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Small-Plot, Low-Dose Treatments of Triclopyr for Selective Control of Eurasian Watermilfoil

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ABSTRACT

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Small-plot treatments of triclopyr were conducted on Lake Minnetonka and Lake Minnewashta, MN, during June 1998 to investigate the herbicide's potential to selectively control Eurasian watermilfoil (*Myriophyllum spicatum* L.) at low doses. Applications were made on 1-ha plots with rates based on plot type: references (0 mg acid equivalent (ae)·L^{-1}), protected plots (0.5 mg ae·L^{-1}), semi-protected plots (1.0 mg ae·L^{-1}), and unprotected plots (1.5 mg ae·L^{-1}). Plot protection was a function of potential mixing in the water column. Herbicide residues were monitored to determine dissipation 1 through 72 h post treatment. Samples were analyzed with both a high performance liquid chromatography technique and an enzyme-linked immunosorbent assay method. Results from these two analytical techniques were compared, and found equivalent (R^2 = 0.96). Triclopyr had a relatively short half-life for each treatment (3.5 hr at 0.5 mg ae·L^{-1}, 2.9 hr at 1.0 mg ae·L^{-1}, and 4.2 hr at 1.5 mg ae·L^{-1}). At 8 weeks post treatment, there was a 30 to 45% reduction in Eurasian watermilfoil distribution. Greatest Eurasian watermilfoil control was achieved in plots using higher triclopyr rates. Frequency of native plants decreased by 24% in the untreated reference plot, 20% in the 0.5 mg ae·L^{-1} plot and 6% in the 1.0 and 1.5 mg ae·L^{-1} plots. Mean species per point, however, either increased or remained unchanged in seven of the nine treated plots. Decline of native plants may be partially attributed to the onset of fall senescence. Larger contiguous areas, higher triclopyr rates, and sequential applications may be required to enhance Eurasian watermilfoil control in small-plot, partial lake treatments.

Key Words: aquatic plant control, *Myriophyllum spicatum*, herbicide, Renovate® 3, ELISA.

The U.S. Environmental Protection Agency (EPA) has recently registered the compound triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid) for use as an aquatic herbicide to selectively control nuisance vegetation in lakes, reservoirs, and wetland sites. This herbicide, formulated as a triethylamine (TEA) salt, has been evaluated via an EPA experimental use permit in aquatic sites across the U.S. since the mid-1980s. Triclopyr and its primary metabolites, 3,5,6-trichloropyridinol and 3,5,6-trichloro-2-methoxypyridine, degrade rapidly in aquatic systems and do not accumulate in sediments, fish, or shellfish (Solomon et al. 1988, Woodburn et al. 1993, Getsinger et al. 2000, Petty et al. 2001, 2003). The product has proved efficacious against invasive aquatic species such as Eurasian watermilfoil (*Myriophyllum spicatum* L.), purple loosestrife (*Lythrum salicaria* L.), water hyacinth (*Eichhornia crassipes* (Mart.) Solms), and alligatorweed (*Alternanthera philoxeroides* (Mart.) Gris.).

Previous triclopyr applications have resulted in excellent control of Eurasian watermilfoil for two growing seasons, with minimal injury to non-target vegetation (Getsinger and Westerdahl 1984, Getsinger et al. 1997, Petty et al. 1998a). However, these applications used the maximum label rate of 2.5 mg acid equivalent (ae) L^{-1} in areas of 4 to 6 ha (10 to 15 acres) located in quiescent bays with extended exposure times. There is no published documentation of triclopyr applications in smaller areas using lower rates.

Treatments at lower doses would lessen the amount of herbicide used for controlling Eurasian watermilfoil, allow for the rapid decline of water residues below the maximum contaminant level (MCL) for potable water intakes imposed by the label (0.4 µg L^{-1}), and minimize injury to non-target plants. Moreover, there is a need for controlling milfoil in small areas of littoral zones in many northern tier lakes. However, as opposed to large contiguous treatment areas, herbicide dispersion in small treatment areas is more readily influenced by water exchange, which may be generated by water temperature and wind velocity (Fox et al. 1991, Getsinger et al. 1996). Quick dispersion results in decreased herbicide concentration and shortened exposure times reducing product effectiveness on target plants. Adequate contact time is necessary for successful milfoil control when using triclopyr at doses 40 to 80% less than the maximum label rate (Netherland and Getsinger 1992).

Measuring herbicide concentrations in treated areas is advantageous in determining if contact time was sufficient for effective control of the target plant; however, analysis for triclopyr residues with high performance liquid chromatography (HPLC) is time consuming and expensive. The use of an enzyme-linked immunosorbent assay (ELISA) would provide rapid analysis of triclopyr residues, thereby increasing the cost-effectiveness of monitoring in operational herbicide applications (Fischer and Michael 1997).

To investigate the potential of low doses of triclopyr to selectively control Eurasian watermilfoil in small treatment areas, a study was conducted on two lakes in Minnesota in 1998. Specific objectives were to: 1) evaluate the potential for triclopyr doses 40 to 80 percent below the maximum label rate to control Eurasian watermilfoil in selected small plots; 2) evaluate impacts of these treatments on non-target native plants; and 3) compare ELISA with the standard HPLC technique for measuring triclopyr residues in water.

Materials and Methods

Plot Description and Herbicide Application

The study was conducted on 5,800 ha (14,325 ac) Lake Minnetonka (Hennepin County, MN, USA) and nearby 300 ha (740 ac) Lake Minnewashta (Carver County, MN, USA) from June through August 1998. A total of 12 plots, eight on Minnetonka (Fig. 1) and four on Minnewashta (Fig. 2) were chosen for evaluation. Each plot was a 1-ha (2.5 ac) square and ~2 m deep (Table 1). These plots were established in regions of the lakes where the target weed, Eurasian watermilfoil (hereafter called milfoil), dominated the submersed plant community with >98% frequency of occurrence (Table 2). Plots receiving chemical applications were chosen to represent different shoreline scenarios: protected; semi-protected; and unprotected (Table 1). Protected plots were located in coves that were shielded from prevailing winds, making them least susceptible to water exchange mechanisms that could move the herbicide off-site and reduce contact time and efficacy. Semi-protected plots were in areas near the shoreline that offered some protection from prevailing winds, making them less susceptible to herbicide dilution via water exchange processes. Unprotected plots were located in areas that were directly exposed to prevailing winds, making them most susceptible to dilution of the herbicide via water exchange mechanisms.

