

Susceptibility of Eurasian Watermilfoil (*Myriophyllum spicatum*) and a Milfoil Hybrid (*M. spicatum* × *M. sibiricum*) to Triclopyr and 2,4-D Amine

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ABSTRACT

Hybridization of the exotic Eurasian watermilfoil (*Myriophyllum spicatum* L.) with the native northern watermilfoil (*M. sibiricum* Komarov) has been verified in the Great Lake and Pacific Northwest regions. To determine if a milfoil hybrid was susceptible to aquatic herbicides typically used to control Eurasian watermilfoil, we conducted a small-scale experiment evaluating the comparative response of *M. spicatum* × *M. sibiricum* and its parental species, *M. spicatum*, to 2,4-D (2,4-dichlorophenoxyacetic acid) and triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid). Plants were field collected from Minnesota, grown in 48-L aquaria, then dosed with 0.01, 0.03, 0.09, 0.27, 0.81, 2.43 acid equivalent (ae) 2,4-D amine and triclopyr for an exposure time of 24 to 28 h. The dose that caused a 50% growth reduction (GR₅₀) in shoot length was calculated from dose-response curves for each herbicide. For triclopyr, the GR₅₀ (±1 SE) for Eurasian watermilfoil was 0.04 ± 0.01 mg ae L⁻¹, while the GR₅₀ for the milfoil hybrid was 0.08 ± 0.01 mg ae L⁻¹. For 2,4-D amine, the GR₅₀ for the Eurasian genotype was 0.11 ± 0.02 mg ae L⁻¹ while the GR₅₀ for the hybrid was 0.12 ± 0.02 mg ae L⁻¹. Rates of ≥0.27 mg ae L⁻¹ triclopyr or 2,4-D amine were effective in reducing shoot biomass by 95 to 100% for both the Eurasian and hybrid milfoils. This initial study showed that Eurasian watermilfoil and the milfoil hybrid responded similarly to the herbicides tested. Additional testing of different milfoil hybrid accessions should be conducted to determine the roles genotypic variation, environmental factors, and plant vigor may play in the operational chemical control of milfoil species.

Key words: submersed aquatic macrophyte, auxin analog herbicide, chemical control, dose-response.

INTRODUCTION

Control of the exotic submersed macrophyte Eurasian watermilfoil (*Myriophyllum spicatum* L.) with the aquatic her-

bicides triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid), and 2,4-D (2,4-dichlorophenoxyacetic acid) has been systematically investigated in the laboratory (Green and Westerdahl 1990, Netherland and Getsinger 1992) and field (Getsinger et al. 1982, Carpentier et al. 1988, Getsinger et al. 1997, Parsons et al. 2001, Poovey et al. 2004). These concentration/exposure time (CET) studies have demonstrated that effective control of Eurasian watermilfoil is dependent upon the length of time plants remain exposed to given concentrations of herbicide, and have proven invaluable in the successful use of 2,4-D and triclopyr in operational field applications throughout the northern U.S.

The hybridization of Eurasian watermilfoil with the native northern watermilfoil (*M. sibiricum* Komarov) has been documented in the Great Lakes (Moody and Les 2002, 2006) and Pacific Northwest regions (Moody and Les 2006). Increased growth rates may give hybrid plants competitive advantages over parental species, eventually displacing them and colonizing new habitats (Les and Philbrick 1993, Ellstrand and Schierenbeck 2000, Vilà et al. 2000). Since several aquatic hybrids persist in areas where parental species have disappeared, they also may possess higher tolerances to extreme environmental conditions or disturbance (Les and Philbrick 1993). Although these traits have been documented for hybrids in many aquatic macrophyte genera (Les and Philbrick 1993), they have not been reported for hybrids in *Myriophyllum* because few studies on milfoil hybrids have been conducted. The life cycle, reproductive capacity, competitive fitness, and survival of *M. spicatum* × *M. sibiricum* are currently unknown (Moody and Les 2002, 2006). Furthermore, there is limited information on the potential response of milfoil hybrids to management techniques, such as biological agents and chemicals.

