J. Aquat. Plant Manage. 41: 124-126

NOTES

Effects of Triclopyr on Variable-Leaf Watermilfoil

KURT D. GETSINGER[1], SUSAN L. SPRECHER[2], AND AMY P. SMAGULA[3]

INTRODUCTION

In North America, the watermilfoil genus *Myriophyllum* contains a number of native and exotic species able to produce dense monotypic stands with the potential to out-compete and displace other submersed plants, degrade water quality, and hinder recreational use of water bodies (Newroth 1985; Nichols and Shaw 1986). One such species, the perennial variable-leaf or two-leaf watermilfoil (*Myriophyllum heterophyllum* Michx.), is native from Canada west to South Dakota and south to Florida (Godfrey and Wooten 1981). However, it is not native in New England where it has become locally abundant and has produced dense populations that hinder boating, fishing, and swimming (Crow and Hellquist 1983). In New Hampshire the species produces vegetation management problems similar to those caused elsewhere by the exotic weed Eurasian watermilfoil (*Myriophyllum spicatum* L.). Eurasian watermilfoil occurs in more alkaline waters of higher conductivity (Nichols and Buchan 1997) and is present in New Hampshire at a limited number of sites. Since its initial discovery in New Hampshire in the 1960s, variable-leaf watermilfoil has spread to 38 bodies of water. It adapts well to the relatively acid, low alkalinity and conductivity, and nutrient-poor conditions in these lakes (Kimball and Baker 1983, Hoyer et al. 1996).

Variable-leaf milfoil spreads primarily via clonal reproduction, seldom forming emergent heterophyllous flower-bearing stems, and does not generate a significant seedbank (McFarland et al. 2003). Thus, in New Hampshire, variable-leaf watermilfoil acts as a softwater analog to Eurasian watermilfoil, that is, as an aggressive non-native species. While Eurasian watermilfoil is effectively controlled by all the aquatic herbicides registered for submersed species control, there is relatively little information on effective rates of these compounds on other *Myriophyllum* species, such as variable-leaf watermilfoil.

The triethylamine salt of triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid), which was recently granted a Section 3 label by the U.S. Environmental Protection Agency for use in aquatic (Renovate®[4], SePRO Corporation, Carmel, IN) and wetland sites (Garlon® 3A, Dow AgroSciences, Indianapolis, IN), may be an option for control of variable-leaf watermilfoil. The mode of action of this systemic herbicide, which is absorbed by leaves and roots and then translocated throughout the plant, is similar to auxin-like herbicides. It targets dicots and seldom affects monocots. Exposure to water concentrations of 1.5 to 2.0 mg ae triclopyr L^{-1} are reported to control the semi-emergent parrotfeather (*Myriophyllum aquaticum* (Vell.) Verdc.) (Anderson 1991, Anderson and Lanini 1993a, Anderson and Lanini 1993b). While the use of low concentrations with adequate exposure times on Eurasian watermilfoil has been shown to maintain certain native dicots and allow their release from competition (Getsinger et al. 1997). This selective activity has potential to be of value in New Hampshire where native submersed monocots such as pondweeds (*Potamogeton* spp.) and horned pondweed (*Zannichellia palustris* L.) need to be maintained, and where sensitive native dicots, including alternate-flowered watermilfoil (*Myriophyllum alterniflorum* DC.) and Farwell's watermilfoil (*Myriophyllum farwellii* Morong), may require protection from competition via invasive species (New Hampshire Natural Heritage Inventory 1998).

The objective of the study described here was to determine the effect on variable-leaf watermilfoil of various combinations of triclopyr concentrations and exposure times using dosage rates that controlled Eurasian watermilfoil under laboratory and field conditions (Netherland and Getsinger 1992, Getsinger et al. 1997, Petty et al. 1998).

MATERIALS AND METHODS

Variable-leaf watermilfoil plants were cultured and treated in a controlled-environment growth chamber at the Army Engineer Research and Development Station (ERDC), Vicksburg, MS. Apical shoot segments of variable-leaf watermilfoil were harvested from a shallow pond with low pH and alkalinity in New Hampshire, and planted in the growth chamber the next day. Plants were clean, robust, and in excellent condition at time of planting. Four 20- to 30-cm apical segments were planted into 300-mL glass beakers that contained a moistened 3:1 (v/v) mixture of sphagnum peat (SunGro Horticulture, Canada, Ltd.) and potting soil (Hyponex® All-Purpose, The Scotts Company, Columbus, OH) and a 1-g dose of 18-6-12 slow-release fertilizer (Osmocote®, The Scotts Company, Columbus, OH). Ten beakers, holding a total of 40 shoots, were placed in each of 51 vertical aquaria (26 by 26 by 77 cm). Each aquarium was filled to a height of 68 cm with deionized water to give a volume of 46 L. Water chemistry measurements were

[1]U.S. Army Engineer Research and Development Center, Environmental Laboratory, 3909 Halls Ferry Road, Vicksburg, MS 39180; e-mail: Kurt.D.Getsinger@erdc.usace.army.mil.