At the time of treatment, milfoil was healthy and shoots were at, or just below, the water surface. Although milfoil was the dominant species in all plots, up to 20 other species were present in the pretreatment evaluation (Table 2). Some of the more common of these included the exotic species curlyleaf pondweed (*Potamogeton crispus* L.), and the native species coontail (*Ceratophyllum demersum* L.), elodea (*Elodea*

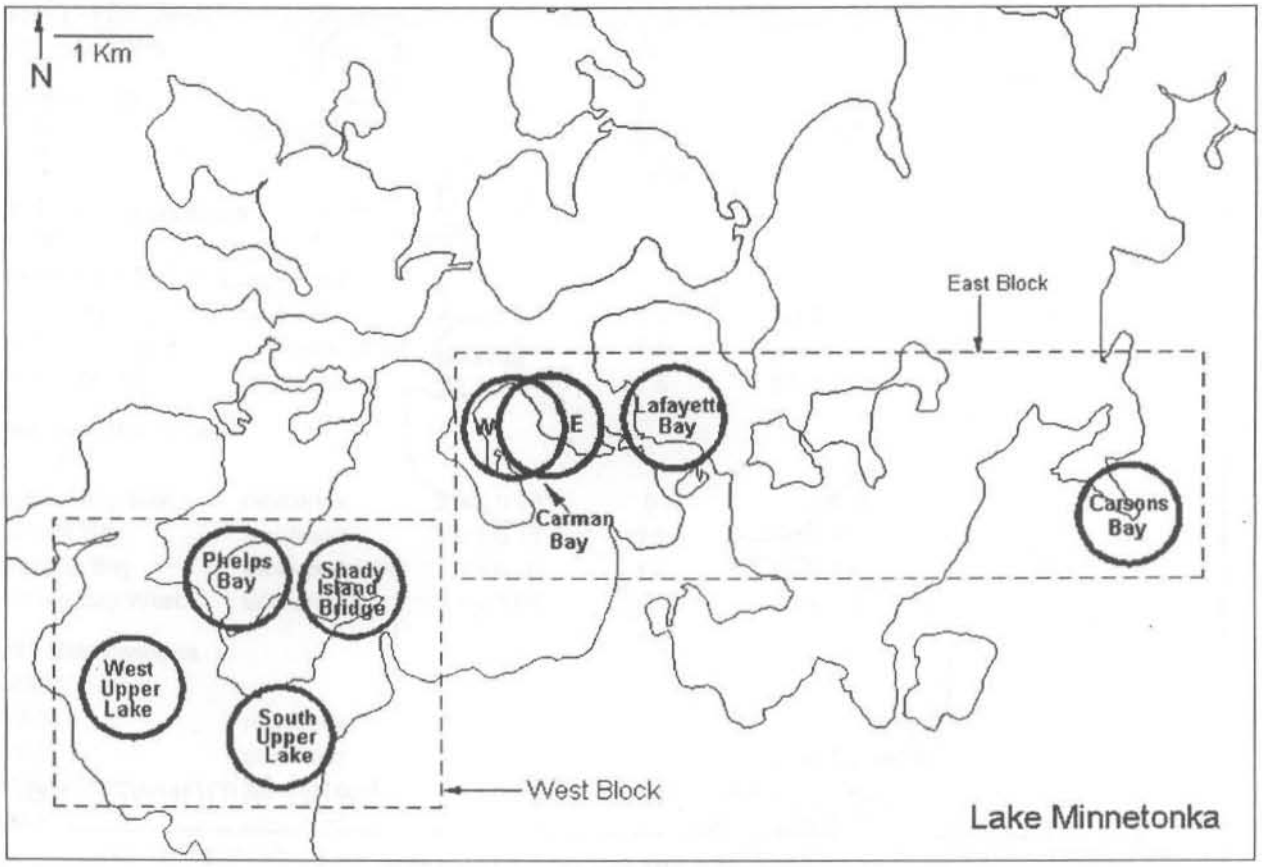

Figure 1.–Location of triclopyr plots on Lake Minnetonka, MN, June through August 1998.

canadensis Michx.), largeleaf pondweed (*Potamogeton amplifolius* Tuckerm.), sago pondweed (*Stuckenia pectinata* (L.) Borner), Richardson's pondweed (*Potamogeton richardsonii* (Ar. Benn.), flatstem pondweed (*Potamogeton zosteriformis* Fern.), and water crowfoot (*Ranunculus* spp).

Triclopyr TEA, as the liquid formulation Renovate® 3[1], was applied to provide nominal application rates below the maximum label rate of 2.5 mg ae L^{-1} (Table 1). These rates were assigned per plot type, with higher rates being applied to the least protected situations: references (0 mg ae L^{-1}), protected plots (0.5 mg ae L^{-1}), semi-protected plots (1.0 mg ae L^{-1}), and unprotected plots (1.5 ae mg L^{-1}). All treatments were replicated three times and blocked by location: West Minnetonka Block, East Minnetonka Block, and Minnewashta Block; with each block containing the full complement of plot types and application rates.

[1] Renovate® 3 is a registered trademark of Dow AgroSciences LLC, Indianapolis, IN, manufactured for SePRO Corporation, Carmel, IN. Mention of trade names is not intended to recommend the use of one product over another.

Applications were made from 23 to 25 June by airboat using subsurface weighted hose injection techniques in all treatment plots except Carsons Bay (Lake Minnetonka), which was inaccessible by airboat. A full tank mix of herbicide plus water (total of 189 L or 50 gal) was evenly applied to each plot with a pump output of 19 L (5 gal) per min to achieve the nominal application rates. Applied in this manner, the treatment of each plot was completed in approximately 10 min. Application in Carsons Bay was made with a small jon boat using weighted hoses for a subsurface injection with a pump output of 6.5 L (1.7 gal) per minute. Herbicide application was made evenly across the plot, and the treatment process for Carsons Bay was completed within 30 min.

