J. Aquat. Plant Manage. 45: 116-118

NOTES

Efficacy of Fluridone on Eurasian and Hybrid Watermilfoil

JEREMY G. SLADE[1,2], ANGELA G. POOVEY[2], AND MICHAEL D. NETHERLAND[3]

INTRODUCTION

A nuisance throughout the United States and Canada, Eurasian watermilfoil (*Myriophyllum spicatum* L.) is an exotic, invasive submersed aquatic weed found in many lakes and rivers. Primarily known for its ability to form dense surface canopies, Eurasian watermilfoil limits recreational activity, reduces native macrophyte diversity (Boylen et al. 1999), and disrupts predator-prey interactions (Crowder and Cooper 1982). Recently, Eurasian watermilfoil was also documented as having hybridized with the native watermilfoil species *M. sibiricum* (Moody and Les 2002).

Control of Eurasian watermilfoil with the systemic herbicide fluridone {1-methyl-3-phenyl-5-[3-(trifluoromethyl) phenyl-4 (1H)-pyridinone} has been well documented in the laboratory and the field (Netherland et al. 1993, Netherland and Getsinger 1995, Smith and Pullman 1997, Sprecher et al. 1998, Poovey et al. 2004). However, herbicide efficacy data for hybrid watermilfoil genotypes (*M. spicatum* × *M. sibiricum*) is unknown. There is concern that hybridization between watermilfoil species could result in the development of enhanced competitive or invasive traits leading to a more herbicide resistant genotype, as described for some terrestrial plants (Ellstrand and Schierenbeck 2000, Barton 2001). Rajguru et al. (2005) documented herbicide resistance transferal to hybrid rice offspring after interspecific crosses between herbicide resistant rice (*Oryza sativa*) and herbicide susceptible rice (*O. sativa*). In a similar study, Wetzel et al. (1999) also documented transferal of herbicide resistance to hybrid offspring from interspecific crosses of two *Amaranthus* species. In addition, anecdotal reports suggest some watermilfoil populations in the upper Midwestern U.S. have shown increased resistance to the herbicide fluridone and there is speculation that this may be related to hybridity. However, there has been no proven linkage, and to date, no empirical evidence to support this claim.

The recent and unexpected development of fluridone resistance by dioecious hydrilla (*Hydrilla verticillata* L.f. Royle) demonstrates that a submersed plant with a high vegetative growth rate, similar to Eurasian watermilfoil, has the capacity for development of increased resistance to fluridone (Michel et al. 2004). The production of viable seed by watermilfoils also affords it another mechanism for resistance development. The changes in efficacy that have been reported in the field suggest a subtle shift in Eurasian watermilfoil susceptibility to fluridone. Nonetheless, current low-use rate strategies for fluridone indicate that minor shifts in susceptibility could result in significant differences in treatment outcomes.

It is uncertain whether watermilfoil hybrids are inherently more tolerant to fluridone applications or that they may overcome herbicide effects due to hybrid vigor, therefore, we conducted a small-scale study to document the impact of low-use rates of fluridone on both the Eurasian and hybrid watermilfoil genotypes.

MATERIALS AND METHODS

A small-scale study was conducted at the U.S. Army Engineer Research and Development Center (ERDC), Vicksburg, MS, in a controlled-environment growth chamber to evaluate efficacy of fluridone on Eurasian and hybrid watermilfoil. Experimental conditions within the chamber were maintained to mimic ambient conditions conducive for submersed plant growth: water temperature of 24 ± 1°C, light intensity of 311 ± 75 µmol/m^2/sec, and a photoperiod of 14:10-hr light:dark cycle. Lighting was provided with 400-watt metal halide bulbs.

Known strains of Eurasian and hybrid watermilfoil were collected from Medicine Lake (Hennepin County) and Otter Lake (Anoka County), MN, respectively. Confirmation of genotypes was performed in a previous study (unpublished data, M. Netherland). Additionally, Moody and Les (2007) documented the presence of hybrid watermilfoil in Otter Lake, and noted in Minnesota lakes where hybrid watermilfoil was present, Eurasian watermilfoil was absent and vice versa. However, this does not imply that hybrid and Eurasian watermilfoil may not co-occur (Moody and Les 2007). Neither lake has a history of fluridone applications.

