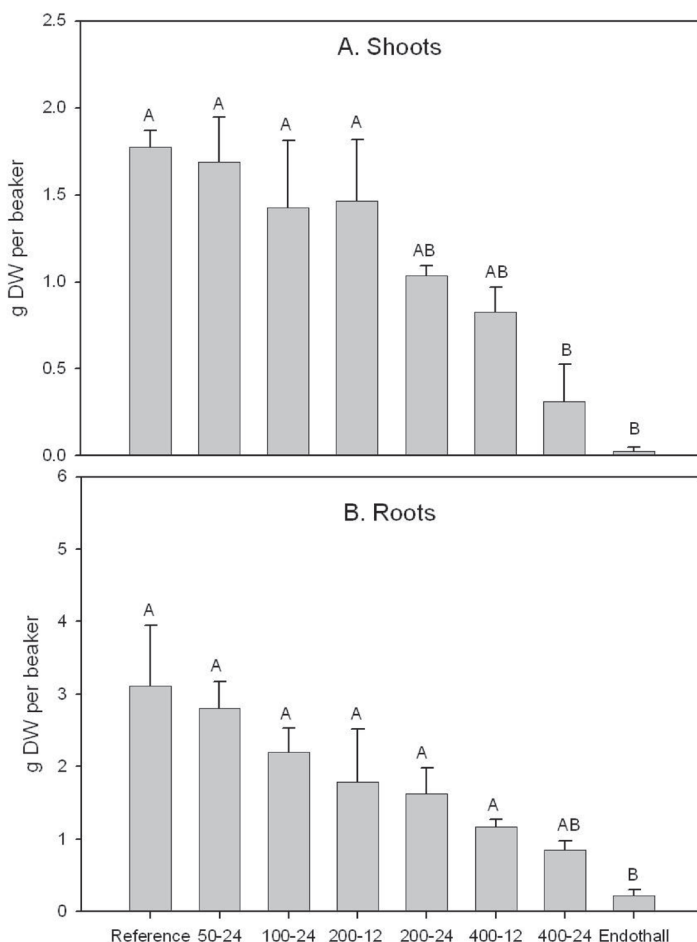


Figure 2. Shoot (A) and root (B) biomass (g DW) of Idaho flowering rush (mean ± 1 SE, n = 4) at 6 weeks after treatment with flumioxazin and endothall (3000 $\mu\text{g ai L}^{-1}$; 24 h exposure time). Flumioxazin concentrations ($\mu\text{g ai L}^{-1}$) are followed by exposure time (h). Treatments with the same letter are not significantly different (Tukey test, $p \leq 0.05$).

et al. 2003, Kliber and Eckert 2005). Guard cell analysis of the four populations not evaluated with herbicides in these experiments indicated that they were also triploid (mean guard cell length range = 52.8 ± 2.0 to 60.1 ± 2.7 μm). Thus, one explanation for the identical genetic profiles is that all six of these flowering rush populations are closely related or identical triploid genotypes, which has implications for their potential expansion and management; however, it is also possible that genetic variation may be tied to the molecular techniques employed, in which variation is lower for AFLPs than for RAPDs. In either case, further study of diploid and triploid flowering rush genotypes is warranted.

As expected for genetically similar plants, flowering rush shoot and root biomass from both populations responded similarly to flumioxazin concentrations and exposure times that were used in both experiments. Conversely, endothall concentrations of 3000 $\mu\text{g ai L}^{-1}$ for a 24 h exposure period greatly reduced root biomass of Idaho plants, but not Minnesota plants. One explanation may be the difference in treatment timing for each experiment. In the Minnesota experiment, herbicide treatments were applied to plants that grew for 3 weeks from sprouted rhizomes, while in the Idaho experiment herbicides were applied to field-collected mature plants that had undergone senescence and regrowth.

The results of these small-scale experiments show that contact herbicides are effective against flowering rush. Concentrations of diquat (370 $\mu\text{g ai L}^{-1}$) for exposure times of 6 and 12 h and endothall (1500 and 3000 $\mu\text{g ai L}^{-1}$) for exposure times of 12 and 24 h reduced shoot biomass of Minnesota submersed flowering rush by >70%; however, these treatments did not significantly impact root biomass. Concentrations of flumioxazin (400 $\mu\text{g ai L}^{-1}$) and endothall (3000 $\mu\text{g ai L}^{-1}$) for exposure times of 24 h controlled Idaho submersed flowering rush by successfully reducing shoot and root biomass by >70%.