The milfoil weevil (*Euhrychiopsis lecontei* Dietz) has been considered a biological agent for Eurasian watermilfoil management (Sheldon and Creed 1995, Sheldon and O'Bryan 1996) and has been associated with declines of Eurasian watermilfoil in the Great Lakes region (Jester et al. 2000, Lillie 2000, Newman and Biesboer 2000). It also may have been associated with declines of milfoil hybrid plant populations in a Minnesota lake (Newman 2004). Roley and Newman (2006) reported that the weevil successfully develops and survives on *M. spicatum* × *M. sibiricum*. Weevil survival rate was intermediate (61%) between its survival rate on Eurasian watermilfoil (88%) and northern milfoil (45%). This study suggests that some hybrid populations may be more

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resistant to herbivory by the milfoil weevil than the Eurasian parental type.

Laboratory or field evaluations that confirm herbicide susceptibility to aquatic plant hybrids have not been conducted; however, herbicide resistance of hybrid genotypes has been documented for terrestrial weeds. Herbicide-resistant parental red rice species (*Oryza sativa* L.) crossed with susceptible parental species resulted in hybrid genotypes resistant to the imidazolinone and glycine herbicides (Gealy et al. 2003, Rajguru et al. 2005, Shivrain et al. 2006). Interspecific hybridization has been theorized to cause herbicide resistance in different *Amaranthus* species. Although small-scale studies have demonstrated the probability of a pigweed parental species conferring herbicide-resistant genes to hybrid progeny (Wetzel et al. 1999, Franssen et al. 2001), this has yet to be corroborated in the field (Trucco et al. 2005). In both of these cases, a herbicide-resistant parent produced a herbicide-resistant hybrid. Because there is no indication that Eurasian watermilfoil or northern milfoil are resistant to aquatic herbicides, it is unlikely that a hybrid progeny of these species would be herbicide-resistant due to heredity alone. Any differential response between Eurasian and hybrid milfoils to systemic herbicides such as triclopyr and 2,4-D may be due to differences in plant physiology, growth, or inherent susceptibility.

A small-scale study was conducted to determine if the milfoil hybrid *M. spicatum* × *M. sibiricum* was susceptible to aquatic herbicides commonly used to control Eurasian watermilfoil, 2,4-D amine and triclopyr. The objective was to quantify any considerable differences between this hybrid and its parental species, using plants that were field-collected from Minnesota, U.S.

MATERIALS AND METHODS

This study was conducted at the U.S. Army Engineer Research and Development Center, Vicksburg, MS, in a walk-in growth chamber (58 m²). Environmental conditions were set with an air temperature of 24 ± 2°C, light intensity of 300 ± 50 μmol m⁻² sec⁻¹, and photoperiod of 14 h:10 h light:dark cycle. Lighting was provided by 400 watt metal halide bulbs with glass plates situated underneath the bulbs.

On 23-24 June 2005, Minnesota populations of hybrid and Eurasian watermilfoils were field-collected from Otter Lake, Ramsey County (Moody and Les 2006), and Pierson Lake, Carver County, (Skogerboe and Getsinger 2006), respectively, and shipped overnight to Vicksburg. Three apical stems (15 ± 0.1 cm in length) of a genotype were planted a 450 ml plastic beaker filled with 400 ml of natural lake sediment (Brown's Lake, Vicksburg, MS) which had been amended with 200 mg L⁻¹ ammonium chloride to provide adequate nutrients for plant growth. Each beaker was then capped with 1-cm layer of coarse-grit sand to prevent suspension of sediment particles in the water column. Fifty-six vertical aquaria (48 L capacity) were filled with culture solution (Smart and Barko 1985) and eight planted beakers, four of each genotype, were placed side by side in each aquarium. After plants had formed a canopy on the water surface (four weeks), they were dosed with herbicide.