[2]18391 Westover Drive, South Bend, IN 46637.

[3]Water Division, New Hampshire Department of Environmental Services, P.O. Box 95, 6 Hazen Drive, Concord, NH 03302. Received for publication September 16, 2002 and in revised form April 17, 2003.

[4]Citation of trade names does not constitute an endorsement or approval of the use of such commercial products.

pH 5.51, 1.75 mg L^{-1} as $CaCO_3$ alkalinity, and 4.1 µS cm^{-1} at 25 C conductivity. The aquaria were maintained at 22 ± 2 C 14 hours of light provided daily by a photosynthetic photon flux density of 378 ± 14.0 µE m^{-2} sec^{-1} at the water surface. The culture solution was constantly aerated, and half of the water volume of each aquarium was exchanged every 2 to 3 days via a flow-through system. In order to mimic growth conditions in water bodies where the plant occurs at weedy levels in New Hampshire, CO_2 was bubbled through the aquaria media via the aeration mechanism from a gas cylinder for a 1.0-minute interval twice a week for 18 days.

Twenty-seven days after planting and a day before triclopyr treatment, the aquatic medium was changed from deionized water to a standard aquatic plant culture solution described by Smart and Barko (1984). The solution pH was 7.9 with alkalinity at 60 mg L^{-1} as $CaCO_3$ and conductivity of 280 µS cm^{-1} at 25 C. At this time, an estimate of dry weight (DW) of plant biomass present in each aquarium at time of treatment was determined by removing one randomly selected beaker of plants from each unit and by harvesting shoot tissue. Harvested shoots were dried at 70 C to a constant weight before determining mass. The average DW value obtained per beaker was 7.6 ± 3.2 g. This value was multiplied by the nine remaining beakers of each aquarium to estimate amount of biomass at the time of application of herbicide.

Triclopyr treatments were applied 28 days after planting. Sixteen treatment combinations of five herbicide concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5 mg ae triclopyr L^{-1} and six exposure times of 12, 24, 36, 48, 60 and 72 hours (Table 1) were applied to three replicate aquaria in a randomized block design. Treatments were blocked on the basis of variation in plant growth as a result of light conditions in the growth chamber and response to CO_2. Three aquaria remained untreated as a reference. Herbicide was applied to the 48 treated aquaria based on a 46-L treatment volume, using a 5-mg ae triclopyr mL^{-1} stock solution made from the Garlon® 3A formulation. After stipulated exposure periods, during which aeration was applied continuously, herbicide was removed from aquaria by draining and refilling two-thirds of each unit's volume three times.

Water samples of 1-L volume were collected from each aquarium 15 min after treatment and following the final draining. Samples were frozen before being analyzed for triclopyr residues at the water chemistry laboratory at the ERDC Lewisville Aquatic Ecosystem Research Facility, Lewisville, TX, using high performance liquid chromatography (HPLC) methods developed by Dow AgroSciences as described in Getsinger et al. (2000). The untreated reference aquaria were subjected to the same draining and re-filling procedure.

Treated plants were maintained in the standard culture solution with flow-through water exchange carried out as before and were monitored visually until 31 days after treatment (DAT). Each aquarium was visually assessed for plant regrowth as shown by the presence (+) or absence (-) of newly emerging apical or axillary shoots (Table 1). Viable shoot tissue in the nine beakers remaining in each experimental unit was then harvested, pooled, and weighed after drying at 70 C. Post-treatment biomass was determined by averaging shoot tissue DW from the three replicate aquaria from each treatment. Analyses of variance were used to examine differences among treatments (SigmaStat 2.0, Jandel 1995). Biomass data were distributed non-normally due to numerous zero quantities among replicates within treatments.

RESULTS AND DISCUSSION

At the time of treatment, DW biomass in the 676 cm^2 surface area of each aquarium comprised 124.7 g m^{-2}. This may be compared to field biomass in Eurasian watermilfoil of 185 g DW m^{-2} determined by Grace and Wetzel (1978) in sites in the Southeast US.

Triclopyr residue analyses of water immediately after treatment showed that applications were close to the nominal rates selected. Treatment concentrations in water samples were 2.5 ± 0.6; 2.2 ± 0.2; 1.6 ± 0.3; 1.1 ± 0.3; and 0.8 ± 0.2 mg L^{-1} for the applied rates of 2.5, 2.0, 1.5, 1.0, and 0.5 mg L^{-1}, respectively.