Three apical meristems (15 cm length) of each genotype were rinsed and planted in 450-ml plastic beakers filled with sediment collected from Brown's Lake, Vicksburg, MS, and amended with ammonium chloride at a rate of 0.2 g/L. A 0.5

[1]Department of Wildlife and Fisheries, Mississippi State University, P.O. Box 9690, Mississippi State, MS 39762.

[2]U.S. Army Engineer Research and Development Center, 3909 Halls Ferry Rd., Vicksburg, MS 39180.

[3]U.S. Army Engineer Research and Development Center, Center for Aquatic and Invasive Plants, 7922 NW 71st Street, Gainesville, FL 32653. Received for publication February 6, 2007 and in revised form April 14, 2007.

cm layer of course grit silica sand was then added to the sediment surface to reduce sediment and nutrient dispersion into the water column. Five planted beakers with either the Eurasian or hybrid genotype were placed in designated aquaria (52 L capacity) containing 48 L of Smart and Barko (1985) culture solution. Twelve tanks were filled with the Eurasian watermilfoil and twelve tanks were filled with hybrid watermilfoil. Tanks were treated three weeks after planting before canopy formation and prior to shoots reaching the water surface. At the time of treatment, plants were healthy and actively growing.

A stock solution of fluridone as Sonar™ A.S. (SePRO Corporation, Carmel, IN) was prepared by diluting 1 ml of formulated herbicide in 1 L of distilled water. From the stock, two rates of fluridone were applied to aquaria to provide rates of 5 and 10 µg ai/L. A static exposure was used. Untreated references were included to evaluate plant growth in absence of herbicide treatment. Pretreatment shoot biomass samples were collected from one beaker, one day prior to herbicide application by cutting all above ground plant material. This study was terminated 45 days after treatment (DAT) and all above ground plant material from the four remaining beakers was collected. All shoot material was oven dried at 70°C for 48 hrs to obtain a dry weight biomass measurement (g DW).

Fluridone's mode of action is disruption of the carotenoid biosynthetic pathway (WSSA 2002), which results in an increase in the carotene precursor, phytoene, and a concomitant reduction of the pigment β-carotene in shoot meristems (Sprecher et al. 1998). Therefore, herbicide injury can be determined through quantification of the pigment β-carotene in plant apices and shoots. Analyses of β-carotene were conducted 5, 10, and 20 DAT. The β-carotene pigment was extracted and quantified according to the protocol established by Sprecher et al. (1998).

Treatments were assigned to aquaria in a completely randomized manner and replicated four times. Means for each replicate were calculated from post-treatment harvest data, and then subjected to a one-way analysis of variance (ANOVA) using Sigmastat (version 3.1, Systat Software, Inc., Point Richmond, CA) to test for herbicide concentration effects of fluridone. If the assumptions of normality and equal variance were not met, data was analyzed using the Kruskal-Wallis one-way ANOVA based on ranks. For shoot biomass, if effects were significant ($p \leq 0.05$), means were separated using the Student-Newman-Kuels (S-N-K) method.

RESULTS AND DISCUSSION

Fluridone effects on β-carotene were documented as early as 5 DAT in both the Eurasian and hybrid watermilfoil genotypes (Table 1). The β-carotene content of treated plants decreased between 5 and 20 DAT for both genotypes and these values were lower than the untreated references indicating that the herbicide effects were phytotoxic. Additionally, fluridone characteristics (i.e., bleached apices) were visible and indistinguishable in treated plants of both genotypes, while there was an obvious difference between treated and untreated plants. Untreated plants remained green with thick growth throughout the study.

TABLE 1. MEAN (± SE) β-CAROTENE CONCENTRATIONS (MG/G FRESH WEIGHT), IN APICES OF EURASIAN AND HYBRID WATERMILFOIL SHOOTS 5, 10, AND 20 DAYS AFTER TREATMENT (DAT) FOLLOWING A STATIC EXPOSURE TO 0, 5, AND 10 µG FLURIDONE/L. WITHIN EACH COLUMN, VALUES FOLLOWED BY A DIFFERENT LETTER ARE SIGNIFICANTLY DIFFERENT ACCORDING TO STUDENT-NEWMAN-KUELS METHOD AT $P \leq 0.05$.