Stocks of 2,4-D dimethylamine salt (amine) as DMATM 4 IVM (39.9% wt acid, Dow AgroSciences, Indianapolis, IN)

and triclopyr as RenovateTM 3 (31.8% wt acid, SePRO Corp., Carmel, IN) were made by diluting 10 g of herbicide in 1 L of distilled water. From the stock, 2,4-D amine and triclopyr, as the acid equivalent (ae), were applied subsurface to aquaria using a pipette to provide rates of 0.01, 0.03, 0.09, 0.27, 0.81, and 2.43 mg ae L⁻¹ for an exposure time of 24 to 28 h. This range of herbicide concentrations was chosen to compare herbicide response in different weed genotypes (Beckie et al. 2000). Untreated references were included to assess plant growth in the absence of herbicide application. Following herbicide exposure, aquaria were completely emptied and refilled with fresh culture solution three times to remove all aqueous herbicide residues. The study continued for five weeks following herbicide applications.

Water temperature, conductivity and pH were measured in each aquarium at the beginning and end of the study with a multi-parameter probe (model 556, YSI, Yellow Springs, OH). Herbicide efficacy was assessed by comparing total shoot length (cm) and shoot biomass (g dry weight; DW) for each treatment. Two beakers were randomly removed from each aquarium one day before herbicide application and five weeks post-treatment. All shoots from each beaker were cut and measured to calculate total shoot length. Afterwards, shoots were dried at 70°C for 48 h and weighed for a biomass estimate.

Treatments were assigned to individual aquaria in a completely randomized manner and replicated four times, including the references. Shoot length and biomass means for both beakers of each genotype in each replicate were calculated from post-treatment harvest data. Data then were subjected a Kruskal-Wallis one-way analysis of variance (ANOVA) based on ranks to test for herbicide concentration effects of either 2,4-D or triclopyr for either Eurasian watermilfoil or the milfoil hybrid ($n = 4$). For shoot biomass, if effects were significant ($p \leq 0.05$), means were separated using the Student-Newman Kuels Method (S-N-K). For shoot length, if effects were significant ($p \leq 0.05$), a 4-parameter log-logistic dose response curve was generated and the GR₅₀ calculated (Seefeldt et al. 1995) with the equation: shoot length = min + (max-min)/(1+(concentration/GR₅₀)^{slope}) for both Eurasian watermilfoil and milfoil hybrid using SigmaPlot 9.0 (Systat Software, Inc., Point Richmond, CA).

RESULTS AND DISCUSSION

Water temperature (mean ± 1 SE) in the aquaria was 24 ± 0.05°C. Conductivity and pH remained constant throughout the study at 0.291 ± 0.004 mS cm⁻¹ and 7.9 ± 0.1, respectively. These conditions were conducive to aquatic plant growth for small-scale experiments (Smart and Barko 1985).

Triclopyr was effective in reducing 100% of shoot biomass for both Eurasian watermilfoil and milfoil hybrid at rates ≥ 0.27 mg ae L⁻¹ following a 24-hour exposure time (Figure 1). Triclopyr is typically applied in the field at rates of 0.75 to 2.5 mg ae L⁻¹ for control of Eurasian watermilfoil (RenovateTM 3 label). Differences in response to triclopyr between the Eurasian and hybrid genotypes occurred at sublethal rates (Figure 2). The calculated GR₅₀ (± 1 SE) based on total shoot length for the Eurasian genotype was 0.04 ± 0.01 mg ae L⁻¹ while the GR₅₀ for the hybrid was 0.08 ± 0.01 mg ae L⁻¹.