Application strategies for complete control of triploid flowering rush with contact herbicides may require repeat applications or higher concentrations (as in the case of endothall and flumioxazin). Combinations of contact herbicides may also improve efficacy, such as the combination of flumioxazin and diquat, or the combination of endothall and diquat, which enhanced hydrilla control in another experiment (Pennington et al. 2001). Combination of contact herbicides with systemic herbicides, perhaps the synthetic auxins, would augment control of both flowering rush populations, with the potential to reduce roots and rhizomes as well as lateral root buds. For example, endothall has been combined with the auxin-type compounds, 2,4-D and triclopyr, for increased control of Eurasian watermilfoil (Madsen et al. 2010). Although synthetic auxin herbicides are typically selective for dicot species, activity against monocots has been reported (Belgers et al. 2007).

Validation of these experimental results in a larger experimental system should be conducted. Using a mixed plant community of nontarget vegetation would help determine species selectivity. Early spring applications would be advantageous when using contact herbicides against submersed flowering rush because impacts on collateral nontarget native vegetation that is still in winter quiescence would be mitigated (Netherland et al. 2000, Poovey et al. 2002, Skogerboe et

al. 2008). Moreover, young triploid flowering rush plants with less biomass than mature plants would be more susceptible to herbicides, and vegetative reproduction potential would be reduced. Early spring applications also would be beneficial for selectively controlling diploid flowering rush to minimize reproduction through seeds and bulbils; however, herbicide response of diploid plants still needs to be determined.

ACKNOWLEDGMENTS

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SOURCES OF MATERIALS

- ¹Black Kow® Topsoil, Black Gold Compost Co., Oxford, FL
- ²Special Kitty, Cat Litter, WalMart, Vicksburg, MS
- ³Aquathol® K, United Phosphorus Inc., King of Prussia, PA
- ⁴Reward® Syngenta Corp., Wilmington, DE
- ⁵Clipper® Valent Corp., Walnut Creek, CA
- ⁶Optic Stowaway® Temperature Probe, Onset Computer Corp., Bourne, MA
- ⁷YSI Model 556, Handheld Multi-Parameter Probe, YSI, Yellow Springs, OH
- ⁸DNeasy® Plant Mini Kits, Qiagen, Valencia, CA
- ⁹ABI 3130xl DNA sequencer, Applied Biosystems, Carlsbad, CA
- ¹⁰MapMarker®, 1000 ROX, BioVentures, Inc., Murfreesboro, TN
- ¹¹GeneMapper®, v4.0, Applied Biosystems, Carlsbad, CA

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Does hydrilla grow an inch per day? Measuring short-term changes in shoot length to describe invasive potential

LEEANN M. GLOMSKI AND MICHAEL D. NETHERLAND*

INTRODUCTION

The ability of invasive plants such as hydrilla (*Hydrilla verticillata* [L.f.] Royle) and Eurasian watermilfoil (*Myriophyllum spicatum* L.) to grow and rapidly expand has been well described in both popular and scientific literature (Steenis 1967, Adams and McCracken 1974, Johnson and Manning 1974, Haller 1976, Sutton et al. 1992). Infestations are often reported in terms of percent cover or percent frequency at the lake scale, while dry weight biomass per square meter is generally reported for scientific studies at laboratory and field scales. Studies have also focused on growth from vegetative propagules and subsequent propagule production as endpoints (Van and Steward 1990, Sutton et al. 1992, Spencer et al. 2000). Nonetheless, when viewed in terms of overall productivity, submersed aquatic plants produce much less biomass when compared to their terrestrial counterparts (Westlake 1963, Grace and Wetzel 1978). Moreover,