At time of application, temperature was measured in 0.5 m increments through the water column using a Hydrolab Surveyor II (Hydrolab Corporation, Austin, TX). Mean water temperature (±1 SE) was 20.6 ±0.2 C in the Lake Minnetonka plots, and 23.3 ±0.6 C in the Lake Minnewashta plots (Table 1). Mean temperature difference between surface and bottom waters in all plots (both lakes) was 1.4±0.2 C, which indicated a near isothermal condition at time of treatment. Wind direction and velocity at time of treatment was measured

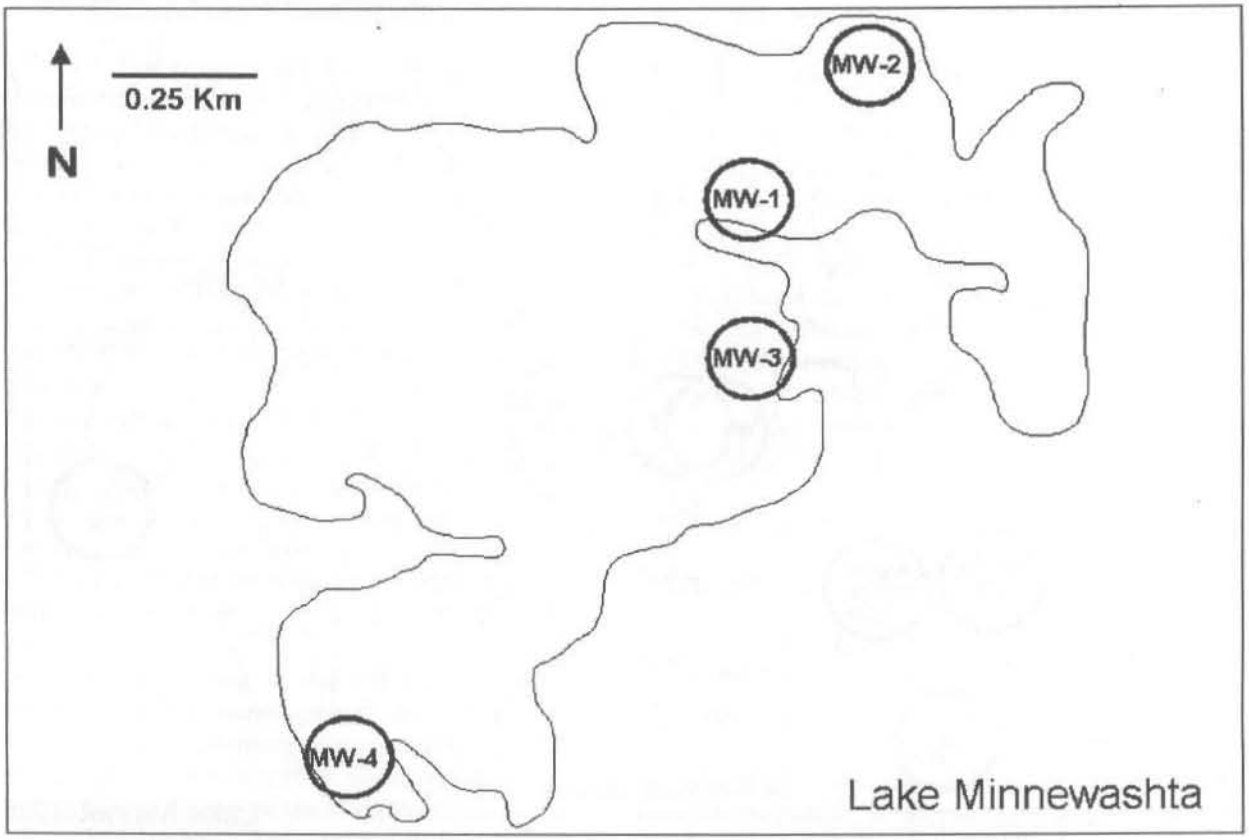

Figure 2.–Location of triclopyr plots on Lake Minnewashta, MN, June through August 1998.

using a Dwyer Wind Meter (Dwyer Instruments, Inc., Michigan City, IN) and ranged from <8 to 16 kph (<5 to 10 mph; Table 1).

Water Residue Sampling and Analysis

To monitor triclopyr residues, duplicate water samples were collected in the center of each plot with submersible pumps at two depths: 0.25 m (subsurface) and 1.0 m (mid-depth). Samples were taken immediately prior to application (PRE), and 1, 4, 8, 24, 48, and 72 hr posttreatment (POST). After collection, samples were stored on ice in the dark until shipped to the analytical laboratories 30 June.

One set of duplicate samples was analyzed using a standard HPLC technique in house at the U.S. Army Engineer Research and Development Center (ERDC). All HPLC procedures were conducted using a Waters HPLC system, made up of the following components: Waters 510 delivery pump, Waters 486 UV detector, Waters 746 data integrator, and incorporating a Waters μ Bondapak C18, 3.9 x 300 mm HPLC column. The method was developed by Dow AgroSciences LLC (Indianapolis, IN) and modified using solid phase extraction (SPE) cartridges as a pretreatment for the cleaning of the water samples as well as concentrating triclopyr. The SPE cartridges were Waters SPE-Pak vac 6 cc (500 mg) C18 cartridges, which were placed on a 12-place SPE-Pak vacuum manifold (JT Baker PN 7018-00).

After column conditioning procedures, a 100 mL aliquot of water sample was filtered through the SPE cartridges to a final elution of 2 mL. Samples were collected and stored in 4-mL amber glass vials and held until analysis. Triclopyr concentrations in water were determined by comparison of the detector response by peak area for the samples against the peak area response obtained from known standard concentrations of triclopyr. Standards were prepared from analytical grade triclopyr obtained from Dow AgroSciences, LLC.

The HPLC conditions were set as follows: eluent for mobile phase, 0.2 N acetic acid plus acetonitrile (1:1 by volume), filtered and degassed prior to use; chart speed at 0.25 cm·min^{-1}; flow rate at 1.5 mL·min^{-1}; wavelength, 280 nm, attenuations were 4 as standard values at 2.5 mg ae·L^{-1}; and sample injection volume was 100 μL. Run time for this compound was 6.5 min, in which the triclopyr peak registered at 5 min.