	β-carotene concentration (mg/g fresh weight)		
Fluridone rate (µg ai/L)	5 DAT	10 DAT	20 DAT
Eurasian watermilfoil			
0 µg ai/L	25.8 ± 2.5 A	26.0 ± 2.7 B	28.8 ± 1.3 B
5 µg ai/L	17.4 ± 1.2 B	17.5 ± 2.8 CD	12.9 ± 1.0 C
10 µg ai/L	15.1 ± 1.5 BC	9.2 ± 0.9 E	10.4 ± 4.1 D
Hybrid watermilfoil			
0 µg ai/L	24.4 ± 2.3 A	32.3 ± 3.4 A	31.2 ± 1.1 A
5 µg ai/L	13.6 ± 1.8 C	19.7 ± 2.7 C	8.2 ± 0.8 D
10 µg ai/L	10.4 ± 1.8 D	13.5 ± 3.0 D	6.5 ± 1.3 E

There was no difference in mean shoot biomass between untreated Eurasian and hybrid genotypes (Figure 1). Both fluridone treatment rates resulted in biomass reductions compared to untreated controls; however, no differences in mean shoot biomass of Eurasian and hybrid watermilfoil were noted between the 5 and 10 µg ai/L treatments (Figure 1). Both rates of fluridone provided 80% or greater control of shoot biomass in both genotypes through 45 DAT compared to the untreated plants. At the end of the study, only small (0.1 m) leafless stems remained in treated tanks of both watermilfoil genotypes.

Eurasian and hybrid watermilfoil responded similarly to the fluridone treatments in this study. For both genotypes, β-carotene levels increased from 5 to 10 DAT and decreased from 10 to 20 DAT (except Eurasian at 10 µg ai/L). In addition, in all hybrid treatments (except hybrid at 10 DAT), β-carotene values were all lower than the Eurasian genotype.

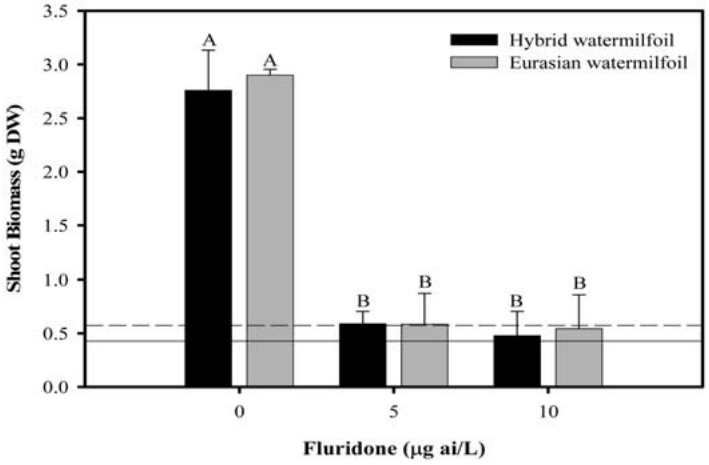

Figure 1. Shoot dry weight biomass (g DW) of hybrid and Eurasian watermilfoil (mean ± SD) treated with fluridone. Biomass harvested 45 days after treatment (DAT). Horizontal lines represent mean pretreatment biomass for each biotype (dashed = Eurasian, solid = Hybrid). Letters above error bars indicate significant differences between treatments (Student-Newman-Kuels method, $p \leq 0.05$, $n = 4$).

While significant differences in β-carotene values were documented early in the study, this did not translate to differences in shoot biomass at the end of the study. Though differences in β-carotene values between the genotypes is notable. Based on the results, it appears that the hybrid genotype may be more sensitive to fluridone than the Eurasian genotype. However, further evaluation of this response is necessary.

Rates selected for this study were similar to operational rates commonly used for selective control of Eurasian watermilfoil (Smith and Pullman 1997, Getsinger et al. 2001). While fluridone exposure requirements in the field usually exceed 45 days (Netherland et al. 1993), trends observed through our study indicate that both Eurasian and hybrid watermilfoil were exposed to lethal concentrations of fluridone.

The major impetus for initiating this study was to determine if there was evidence of a response difference between Eurasian and hybrid watermilfoil to fluridone. Initially, based on β-carotene values we suspected there might be a response difference between genotypes, but by the end of the study this did not result in differences in shoot biomass. In future trials, the testing of a larger sample of Eurasian and hybrid populations is recommended. In addition, the comparison of milfoil populations with a significant history of fluridone exposure compared to populations that have not been treated with fluridone is also suggested.