prior studies have demonstrated that Eurasian watermilfoil is not particularly productive when biomass production is compared to other submersed native species (Grace and Wetzel 1978, Smith and Barko 1990). While the vast majority of scientific trials have focused on changes in biomass or propagule production as an endpoint for evaluating growth or response of hydrilla or Eurasian watermilfoil to control methods, measuring the change in total stem length may be a useful technique for explaining rapid rates of lateral plant spread in a water body. Emphasis has often been placed on hydrilla and Eurasian watermilfoil concentrating biomass in a surface canopy (Haller and Sutton 1975, Grace and Wetzel 1978, Madsen 1997), yet production of numerous lateral stems and stolons and the subsequent rates of extensions may better explain rapid radial expansion. Hydrilla stolons, for example, have been documented to expand a colony radially at a rate of 4 cm d⁻¹ (Madsen and Smith 1999). While the statement that “hydrilla can grow an inch a day” (Langeland 1996) initially sounds impressive, it is unlikely this rate of extension would explain the ability of hydrilla to form large contiguous surface canopies on hundreds or thousands of acres in lakes and reservoirs.

To evaluate the rate of growth of hydrilla and Eurasian watermilfoil, a series of mesocosm trials were conducted to mea-

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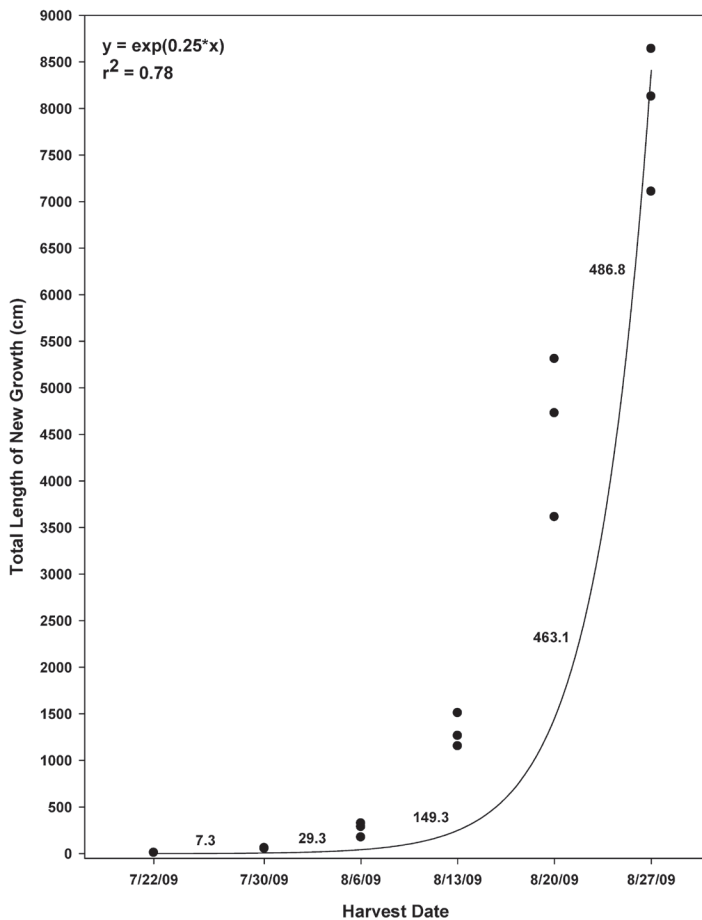


Figure 1. Mean hydrilla growth (cm) \pm SE. Values reported directly on the graph represent the daily expansion rate in cm day⁻¹ for weeks 1 through 5 of the study.

sure the change in shoot length over a 35-day period during summer growth conditions. The objective of this study was to compare the shoot growth of two introduced and two native plant species and evaluate the data to determine if rates of overall shoot extension may help explain invasive properties.

MATERIALS AND METHODS

To determine the rate of shoot extension of hydrilla, Eurasian watermilfoil, American pondweed (*Potamogeton nodosus* Poir.), and water stargrass (*Heteranthera dubia* [Jacq.] MacMill.), mesocosm studies were conducted at the U.S. Army Engineer Research and Development Center, Lewisville Aquatic Ecosystem Research Facility (LAERF) in Lewisville, Texas. Hydrilla was evaluated 22 July through 27 August 2009. Eurasian watermilfoil was evaluated twice, 26 March through 3 May and 7 May through 14 June 2010, and temperature was recorded throughout both study periods. Both native species were evaluated 23 June through 2 August 2010. Eurasian watermilfoil exists as an evergreen and is typically active in the fall, winter, and spring in Texas. Therefore this portion of the study was conducted to compare early spring and late spring rates of growth. Hydrilla and both native species were evaluated during summer months when growth rates are expected