Table 1.–Plot conditions and rates of application for triclopyr evaluations on Lakes Minnetonka and Minnewashta, MN, June 1998.

Treatment Block Date	Plot Description	Mean Depth m±1 SE	Triclopyr Rate mg ae·L^{-1}	Renovate® used L (gal)	Wind Velocity Direction kph	Mean Water Temperature °C ± 1 SE
West Lake Minnetonka						
6/23/98						
Shady Island Bridge	reference	2.6 ± 0.06	0	0 (0)	na	20.2 ± 0.3
Phelps Bay	protected	2.1 ± 0.04	0.5	28.8 (7.6)	<8 SE	20.9 ± 0.1
South Upper Bay	semi-protected	2.0 ± 0.03	1.0	54.5 (14.4)	<8 SE	20.6 ± 0.2
West Upper Bay	unprotected	2.1 ± 0.04	1.5	87.1 (23.0)	<8 SE	20.8 ± 0.2
East Lake Minnetonka						
6/24/98						
Carmen Bay East	reference	2.8 ± 0.08	0	0 (0)	na	21.1 ± 0.2
Carsons Bay	protected	1.8 ± 0.11	0.5	24.6 (6.5)	11-16 SE	22.6 ± 0.9
Lafayette Bay	semi-protected	1.9 ± 0.05	1.0	53.0 (14.0)	8-11 SE	20.0 ± 0.2
Carmen Bay West	unprotected	1.7 ± 0.04	1.5	71.9 (19.0)	8-11 SE	20.9 ± 0.2
Lake Minnewashta						
6/25/98						
MW-3	reference	2.4 ± 0.04	0	0 (0)	na	22.0 ± 0.3
MW-2	protected	1.9 ± 0.03	0.5	25.9 (6.85)	16 SW	24.1 ± 0.2
MW-4	semi-protected	1.7 ± 0.03	1.0	46.2 (12.2)	8-11 SSE	25.5 ± 0.5
MW-1	unprotected	2.1 ± 0.02	1.5	41.6 (23.3)	8-11 SSW	24.5 ± 0.2

Reporting limit for this method is 5.0 µg·ae L^{-1}. Quality control samples were analyzed at 10% of the total number of samples. The range of recovery for sample spikes was 93 to 105% with an average of 103.5%.

The other set of duplicate samples was analyzed using a newly developed ELISA technique at SePRO Corporation (Carmel, IN). The applications of ELISA principles are summarized by Netherland et al. (2002). Samples were analyzed using ELISA kits (Strategic Diagnostics Incorporated (SDI), Newark, DE). Absorbance was measured using a RPA-1 RaPID Analyzer™ (SDI, Newark, DE). Duplicate analyses of four standards (0 mg·mL^{-1}, 0.5 mg·mL^{-1}, 2.5 mg·mL^{-1}, 6.0 mg·mL^{-1}) were used for the calibration curve. Quality control samples were 10% of total number of samples. The average recoveries of triclopyr ranged from 89 to 118% with an average of 101.8%. The lower limit of detection for this method was 0.1 mg·mL^{-1}. Analytical methods, HPLC and ELISA, were compared using a linear regression with a 1:1 relation between variables.

Plant Surveys

The submersed plant community was assessed in each plot using a point intercept method to determine frequency of species occurrence (Madsen 1999). Thirty-six points were mapped on a 20 m by 20 m grid in each plot. At each point, plant species were identified (Crow and Hellquist 2000a, Crow and Hellquist 2000b) and recorded 3 days PRE (20 June) and 8 weeks POST (3 August). An aquascope was used to aid in underwater viewing of plants. If plants could not be readily identified from the surface, or if plants at the bottom could not be clearly seen, a rake-head device was lowered through the water column and plants were collected and brought to the surface for species verification. Voucher specimens representing all submersed plant species observed were collected and archived at the ERDC. Frequency of species PRE and POST were compared using Chi-square analyses of two-by-two comparisons of the actual number of points with and without that species in a plot. Because plant frequency data were statistically homogenous for plots with the same herbicide application rate, these data were pooled and re-analyzed using Chi-square. Mean species per point was calculated as average number of species present at points in a plot; treatments were compared PRE and POST using the Mann-Whitney Rank Sum Test (Rank Sum Test; $p \leq 0.05$).

Table 2.–Percent frequency of plant species in study plots for each treatment before (PRE) and after 8 weeks (POST) triclopyr application on Lakes Minnetonka and Minnewashta, MN in 1998. There were 36 sample points mapped in each plot for a total of 108 points per treatment.

Species	Triclopyr Treatment Rate							
	0 mg ae L^{-1}		0.5 mg ae L^{-1}		1.0 mg ae L^{-1}		1.5 mg ae L^{-1}	
	PRE	POST	PRE	POST	PRE	POST	PRE	POST
Ceratophyllum demersum L. Coontail	9	9	91	93	46	43	43	52
Chara spp Muskgrass	0	0	0	2	0	2	0	4
Elodea canadensis Michx. Elodea	2	2	36	18	5	8	6	5
Lemna trisulca L. Forked duckweed	0	0	0	0	0	17	0	0
Megalodonta beckii (Torr. ex Spreng.) Greene Water marigold	0	4	0	0	0	5	4	7
Myriophyllm sibiricum Komarov Northern watermilfoil	0	0	0	0	1	0	6	0
Myriophyllum spicatum L. Eurasian watermilfoil	99	100	100	73	98	65	99	56
Najas guadalupensis (Spreng.) Magnus Common water nymph	0	7	0	8	0	18	0	15
Nuphar advena (Ait.) Ait. f. Spatterdock	0	0	6	13	8	8	13	15
Nymphaea odorata Ait. Fragrant waterlily	0	0	7	15	7	5	6	3
Potamogeton amplifolius Tuckerm. Largeleaf pondweed	15	10	29	32	12	8	6	4
Potamogeton diversifolius Raf. Variableleaf pondweed	0	0	3	0	0	0	4	0
Potamogeton crispus L. Curlyleaf pondweed	69	0	61	0	51	0	32	0
Potamogeton gramineus L. Variable pondweed	0	0	0	0	0	0	1	9
Potamogeton illinoensis Morong Illinois pondweed	0	6	2	2	0	0	0	1
Potamogeton natans L. Floatingleaf pondweed	0	0	1	2	0	0	10	8
Potamogeton praelongus Wulf. Whitestem pondweed	16	1	5	0	15	1	5	0
Potamogeton richardsonii (Ar. Benn.) Rydb. Claspingleaf pondweed	39	6	22	5	47	22	24	7
Potamogeton robbinsii Oakes Robbins' pondweed	0	0	0	0	0	0	0	1
Potamogeton zosteriformis Fern. Flatstem pondweed	49	18	71	23	45	42	32	17
Ranunculus spp Water crowfoot	2	0	22	0	8	3	6	0
Stuckenia pectinata (L.) Borner Sago pondweed	18	8	31	30	18	24	12	12
Utricularia vulgaris L. Common bladderwort	0	0	3	18	2	0	1	12
Vallisneria americana Michx. Wild celery	0	5	0	0	0	12	1	4
Zosterella dubia (Jacq.) Small Water stargrass	0	1	1	4	0	12	1	0