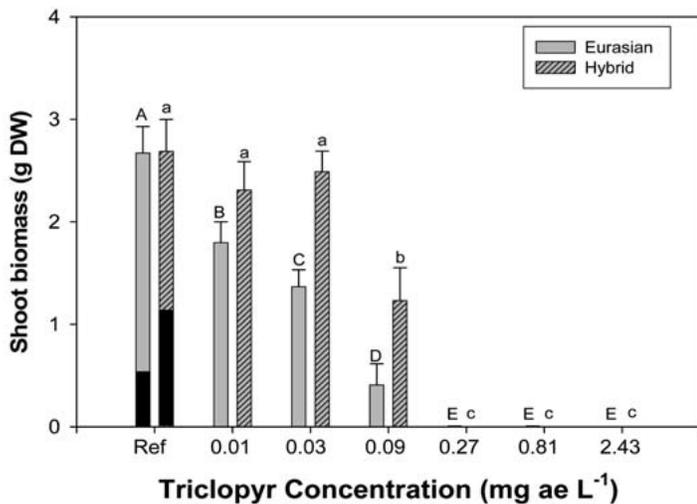


Figure 1. Shoot biomass (g DW) of Eurasian watermilfoil and a milfoil hybrid (*M. spicatum* × *M. sibiricum*) five weeks following triclopyr applications of 0.01, 0.03, 0.09, 0.27, 0.81, and 2.43 mg ae L⁻¹. Black shading indicates pretreatment biomass. Means are ±1 SE (n = 4). Upper case letters denote significant differences among treatments for Eurasian watermilfoil; lower case letters denote significant differences among treatments for the milfoil hybrid (S-N-K, p ≤ 0.05).

Visually, the Eurasian and hybrid milfoils responded similarly to triclopyr. Plants from all herbicide treatments exhibited epinasty one week after treatment. Three weeks after treatment, plants treated with rates ≥0.27 mg ae L⁻¹ had turned black and were deteriorating, while plants treated with the lower rates were green with epinastic leaves and stems. By the end of the study, plants treated with 0.27 to 2.43 mg ae L⁻¹ were dead, and plants treated with 0.09 mg ae L⁻¹ were deteriorating. Plants treated with 0.01 and 0.03 mg ae L⁻¹ had epinastic and/or brown apices and stems. That symptoms remained five weeks after a 24-hour exposure to

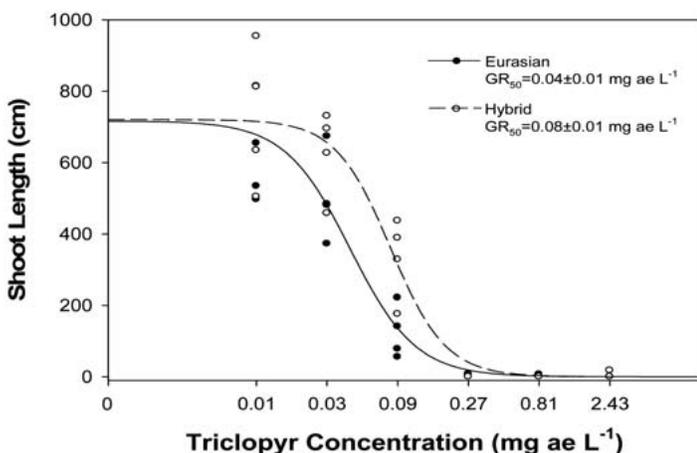


Figure 2. Total shoot length (cm) of Eurasian watermilfoil and a milfoil hybrid (*M. spicatum* × *M. sibiricum*) five weeks following a triclopyr application, including references (0 mg ae L⁻¹ triclopyr concentration). Dose-response curve for Eurasian watermilfoil was $y = 9.34 + (717-9.34) / [1 + (x/0.04)^{1.97}]$, $R^2 = 0.93$, $p < 0.0001$ and dose-response curve for milfoil hybrid was $y = 1.38 + (721-1.38) / [1 + (x/0.08)^{2.34}]$, $R^2 = 0.93$, $p < 0.0001$.

triclopyr concentrations 80 to 250-fold less than the maximum label rate of 2.5 mg ae L⁻¹ demonstrates the extreme sensitivity of these milfoil species to this herbicide.

Like triclopyr, 2,4-D amine was effective in reducing shoot biomass by 95 to 100% for both Eurasian watermilfoil and the milfoil hybrid at rates ≥0.27 mg ae L⁻¹ (Figure 3). Differences in response to 2,4-D amine between the Eurasian and hybrid milfoils also occurred at sublethal rates (Figure 4). The calculated GR₅₀ (±1 SE) based on total shoot length for Eurasian watermilfoil was 0.11 ± 0.02 mg ae L⁻¹, while the GR₅₀ for the milfoil hybrid was 0.12 ± 0.01 mg ae L⁻¹. The liquid 2,4-D amine label (DMATM 4 IVM) states that rates of 2 to 4 mg ae L⁻¹ are effective for control of Eurasian watermilfoil; however, lower rates (0.5 to 1 mg ae L⁻¹) have been effective in reducing plant populations (Skogerboe and Getsinger 2006).