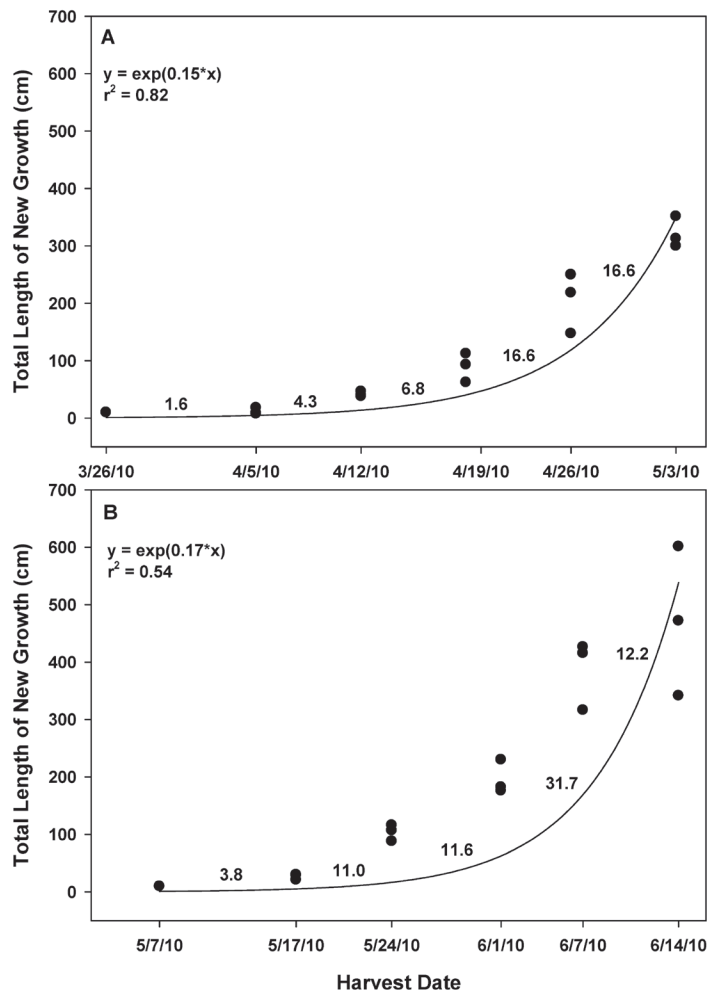


Figure 2. Mean Eurasian watermilfoil growth (cm) \pm SE in A) study 1 and B) study 2. Values reported directly on the graph represent the daily expansion rate in cm day⁻¹ for weeks 1 through 5 of the study.

to be at a maximum due to the increased photoperiod and mesocosm temperatures in the range of 28 to 30 C. Plants were obtained from LAERF ponds, and one 15 cm apical stem section was planted in pots (3.78 L) filled with LAERF pond sediment amended with 3 g L⁻¹ Osmocote (16-8-12). Apical stem sections were planted so that 10 cm was initially above the sediment surface. Ten pots were placed into each of three 7000 L outdoor mesocosms, which were filled with lake water. Two pots from each replicate tank were harvested each week for 5 weeks, and all stems in the pot were measured for total length. To determine centimeters of growth per day for a given week, the previous week's average was subtracted from the current week's average and then divided by the number of days between the two harvests. The number of lateral branches, new stems, and stolons was also recorded. Lateral branches were defined as secondary branches, new stems as stems originating from the root crown or from nodes along the stolons, and stolons as horizontal stems atop the sediment surface originating from the root crown (Haller 1976, Yeo et al. 1984). Plant length data were subjected to regression analysis.

TABLE 1. NUMBER OF LATERAL BRANCHES, NEW STEMS AND STOLONS (\pm SE) PRODUCED BY HYDRILLA, EURASIAN WATERMILFOIL (STUDY 1 AND 2), AMERICAN PONDWEED, AND WATER STARGRASS EACH WEEK.

