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Case Study

Species Selectivity of Granular 2,4-D Herbicide When Used to Control Eurasian Watermilfoil (*Myriophyllum spicatum*) in Wisconsin Lakes

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A total of 24 pre-and posttreatment plant frequency data sets were analyzed from 15 Wisconsin lakes treated with granular 2,4-D BEE herbicide for the control of Eurasian watermilfoil (*Myriophyllum spicatum*). Six data sets from four untreated control lakes were analyzed for comparison. The data sets included the results of line-transect aquatic plant surveys and point-intercept aquatic plant surveys. The results from these two survey methods were analyzed separately. Analysis of pre-and posttreatment changes in frequency of occurence for 46 species of aquatic plants indicated Eurasian watermilfoil was the only species to show significant declines in all the surveys. At application rates of 112 kg ha^{-1}, Eurasian watermilfoil declined an average 65.9% among the line-transect surveys; and 58.0% among the point-intercept surveys. At application rates of 168 kg ha^{-1}, Eurasian watermilfoil declined by 94.4% and 76.5% among line-transect and point-intercept surveys, respectively. Among the control lakes, Eurasian watermilfoil increased an average of 77% in year 1 and 24% in year 2. Northern watermilfoil (*Myriophyllum sibiricum*), a closely related native plant, underwent declines in frequency at the higher 2,4-D application rate (20.0%) but showed an increase (88.9%) at the lower rate among the line-transect surveys. Northern watermilfoil exhibited declines at both rates among the point-intercept surveys (48 and 50%, respectively); however, the plant also exhibited declines in the control lakes in year 2. Most other native aquatic plant species were unaffected or showed increases following treatment with 2,4-D BEE. The high degree of selectivity to Eurasian watermilfoil found in this survey of operational treatments with 2,4-D BEE suggests that this herbicide is an important tool for restoring plant communities that have been degraded by Eurasian watermilfoil.

Nomenclature: Granular 2,4-D BEE, 2,4-dichlorophenoxyacetic acid-butoxyethyl ester; Eurasian watermilfoil, *Myriophyllum spicatum* L.; northern watermilfoil, *Myriophyllum sibiricum* Kom.

Key words: Exotic aquatic plants, native aquatic plants, species selectivity, selective herbicide, Navigate®, nuisance weeds.

Eurasian Watermilfoil. Eurasian watermilfoil (*Myriophyllum spicatum* L.) is an exotic submersed plant that can form dense surface canopies that impede boating, swimming, and fishing. Of the eight milfoil (genus *Myriophyllum*) species found in Wisconsin, Eurasian watermilfoil is the only exotic species. The plant was first introduced into U.S. waters in 1940, and by 1960, it had reached Wisconsin's lakes where its expansion has been exponential. Currently Eurasian watermilfoil has been documented in over 600 Wisconsin lakes (Figure 1).

The spread of Eurasian watermilfoil has significant economic consequences throughout the United States. In the Truckee River watershed below Lake Tahoe, economic damages caused by Eurasian watermilfoil to the recreation industry have been projected at $30 to $45 million annually (Eiswerth et al. 2003). In the Tennessee Valley Authority reservoirs, Eurasian watermilfoil was found to depress nearby real estate values, stop recreational activities, clog municipal and industrial water intakes, and increase mosquito production (Smith 1971). A number of studies have linked declines in real estate values with infestation of Eurasian watermilfoil (Zhang and Boyle 2010). In a study of 170 Vilas