Visually, Eurasian watermilfoil and the milfoil hybrid responded similarly to 2,4-D amine. Plants from all herbicide treatments exhibited epinasty one week after treatment, with browning of leaves and stems occurring in treatments with herbicide concentrations ≥0.27 mg ae L⁻¹. Three weeks after treatment, plants displayed varying degrees of injury corresponding to herbicide dose. Plants treated with 0.81 and 2.43 mg ae L⁻¹ had turned black and were deteriorating, while plants treated with 0.27 mg ae L⁻¹ were starting to fragment. Plants treated with 0.03 and 0.09 mg ae L⁻¹ had some brown stems with epinastic apices. Plants treated with 0.01 mg ae L⁻¹ displayed no visual injury. By the end of the study, plants treated with ≥0.27 mg ae L⁻¹ were dead, and plants treated with 0.09 mg ae L⁻¹ were deteriorating. Plants treated with 0.03 mg ae L⁻¹ still had some epinastic and brown apices and stems, while plants treated with 0.01 mg ae L⁻¹ were green with no visual injury.

According to the dose-response curves and calculated GR₅₀ values, Eurasian watermilfoil and the milfoil hybrid responded similarly to 2,4-D and triclopyr. The lower GR₅₀ val-

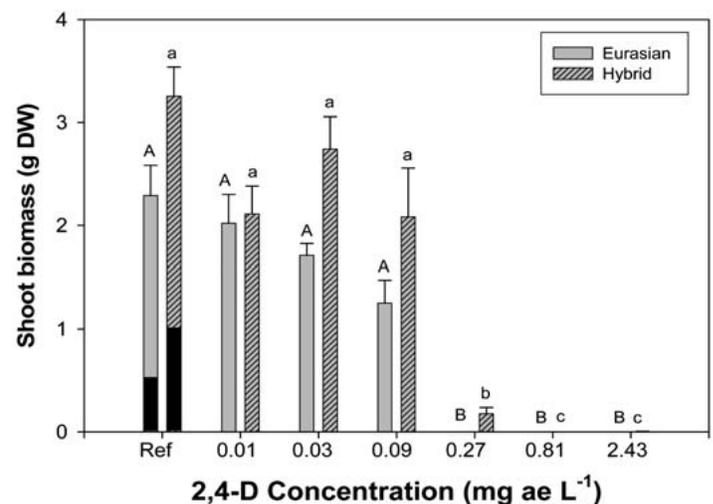


Figure 3. Shoot biomass (g DW) of Eurasian and a milfoil hybrid (*M. spicatum* × *M. sibiricum*) five weeks following 2,4-D amine applications of 0.01, 0.03, 0.09, 0.27, 0.81, and 2.43 mg ae L⁻¹. Black shading indicates pretreatment biomass. Means are ±1 SE (n = 4). Upper case letters denote significant differences among treatments for Eurasian watermilfoil; lower case letters denote significant differences among treatments for the milfoil hybrid (S-N-K, p ≤ 0.05).

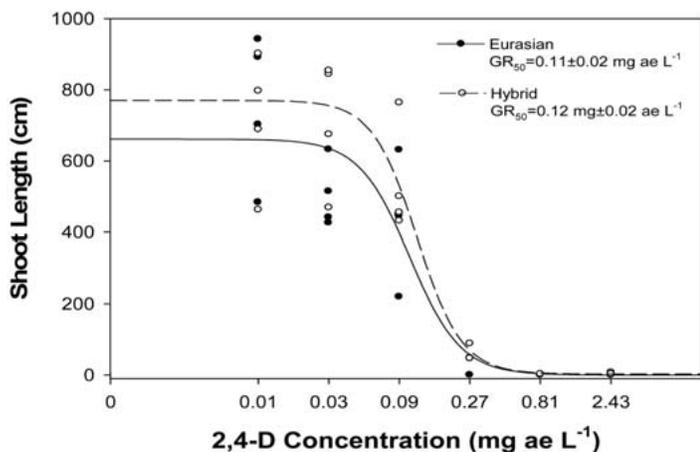


Figure 4. Total shoot length (cm) of Eurasian watermilfoil and a milfoil hybrid (*M. spicatum* × *M. sibiricum*) five weeks following a 2,4-D amine application, including references (0 mg ae L⁻¹ 2,4-D concentration). Dose-response curve for Eurasian watermilfoil was $y = 2.52 + (661-2.52)/[1 + (x/0.11)^{2.53}]$, $R^2 = 0.85$, $p < 0.0001$ and dose-response curve for milfoil hybrid was $y = 6.09 + (767-6.09)/[1 + (x/0.12)^{2.85}]$, $R^2 = 0.91$, $p < 0.0001$.