	Week 1	Week 2	Week 3	Week 4	Week 5
Hydrilla					
Lateral Branches	2 \pm 2	13 \pm 6	43 \pm 11	129 \pm 29	157 \pm 38
New Stems	2 \pm 0	6 \pm 1	34 \pm 13	71 \pm 16	190 \pm 63
Stolons	0 \pm 0	1 \pm 1	3 \pm 2	9 \pm 6	35 \pm 20
Eurasian watermilfoil 1					
Lateral Branches	1 \pm 1	3 \pm 1	4 \pm 0	10 \pm 2	12 \pm 0
New Stems	0 \pm 0	0 \pm 0	0 \pm 0	1 \pm 0	1 \pm 0
Stolons	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
Eurasian watermilfoil 2					
Lateral Branches	2 \pm 0	3 \pm 1	5 \pm 1	8 \pm 2	11 \pm 4
New Stems	0 \pm 0	0 \pm 0	1 \pm 0	2 \pm 0	3 \pm 0
Stolons	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
American pondweed					
Lateral Branches	1 \pm 1	1 \pm 1	1 \pm 0	2 \pm 1	1 \pm 0
New Stems	0 \pm 0	0 \pm 0	2 \pm 0	4 \pm 0	8 \pm 2
Stolons	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
Water stargrass					
Lateral Branches	1 \pm 0	1 \pm 0	1 \pm 0	1 \pm 1	1 \pm 1
New Stems	0 \pm 0	0 \pm 0	1 \pm 1	1 \pm 0	3 \pm 0
Stolons	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0

RESULTS AND DISCUSSION

Hydrilla increased by 7.3 cm d⁻¹ in the first week of the study (Figure 1). New growth was primarily from elongation of the initial stem as well as the formation of lateral stems. In week 2, new stems originating from the base of the initial plant accounted for most of the new growth, and hydrilla grew 29.3 cm d⁻¹. By week 3, stolons and new stems along the stolon had formed, and the growth rate of hydrilla increased to 149.3 cm d⁻¹. The growth rates for hydrilla in weeks 4 and 5 were 463.1 and 486.8 cm d⁻¹, respectively (Figure 1). Hydrilla had also produced 190 new stems by week 5 (Table 1). From the original 10 cm shoot, hydrilla had increased to over 8000 cm of shoot tissue in a 35-day period. The early lag period was followed by rapid expansion in weeks 4 and 5. In terms of answering the question of whether hydrilla grows an inch a day, the current results would paint a more complex picture that suggests the initial 10 cm shoot was extending both vertically and horizontally by up to 191 inches per day. While growth of individual shoots may be closer to the 1 to 4 inches per day commonly cited, hydrilla was producing numerous stolons and lateral stems that resulted in a rapid three dimensional or radial expansion from the original single 10 cm shoot.

Compared to hydrilla, Eurasian watermilfoil shoot extension was considerably reduced in both trials. Overall, Eurasian watermilfoil growth was greater in study 2 than in study 1 (Figure 2A and B). This difference may have been related to temperatures, which ranged from 13 to 23 C in study 1 and 18 to 36 C in study 2. Eurasian watermilfoil can photosynthesize at temperatures as low as 10 C, with the optimal range occurring between 30 and 35 C (Stanley and Naylor 1972). The maximum growth rate for Eurasian watermilfoil was 16.6 and 31.7 cm d⁻¹ in week 4 of study 1 and 2 respectively. The number of lateral branches produced by Eurasian watermil-

foil was similar in both studies and along with elongation of the main stem accounted for most of the new growth (Table 1). While interspecific competition was not evaluated in these trials, such a large disparity between total stem lengths of hydrilla versus Eurasian watermilfoil suggests that hydrilla would have a strong competitive advantage under these growth conditions.

The growth rate of American pondweed ranged from 1.8 to 32.9 cm d⁻¹ with the maximum during week 4 (Figure 3A). Water stargrass growth ranged from 2.4 to 12.6 cm d⁻¹ with the maximum also occurring during week 4 (Figure 3B). The increase in shoot length was generally linear through the 5-week study, and the total stem values of approximately 200 and 400 cm was 20 to 40 times less than observed for hydrilla, but similar to that of Eurasian watermilfoil.