ACKNOWLEDGMENTS

The authors would like to thank the Minnesota Department of Natural Resources, Aquatic Plant Division and the U.S. Army Corps of Engineers Aquatic Plant Control Research Program. Thanks to John Skogerboe for collecting the watermilfoil samples used in these studies and Kurt Getsinger and Linda Nelson for early reviews of this manuscript. Permission was granted by the Chief of Engineers to publish this information. Citation of trade names does not constitute an official endorsement or approval of the use of such commercial products.

LITERATURE CITED

Barton, N. H. 2001. The role of hybridization in evolution. Molecular Ecol. 10:551-568.

Boylen, C. W., L. W. Eichler and J. D. Madsen. 1999. Loss of native aquatic plant species in a community dominated by Eurasian watermilfoil. Hydrobiologia 415:208-211.

Crowder, L. B. and W. E. Cooper. 1982. Habitat structural complexity and the interaction between bluegill and their prey. Ecology 63:1802-1813.

Ellstrand, N. C. and K. A. Schierenbeck. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants. Proc. Natl. Acad. Sci. 97:7043-7050.

Getsinger, K. D, J. D. Madsen, T. J. Koschnick, M. D. Netherland, R. M. Stewart, D. R. Honnell, A. G. Staddon and C. S. Owens. 2001. Whole-lake applications of Sonar for selective control of Eurasian watermilfoil. Tech Report ERDC/EL TR-01-7, U.S. Army Engineer Research and Development Center, Vicksburg, MS. 52 pp.

Michel, A., B. E. Scheffler, R. S. Arias, S. O. Duke, M. D. Netherland and F. E. Dayan. 2004. Somatic mutation-mediated evolution of herbicide resistance in the non-indigenous invasive plant hydrilla (*Hydrilla verticillata*). Molecular Ecol. 13:3229-3237.

Moody, M. L. and D. H. Les. 2007. Geographic distribution and genotypic composition of invasive hybrid watermilfoil (*Myriophyllum spicatum* × *M. sibiricum*). Biol. Invasions 9:559-570.

Moody, M. L. and D. H. Les. 2002. Evidence of hybridity in invasive watermilfoil (*Myriophyllum*) populations. Proc. Natl. Acad. Sci. 99:14867-14871.

Netherland, M. D. and K. D. Getsinger. 1995. Laboratory evaluation of threshold fluridone concentrations under static conditions for controlling hydrilla and Eurasian watermilfoil. J. Aquat. Plant Manage. 33:33-36.

Netherland, M. D., K. D. Getsinger and E. G. Turner. 1993. Fluridone concentration and exposure time requirements for control of Eurasian watermilfoil and hydrilla. J. Aquat. Plant Manage. 31:189-194.

Poovey, A. G., J. G. Skogerboe and K. D. Getsinger. 2004. Efficacy of AVAST! fluridone formulation against Eurasian watermilfoil and nontarget submersed plants. Technical report ERDC/EL TR-04-9, U.S. Army Engineer Research and Development Center, Vicksburg, MS. 30 pp.

Rajguru, S. N., N. R. Burgos, V. K. Shivrain and J. M. Stewart. 2005. Mutations in the red rice ALS gene associated with resistance to imazethapyr. Weed Sci. 53:567-577.

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

Smith, C. S. and G. D. Pullman. 1997. Experiences using Sonar A.S. aquatic herbicide in Michigan. J. Lake and Reserv. Manage. 13:338-346.

Sprecher, S. L., M. D. Netherland and A. B. Stewart. 1998. Phytoene and carotene response to fluridone under laboratory conditions. J. Aquat. Plant Manage. 36:111-120.

Weed Science Society of America (WSSA). 2002. Herbicide Handbook—Eighth edition. W. K. Vencill (ed.). Lawrence, KS. 493 pp.

Wetzel, D. K., M. J. Horak, D. Z. Skinner and P. A. Kulakow. 1999. Transferal of herbicide resistance traits from *Amarathus palmeri* to *Amaranthus rudis*. Weed Sci. 47:538-543.