ues for triclopyr (0.04 to 0.08 mg ae L⁻¹) compared to those for 2,4-D amine (0.11 to 0.12 mg ae L⁻¹) indicate the differences in recommended initial and maximum label rates. Initial rates of these products range from 0.75 for triclopyr to 2.0 mg ae L⁻¹ for 2,4-D; therefore, the difference in the calculated GR₅₀ values (0.01 to 0.04 mg ae L⁻¹) has limited implications for operational use.

Results from this study suggest that environmental factors may play a bigger role than genotypic variation in the operational chemical control of Eurasian and hybrid milfoils. Sterling et al. (2004) concluded that environmental factors were more important than genotypic variation when examining varying responses of invasive rangeland weeds to chemical management in the field. Numerous environmental factors complicate submersed aquatic herbicide applications. Factors such as water exchange, water temperature, pH, turbidity, and conductivity, can affect herbicide efficacy against a target plant.

Plant age, growth, and density also contribute to effectiveness of submersed herbicide applications, particularly with systemic herbicides like 2,4-D and triclopyr (Westerdahl and Getsinger 1988, also check product labels). Although pretreatment biomass for the milfoil hybrid was slightly greater than for Eurasian watermilfoil (Figures 1 and 3), all plants were young, actively growing, and had just formed a thin canopy when dosed with herbicide. A rate of 0.27 mg ae L⁻¹ with a 24-hour exposure time was sufficient for complete growth inhibition for five weeks with both products. This outcome was better than that predicted from CET studies for either triclopyr (70-85% control; Netherland and Getsinger 1992) or 2,4-D amine (<70% control; Green and Westerdahl 1990). A possible explanation could be that plants with less biomass and rapid growth due to warm water temperatures may have absorbed and translocated more herbicide than slower-growing plants with more biomass in cooler water used in the CET studies. Vegetation density in those studies (123 to 158 g DW m⁻²) represented maximum summer biomass (Grace

and Wetzel 1978) with water temperatures ranging from 21 to 22°C, while density in this study was 36 g DW m⁻² with water temperatures of 24°C. The increase from 22 to 24°C significantly increases Eurasian watermilfoil shoot length, shoot biomass production, and canopy expansion (Barko and Smart 1981, Barko et al. 1982).

Further testing of Eurasian watermilfoil, northern watermilfoil and its hybrid progeny should be conducted using plant accessions from other lakes and those with known management history of 2,4-D and triclopyr. Different hybrid biotypes may respond differently to herbicides. Bultemeier and Netherland (2007) found that different biotypes of fanwort (*Cabomba caroliniana* Gray) varied in response to both systemic and contact herbicides.

Although there were minor differences in response between Eurasian watermilfoil and the milfoil hybrid *M. spicatum* × *M. sibiricum* to triclopyr under the controlled-experimental conditions in this initial study, current recommended use rates should be effective when treating either genotype with this product or 2,4-D amine. Identification of the genotype present in treatment areas followed by intensive monitoring of herbicide applications could determine whether genotypic variation or environmental factors influence herbicide efficacy.

ACKNOWLEDGMENTS

We thank C. Poovey, C. McNemar, and C. Grutzmacher for technical assistance. J. Skogerboe collected the watermilfoil samples used in these studies. We appreciate reviews of this manuscript by K. Getsinger, J. Skogerboe, C. Welling, and C. Poovey. Herbicide formulations were supplied by Dow AgroSciences LLC and SePRO Corporation. This research was supported by the US Army Aquatic Plant Control Research Program and the Minnesota Department of Natural Resources. 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.

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