The use of large mesocosm tanks and the short-term nature of these studies prevented hydrilla from approaching carrying capacity. Moreover, interspecific competition was not a factor reducing the rates of growth. While the shoot expansion rates reported would slow greatly as hydrilla formed a canopy, this study demonstrates that following a short initial lag, hydrilla increased rapidly, and the production of numerous lateral stems and stolons helps to explain radial expansion as a key invasive trait. While this 35-day study was conducted during a period when maximum rates of growth would be expected, similar environmental conditions can generally exist from May through September in many southern states. In a Brazilian trial, hydrilla doubling times of 2.5 to 19 days were reported (Bianchini et al. 2010), and our current results with shoot extension would support this rate of expansion. While measuring stem extension is quite labor intensive compared to collecting plants for dry weight biomass, this type of data provides a unique look at how hydrilla expansion can be rapid as the plant fills the available water column with biomass.

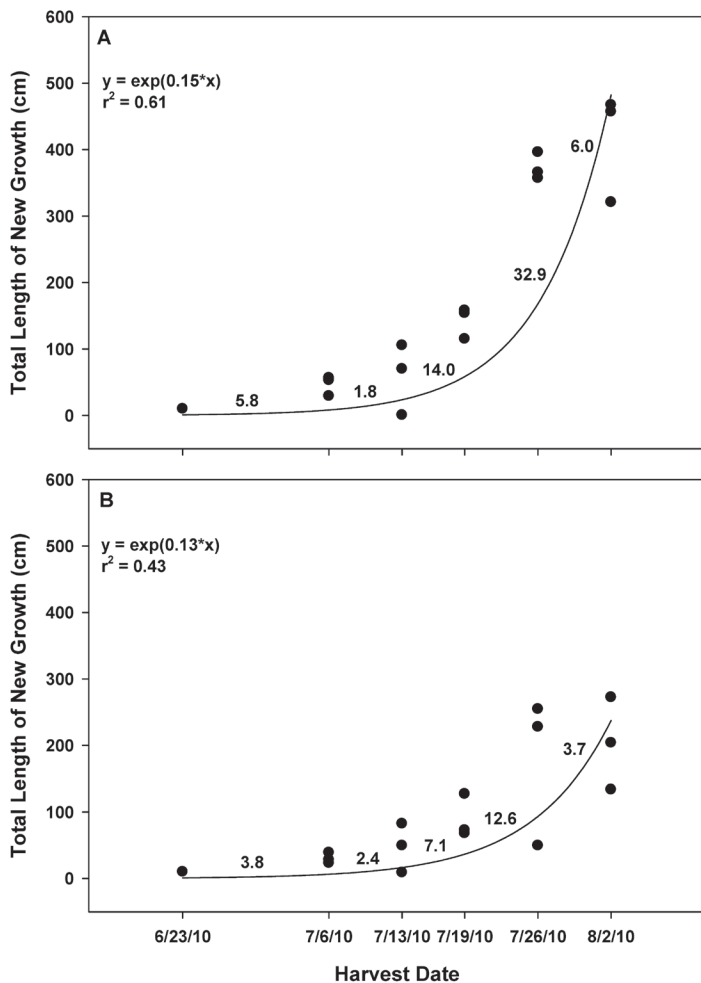


Figure 3. Mean (A) American pondweed and (B) water stargrass growth (cm) \pm SE. Values reported directly on the graph represent the daily expansion rate in cm day⁻¹ for weeks 1 through 5 of the study.

The reduced shoot extension rate for the native plants when compared to hydrilla was generally expected; however, the comparative performance of the Eurasian watermilfoil was much lower than expected. The lack of lateral shoot and new stem production by Eurasian watermilfoil during the two studies was in contrast to hydrilla and may, in part, explain lower shoot extension values observed during these short-term studies. Eurasian watermilfoil will overwinter as an evergreen, and new growth is often observed from large and well-established root crowns (Smith and Barko 1990). In this study, we were evaluating growth from single apical shoot fragments that do not contain starch reserves to support fast, copious shoot growth. Future studies evaluating rates of Eurasian watermilfoil expansion from established root crowns that have overwintered are planned.

Data generated from this trial may be useful in conveying the potential invasiveness of hydrilla to public and pri-

vate stakeholder groups that have an interest, but not an expertise, in aquatic plant management. When describing hydrilla growth, a term such as “300 g dry weight m⁻³” can be confusing and lack context, whereas reporting changes in length over a short period of time is intuitive and may better describe how hydrilla can readily dominate a given marina, cove, or waterbody.

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