TABLE 1. MEAN (±SD) BIOMASS (G DW) OF VARIABLE-LEAF WATERMILFOIL IN EXPERIMENTAL UNITS AT 31 DAYS AFTER TREATMENT WITH TRICLOPYR[a]. NEGATIVE SIGN (-) INDICATES NO NEW GROWTH OF APICAL MERISTEMS OR AXILLARY BUDS IN AN INDIVIDUAL REPLICATE; POSITIVE SIGN (+) INDICATES NEW GROWTH ON ONE OR MORE PLANTS.

Triclopyr (mg ae L^{-1})	Exposure time (hours)						
	0	12	24	36	48	60	72
2.5		0.09 ± 0.13 (- - +)	0.01 ± 0.01 (- - +)	0.01 ± 0.01 (- - -)	0.0 ± 0.0 (- - -)		
2.0			0.03 ± 0.30 (- - +)	0.0 ± 0.0 (- - -)	0.0 ± 0.0 (- - -)		
1.5			0.0 ± 0.0 (- - -)	0.86 ± 1.25 (+ + +)	0.02 ± 0.04 (- - +)	0.0 ± 0.0 (- - -)	
1.0				0.08 ± 0.07 (- + +)	0.19 ± 0.32 (- - +)	0.07 ± 0.12 (- - +)	
0.5						0.35 ± 0.44 (- + +)	0.02 ± 0.02 (- + +)
0	5.3 ± 2.2 (+ + +)						

[a]Biomass was significantly different among the untreated reference and all of the triclopyr-treated units (p = <0.001, Tukey's multiple comparison). There were no significant differences among the herbicide treatments (p = 0.289, ANOVA on raw data; p = 0.136, Kruskal-Wallis ANOVA on ranks).

Aqueous triclopyr levels after aquaria were drained were all below detection, indicating that the procedure was successful in removing herbicide residues following treatment.

Plants treated at all herbicide concentrations and exposure times had typical auxin-like epinastic symptoms of twisting and bending in leaves, stems and apical meristems 4 days after application of triclopyr. By this time plants in some of the replicate units treated at 1.5 mg L^{-1} for 36, 48 and 60 hr and at 2.5 mg L^{-1} for 24 hr had sunk half-way down the water column due to partial loss of buoyancy. At 7 DAT, most treatments remained symptomatic, and plants treated with 1.0 mg L^{-1} for 36 hr, 2.0 mg L^{-1} for 24 hr, and 2.5 mg L^{-1} for 36 hr, had also lost buoyancy and had settled low in the water column. However, newly emerging apical growth in plants treated at 0.5 mg L^{-1} for 72 hr lacked epinasty and looked normal. Untreated reference plants remained upright with normal leaf and stem morphology.

At 17 DAT, plants treated at 1.0 mg L^{-1} for 48 hr had sunk below the surface, and those that had previously dropped out of the water column had not regained buoyancy. Older leaf tissue in treated units was decomposing and colonized by algae, but continued stem viability was indicated where plants had newly emerging axillary shoots or regrowth of apical meristems. Some algal infestation was also present in two reference units. Auto-fragmentation of viable apical shoots was not observed during the post-treatment period.

At the time of post-treatment harvest at 31 DAT, 10 of the 16 treatments had produced some new growth although DW data showed that no treatment had recovered to more than 10% of pretreatment biomass levels (Table 1). Untreated plants had decreased 30% in biomass from time of treatment (i.e., 7.6 g to 5.3 g), due to decline in older shoot tissue during the course of the study. However, at the conclusion of the study all of the untreated plants exhibited healthy, actively growing shoots, and the differences in biomass between untreated and all treated units were highly significant.

Analysis of variance on post-treatment DW biomass showed no block effect on treatment results ($p = 0.42$), and further analysis was based on one-way ANOVA of a completely randomized design. While all treated material was significantly lower than untreated reference plants ($p = < 0.001$, Tukey's multiple comparison versus control), there were no statistical differences in biomass among treatments ($p = 0.289$, ANOVA on raw data; $p = 0.136$, Kruskal-Wallis ANOVA on ranks). Results from this study suggest that triclopyr may be efficacious against variable-leaf watermilfoil in the field over a wide range of concentrations and exposure times. These data indicate a broad dose/response range that could allow for maximum flexibility in controlling *M. heterophyllum* with triclopyr under field situations where water exchange characteristics and size of plant infestations dictate application strategies.

ACKNOWLEGMENTS

This research was conducted under the U.S. Army Corps of Engineers Aquatic Plant Control Research Program, U.S. Army Engineer Research and Development Center. Permission to publish this information was granted by the Chief of Engineers. Financial support was also provided by the New Hampshire Department of Environmental Sciences. Product used in this study was supplied by Dow AgroSciences. The authors thank D. Honnell for analytical support, and A. Poovey and L. Nelson for earlier reviews of the manuscript.