Results and Discussion

Correlation of HPLC and ELISA Techniques

There was a significant and direct correlation between HPLC and ELISA analytical techniques in this study ($y = 0.034 + 0.952x$, $p<0.0001$, $R^2 = 0.96$; Fig. 3). The average percent recovery from the ELISA technique was 101.8% compared to 103.5% from the HPLC method. These results indicate that this immunoassay technique is a valid method to test for the presence and dissipation of triclopyr in lakes and reservoirs. A significant comparison of ELISA with HPLC has also been reported in the analysis of trace amounts of triclopyr (10 to 80 µg ae·L^{-1}) found in forest streams ($y = 4.1 + 1.01x$, $R^2 = 0.92$; Fischer and Michael 1997). Using ELISA can provide for relatively rapid (24 to 48 hr POST) assessment of water residues, both within and outside of the treated area (Netherland et al. 2002). Moreover, ELISA may be more cost-effective compared to HPLC when large numbers of samples are collected for analysis (Fischer and Michael 1997).

Based on the high correlation between ELISA and HPLC methods, data from each method were combined and subjected to a three-way analysis of variance (ANOVA) to test for significant effects of rate, sample time, and depth. Because there were no statistical differences between subsurface and mid-depth samples ($MS = 0.0116$, $F = 0.128$, $p = 0.721$), these data also were combined for correlating sample time with water residues for each treatment using a nonlinear regression. The triclopyr half-life ($t_{1/2}$) for each treatment was then calculated using the slope (m) of each significant regression ($p \leq 0.05$) in the equation: $t_{1/2} = -1n(0.5)/m$.

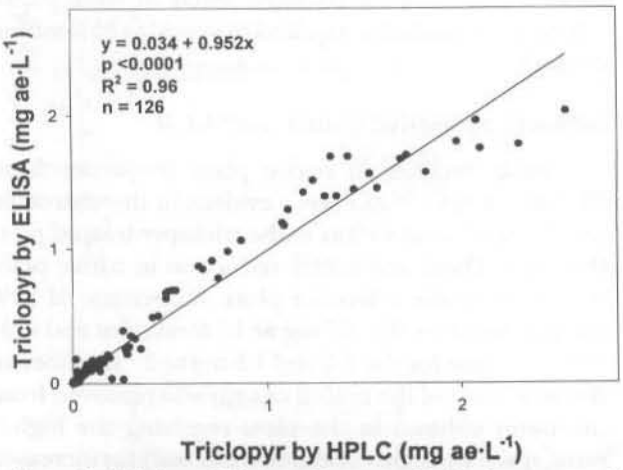

Figure 3.–Relation of high performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA) analytical techniques for determining triclopyr concentration in water samples from Lake Minnetonka and Lake Minnewashta, MN after herbicide application in June 1998.

Water Residues

Water temperatures were essentially isothermal in all plots at the time of treatment (Table 1). Mean triclopyr water residues at 1 hr POST indicated that the herbicide was relatively well distributed in the upper half (top and mid) of the water column for each treatment regime (Table 3). Measured rates were 12 to 59% greater than target concentrations which was probably an artifact of the time it took for the concentrated material to mix into the water column. By 4 hr POST, there was complete mixing of residues through the water column, thus lowering triclopyr concentrations. By 8 hr POST, water residues were at, or near, the product's labeled MCL of 0.4 mg ae L^{-1} in all plots. Triclopyr in the water column had dissipated to levels <0.15 mg ae L^{-1} by 24 hr POST for all treatments, representing a 70 to 90% decline in aqueous concentrations from the 1-hr POST measurements.

The half-life for the 1.0 mg ae L^{-1} treatment was 2.85 hr, which was shorter than the other treatments, 3.48 hr and 4.15 hr for the 0.5 and 1.5 mg ae L^{-1}, respectively (Fig. 4). Because photolysis and hydrolysis are not significant routes of degradation in the field (reviewed in Petty et al. 2003), these short half-lives may be attributed to dilution via the large fringe area of untreated water associated with the small plot size used in this study. Half-lives in ponds where triclopyr was applied to the entire water bodies with no water exchange averaged 6.5 days (Petty et al. 1998b, Petty et al. 2001). Half-lives reported from larger treatment plots (4 to 6 ha) in Lake Minnetonka, MN, and the Pend Oreille River, WA, ranged from 0.8 to 4.7 days (Getsinger et al. 1997, Getsinger et al. 2000).

Plots receiving the highest application rate of 1.5 mg ae L^{-1} were established in unprotected shoreline situations. Winds during the application period were 8 to 11 kph (Table 1), yet the mean triclopyr half-life was 4.15 hours (Fig. 4), greater than the other plot locations. Plots receiving the lowest application rate of 0.5 mg ae L^{-1} were established in protected shoreline situations with a mean triclopyr half-life of 3.86 hours (Fig. 4); however, wind gusts of 11 to 16 kph occurred during the application period (Table 1). Regardless of plot location, winds may have played a role in moving aqueous triclopyr residues off-target more quickly than anticipated, thereby reducing herbicide contact time (Fig. 4).

Table 3.–Triclopyr concentrations (mg ae·L^{-1}) analyzed with high performance liquid chromatography (HPLC) and immunoassay (ELISA). Samples were taken subsurface (top) and mid-depth (mid) in the water column for each sample time (mean ± 1 SE, $N = 3$) after application in Lake Minnewashta, MN, June 1998.

	Hours after application													
	1		4		8		24		48		72			
	top	mid	top	mid	top	mid	top	mid	top	mid	top	mid	top	mid
HPLC														
0.5[a,b]	0.60±0.14	0.52±0.21	0.31±0.12	0.26±0.11	0.20±0.08	0.35±0.20	0.11±0.04	0.06±0.04	0.13±0.03	0.07±0.01	0.05±0.01	0.06±0.0		
1.0	1.50±0.30	1.58±0.20	0.62±0.25	0.77±0.18	0.28±0.09	0.33±0.18	0.04±0.01	0.03±0.02	0.02±0.01	0.01±0.01	0.01±0.01	0.06±0.0		
1.5	1.98±0.32	1.76±0.29	1.28±0.53	1.07±0.40	0.59±0.47	0.46±0.39	0.12±0.01	0.15±0.02	0.03±0.01	0.02±0.0	BD[c]	BD		
ELISA														
0.5	0.75±0.17	0.70±0.27	0.37±0.17	0.31±0.16	0.18±0.04	0.41±0.25	0.12±0.05	0.13±0.05	0.09±0.03	0.08±0.02	0.06±0.01	0.06±0.01		
1.0	1.61±0.23	1.56±0.08	0.79±0.26	0.87±0.15	0.30±0.10	0.34±0.026	0.08±0.02	0.04±0.03	0.03±0.01	0.03±0.02	0.01±0.0	0.02±0.01		
1.5	1.84±0.10	1.61±0.12	1.18±0.42	1.04±0.38	0.59±0.49	0.56±0.48	0.12±0.0	0.16±0.02	0.03±0.01	0.03±0.0	BD	BD		

[a]Nominal application rates (mg ae·L^{-1}).
[b]Triclopyr maximum contaminant level (MCL) = 0.4 mg ae·L^{-1}.
[c]Below detection (<0.01 mg ae·L^{-1}).

Assessment of Plant Community

Efficacy on Eurasian watermilfoil

By 3 days POST, milfoil shoots were epinastic with chlorotic apices. By 14 days POST, milfoil shoots were defoliated and lying on the sediment surface. Plant necrosis followed in the next two weeks. By 8 weeks POST, mean milfoil frequency in the triclopyr treatments was reduced by 27 to 43% compared to untreated reference plots, and was significantly reduced compared to pretreatment levels (Table 2; Fig. 5A). Moreover, milfoil plants present in the treated plots were small, only 5 to 20 cm in height, which indicated they were probably resprouting from rootcrowns that survived herbicide exposure. It is uncertain whether these small plants would continue to survive and successfully overwinter.

Plots receiving the highest application rate of 1.5 mg ae L^{-1} yielded the most effective milfoil control with a 43% reduction in frequency. Semi-protected plots receiving the middle application rate of 1.0 mg ae L^{-1} yielded the next greatest control with a 33% reduction in milfoil frequency. Plots receiving the lowest application rate of 0.5 mg ae L^{-1} yielded the least effective control with a 27% reduction in milfoil frequency.

While the level of milfoil control in this study was less than that achieved in previously reported field studies (Getsinger et al. 1997, Petty et al. 1998a), it matches that predicted from small-scale triclopyr concentration/exposure time (CET) studies (Netherland and Getsinger 1992). Results from those growth chamber evaluations indicated that contact times similar to the half-lives measured in this study ($t_{1/2}$ = 2.85 to 4.15 hours) would provide <70% control of milfoil, and that exposure times > 24 hours for triclopyr levels of from 0.5 to 1.5 mg ae L^{-1} would be required to provide >85% milfoil control.

Impacts on Native Plants

Small declines in native plant frequency from PRE to 8 weeks POST were evident in the untreated reference plots as well as in the triclopyr-treated plots (Fig. 5B). There was a 24% reduction in native plant frequency in the reference plots. A decrease of 20% was recorded for the 0.5 mg ae L^{-1} treatment and only a 6% decrease for the 1.0 and 1.5 mg ae L^{-1} treatments. Because most of the milfoil canopy was removed from the water column in the plots receiving the higher rates, space and light conditions necessary for increased growth and competition from native plants were created (discussed by Madsen 1997).

In 7 of the 9 treatment plots, mean species per point either significantly increased (2 plots; Fig. 6) or

remained unchanged (5 plots; Fig. 6). Water marigold (*Megalodonta beckii* (Torr. Ex Spreng.), a protected species in many Midwestern and Northeastern states (Nelson et al. 2002), increased slightly in the 1.0 and 1.5 mg ae L^{-1} treatments. Distribution increases of common bladderwort (*Utricularia vulgaris* L.), common water nymph (*Najas guadalupensis* (Spreng.) Magnus), wild celery (*Vallisneria americana* Michx), and water stargrass (*Zosterella dubia* (Jacq.) Small) were greater in the triclopyr treatment plots than in the untreated reference plots. Two of the three reference plots exhibited significant decreases in species, while the third plot remained unchanged (Fig. 6).

Declines in native plant frequency were most likely due to the normal late season senescence of the pondweeds typical in northern tier shallow lakes rather than to herbicide injury (Fig. 5, Table 2). Pondweeds reportedly have tolerated triclopyr in the laboratory (Sprecher and Stewart 1995) and the field (Getsinger et al. 1997). Northern watermilfoil (*Myriophyllum sibiricum* Komarov), a close relative of the target milfoil in this study, was absent at 8 weeks POST in the 1.0 and 1.5 mg ae L^{-1} treatments (Table 2). Although elodea was not affected by triclopyr rates of 2.5 mg ae L^{-1} in a laboratory study (Sprecher and Stewart 1995), distribution was variable in this study (Table 2).