LITERATURE CITED

Anderson, L. 1991. Control of parrotfeather with Garlon 3a: Foliar and in-water application. USDA-ARS Ann. Rep. Aquat. Weed Contr. Investigations 1993. p. 21-23.

Anderson, L. and T. Lanini. 1993a. Foliar application of Garlon 3a for control of parrotfeather (*Myriophyllum aquaticum*). USDA-ARS Ann. Rep. Aquat. Weed Contr. Investigations 1993. p. 11-12.

Anderson, L. and T. Lanini. 1993b. Effect of in-water application of Garlon 3a on parrotfeather (*Myriophyllum aquaticum*). USDA-ARS Ann. Rep. Aquat. Weed Contr. Investigations. p. 13-14.

Crow, G. E. and C. B. Hellquist. 1983. Aquatic Vascular Plants of New England: Part 6. Trapaceae, Haloragaceae, Hippuridaceae. New Hampshire Ag. Exp. Sta., Univ. New Hampshire, Durham, NH.

Getsinger, K. D., E. G. Turner, J. D. Madsen and M. D. Netherland. 1997. Restoring native vegetation in a Eurasian water milfoil-dominated plant community using the herbicide triclopyr. Reg. Rivers: Res. Manage. 13:357-375.

Getsinger, K. D., D. G. Petty, J. D. Madsen, J. G. Skogerboe, B. A. Houtman, W. T. Haller and A. M. Fox. 2000. Aquatic dissipation of the herbicide triclopyr in Lake Minnetonka, Minnesota. Pest Manage. Sci. 56:388-400.

Godfrey, R. K. and J. W. Wooten. 1981. Aquatic and Wetland Plants of Southeastern United States: Dicotyledons. Univ. Georgia Press, Athens, GA. 933 pp.

Grace, J. B. and Wetzel, R. G. 1978. The production biology of Eurasian watermilfoil (*Myriophyllum spicatum* L.): A review. J. Aquat. Plant Manage. 16:1-11.

Hoyer, M. V., D. E. Canfield, Jr., C. A. Horsburgh and K. Brown. 1996. Florida Freshwater Plants: A Handbook of Common Aquatic Plants in Florida Lakes. Univ. Florida, Inst. Food Ag. Sci., Gainesville, FL. 264 pp.

Jandel Corporation. 1995. SigmaStat 2.0 for Windows. Version 2.0. Jandel Corporation, San Rafael, CA. 800 pp.

Kimball, K. D. and A. L. Baker. 1983. Temporal and morphological factors related to mineral composition in *Myriophyllum heterophyllum* Michx. Aquat. Bot. 16:189-205.

McFarland, D. G., A. G. Poovey and J. D. Madsen. 2003. Evaluation of the potential of selected nonindigenous aquatic plant species to colonize Minnesota water resources. Tech. Rep. ERDC/EL-03-, U.S. Army Eng. Res. Develop. Center, Vicksburg, MS. 100 pp. (in press).

Netherland, M. D. and K. D. Getsinger.1992. Efficacy of triclopyr on Eurasian watermilfoil: Concentration and exposure time effects. J. Aquat. Plant Manage. 30:1-5.

New Hampshire Natural Heritage Inventory 1998. http://webster.state.nh.us/nhinfo/plant_1.html. Rare Plant Species in New Hampshire. Accessed: January 1, 1998.

Newroth, P. R. 1985. A review of Eurasian watermilfoil impacts and management in British Columbia. Proc. 1st Int. Symp. Watermilfoil (*Myriophyllum spicatum*) and related Haloragaceae Species, Vancouver, British Columbia, July 23-24, 1985, Aquat. Plant Manage. Soc., Clermont, FL. p. 139-153.

Nichols, S. A. and L. A. J. Buchan. 1997. Use of native macrophytes as indicators of suitable Eurasian watermilfoil habitat in Wisconsin lakes. J. Aquat. Plant Manage. 35:21-24.

Nichols, S. A. and B. H. Shaw. 1986. Ecological life histories of three aquatic nuisance plants, *Myriophyllum spicatum*, *Potamogeton crispus*, and *Elodea canadensis*. Hydrobiol. 131:3-21.

Petty, D. G., K. D. Getsinger, J. D. Madsen, J. G. Skogerboe, W. T. Haller, A. M. Fox and B. A. Houtman. 1998. Aquatic dissipation of the herbicide triclopyr in Lake Minnetonka, Minnesota. Tech. Rep. A-98-01, U.S. Army Eng. Waterways Exp. Sta., Vicksburg, MS. 96 pp.

Smart, R. M. and J. W. Barko. 1984. Culture Methodology for Experimental Investigations Involving Rooted Submersed Aquatic Plants. Misc. Paper A-84-6, U.S. Army Eng. Waterways Exp. Sta., Vicksburg, MS. 20 pp.