Spatterdock (*Nuphar advena* Ait.) increased in all treatments while fragrant waterlily (*Nymphaea odorata* Ait.) was only slightly reduced at the higher rates (Table 2). These ecologically important floating-leaved species may be susceptible to systemic herbicides when sprayed directly on the leaf surface; however, low triclopyr rates in a submersed application technique should have little to no effect on these plants within and adjacent to the treated area.

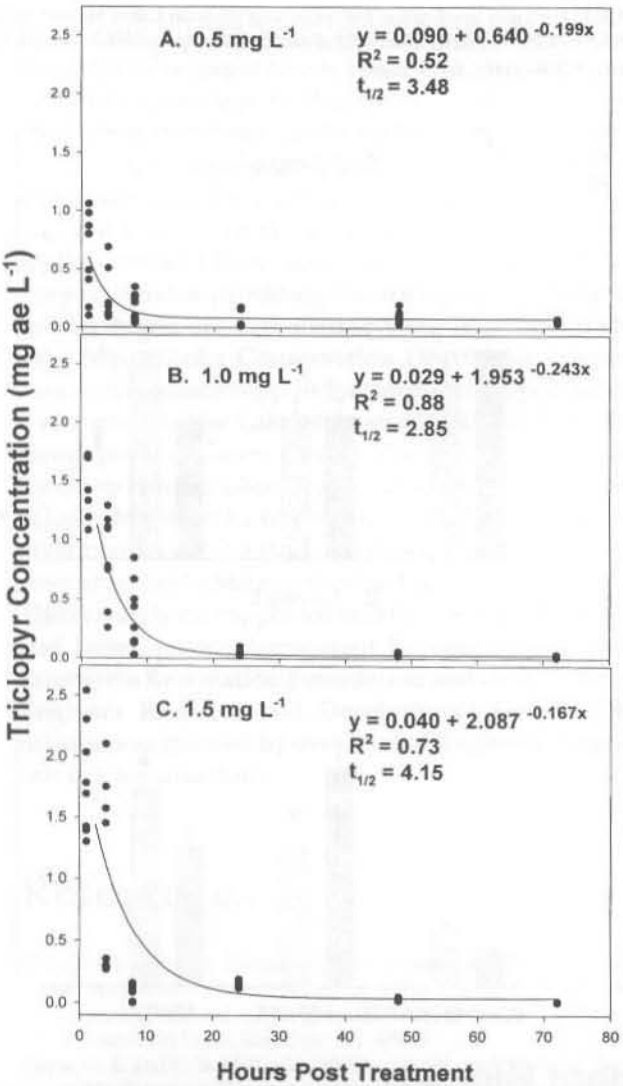

Figure 4.–Regression equations and half-lives for triclopyr dissipation in treatment plots on Lake Minnetonka and Lake Minnewashta, MN during June 1998: A. 0.5 mg ae L^{-1}; B. 1.0 mg ae L^{-1}; C. 1.5 mg ae L^{-1}.

Management Implications

Considering the low rates of triclopyr applied and the small plots used, these treatments would be recognized as a short-term (seasonal) success. Most of the milfoil shoot mass was selectively removed from the water column and the frequency of milfoil occurrence was significantly reduced in the year-of-treatment. By greatly reducing the milfoil canopy, the non-target plant community was maintained and/or enhanced, creating a more open architecture for fish and wildlife habitat, water exchange and circulation, and recreational use of the water. However, many of the milfoil rootcrowns were not killed at any of the triclopyr rates evaluated in these spot-treatment scenarios. Therefore, without follow-up applications, recovery of milfoil to nuisance levels would be anticipated by the next growing season.

It is apparent that aqueous dissipation of triclopyr can be rapid (3 to 4 hr half-life) within small plots, regardless of plot location. Plot selection with respect to wind vectors did not seem to drive herbicide efficacy, but rather greatest milfoil control was achieved in plots with the highest triclopyr rate. While substantial removal of standing milfoil shoots and reduced frequency of the plant can be obtained in the year-of-treatment with low doses in small plots, complete kill of rootcrowns may not occur due to short herbicide exposure time resulting from rapid aqueous dissipation of triclopyr. Therefore, several strategies should be considered to mitigate rapid dissipation of triclopyr when applied as spot-treatments, thereby increasing herbicide contact time and improving con-

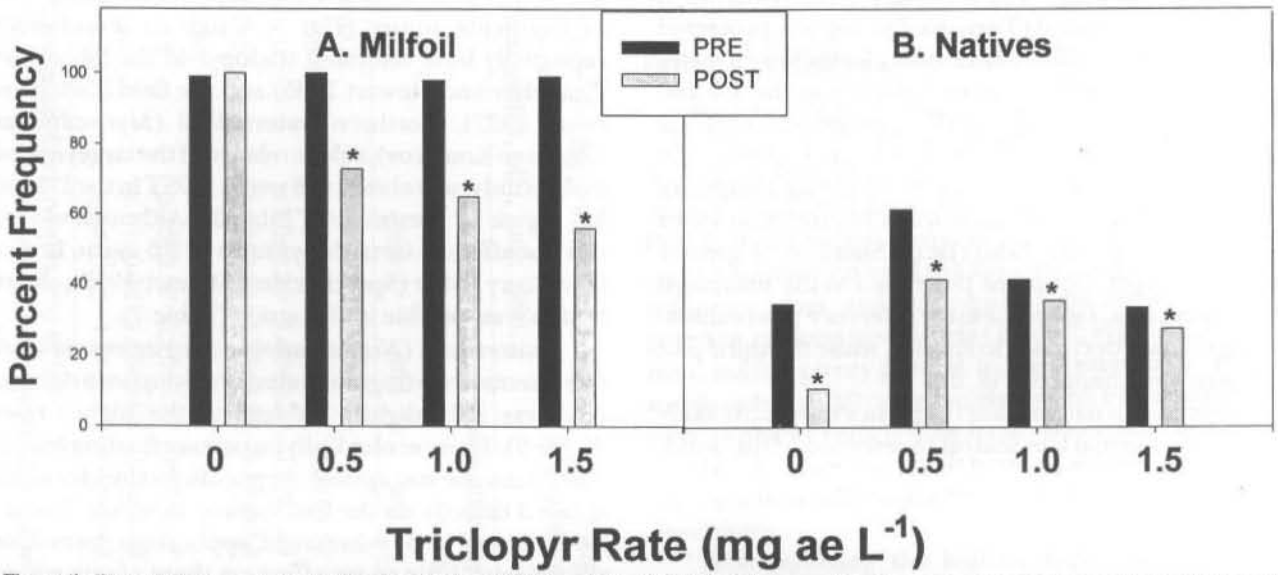

Figure 5.–Percent frequency of aquatic plants before (PRE) and after (POST) triclopyr application for treatment plots on Lakes Minnetonka and Minnewashta, MN during 1998: A. Milfoil; B. Native species (without the exotics, Eurasian watermilfoil and curlyleaf pondweed). Asterisks denote significant differences between PRE and POST for each treatment (Chi-square; $p < 0.05$).

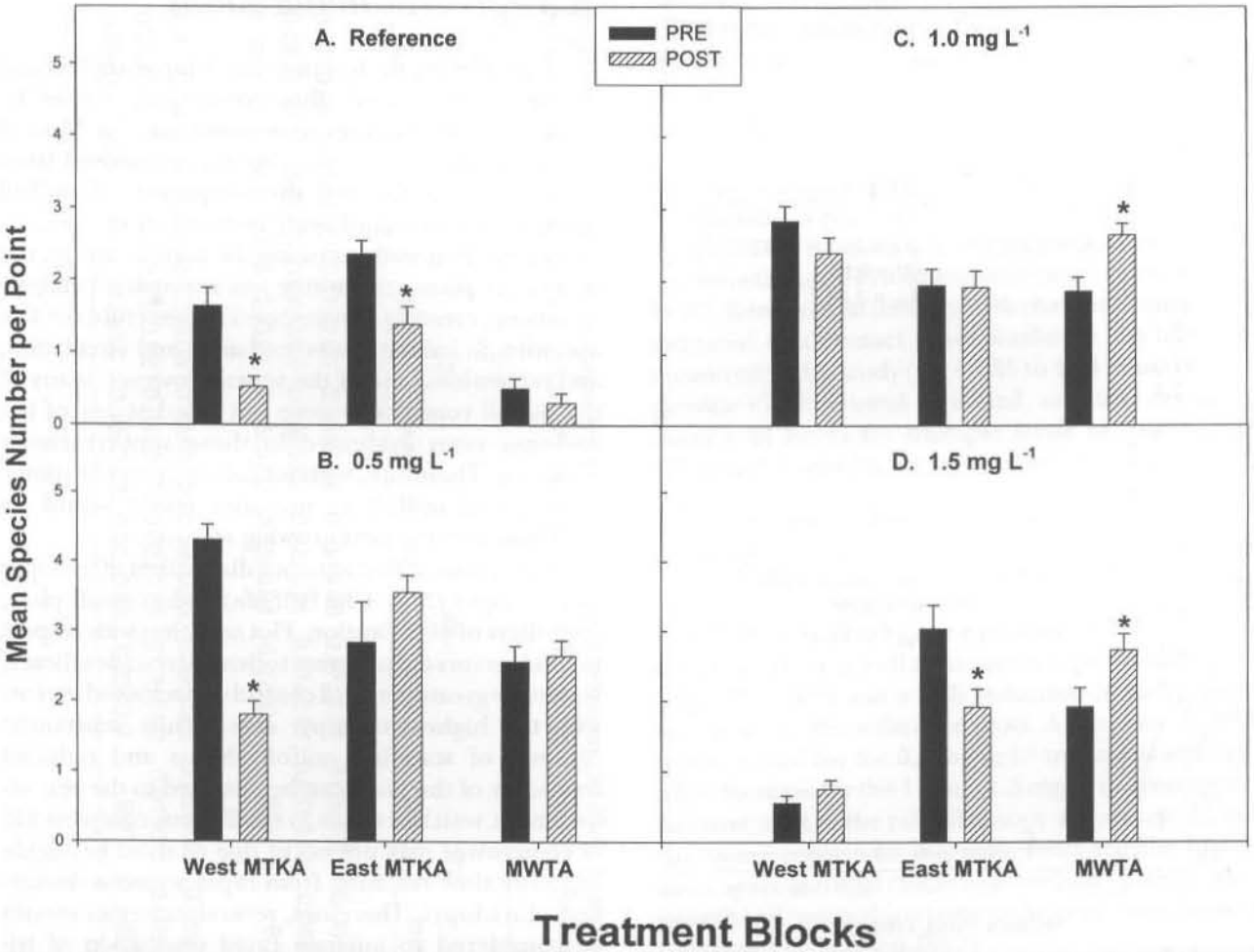

Figure 6.–Mean number of species per point before (PRE) and after (POST) triclopyr application for treatment plots on Lake Minnetonka and Minnewashta, MN during 1998: A. Reference; B. 0.5 ae mg L^{-1}; C. 1.0 mg ae L^{-1}; D 1.5 ae mg L^{-1}. Asterisks denote significant differences between PRE and POST (Rank Sum Test, $p \leq 0.05$).

trol of milfoil. These considerations should include the use of application rates >1.5 mg ae L^{-1} (e.g., 1.75 to 2.5 mg ae L^{-1}); the use of treatment areas > 1 ha in size (e.g., 2 to 4 ha); and the use of sequential applications in the same treatment area (e.g., multiple applications 2 to 8 hr apart, to not exceed a total treatment dose of 2.5 mg ae L^{-1} triclopyr).

The newly developed immunoassay technique is a promising tool for measurement of triclopyr residues in water. This rapid analytical technique will be useful for monitoring triclopyr residues within treated areas, at potable water and irrigation intakes, and in areas outside of treatment boundaries, where a short turn around time for monitoring data is valued. Additional studies should be conducted in varying geographical sites to further validate the correlation between the ELISA and HPLC techniques of measuring aqueous triclopyr residues.

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