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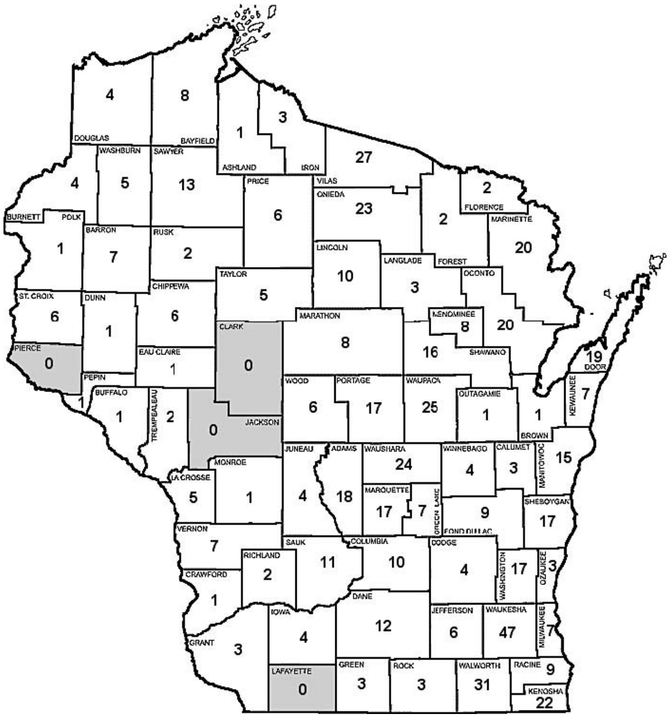


Figure 1. Map showing the number of water bodies in Wisconsin (by county) that have been confirmed to contain Eurasian watermilfoil as of 2010.

County, Wisconsin lakes, Horsch and Lewis (2009) found that Eurasian watermilfoil infestation depressed lakefront property values by 8%, and vacant land values by 13%.

The more significant impacts of Eurasian watermilfoil invasion have been the plant's impacts to aquatic ecosystems. Dense beds of Eurasian watermilfoil have been found to reduce predatory success of fish such as largemouth bass (*Micropterus salmoides* L.) (Engel 1985), and spawning success for trout (*Salmonidae* spp.) (Newroth 1985). Dense mats of Eurasian watermilfoil alter temperature and oxygen profiles and cause anoxic conditions in bottom water layers (Unmuth et al. 2000) resulting in localized mortality to mollusks and other invertebrates. Eurasian watermilfoil has also been found to increase phosphorus concentrations in lakes through accelerated internal nutrient cycling (Smith and Adams 1986).

Perhaps the greatest ecological impacts, though, have been to aquatic plant communities. Eurasian watermilfoil grows very rapidly in the spring, which gives it a competitive advantage over most native plant species. The dense surface mats formed by the plant block sunlight, thereby inhibiting the growth of important native plants (Borman et al. 1997). Over 200 studies link declines in native plants with increases in Eurasian watermilfoil (Madsen et al. 2002). The resultant loss of plant diversity degrades fishery habitat and reduces foraging opportunities for waterfowl and aquatic mammals.

Protecting and restoring native plant communities should be a goal of any Eurasian watermilfoil control program. The goal of protecting and restoring native plants emphasizes the need for selective control methods. A selective management alternative effectively targets Eurasian watermilfoil while having minimal negative impacts to native plants.

Management Alternatives. A number of different methods are used to manage Eurasian watermilfoil throughout the United States. Physical control methods such as benthic barriers, mechanical harvesting, and rotovation of bottom sediments are nonselective. Biological methods include stocking of milfoil weevils (*Euhrychiopsis lecontei* Dietz) (Newman et al. 2001) (Creed and Sheldon 1995). This method is highly selective, but has provided inconsistent results. Chemical methods include Environmental Protection Agency–labeled aquatic herbicides, such as diquat dibromide (6,7-dihydrodipyrido [1,2-a:2′,1-c′] pyrazinediium dibromide), fluridone, triclopyr, endothall, and several formulations of 2,4-D.

Several of the herbicides commonly used for Eurasian watermilfoil management are considered nonselective, meaning that they will affect numerous plant species. When using these herbicides, selectivity to Eurasian watermilfoil may be dependent on treatment timing (endothall), or on applications at very low rates (fluridone) (Wagner et al. 2007). Other herbicides that are commonly used to control Eurasian watermilfoil include triclopyr and 2,4-D. These herbicides are considered selective, because they only affect certain plant taxa. 2,4-D typically only affects dicotyledonous plants. Monocotyledons, such as the pondweed species (*Potamogeton* spp.), are considered tolerant to 2,4-D (Sprecher, et al. 1998).

The Navigate®[1] (2,4-D BEE) label lists watermilfoils (*Myriophyllum* spp.), water stargrass [*Heteranthera dubia* (Jacq.) MacMill.], bladderworts (*Utricularia* spp.), white water lily (*Nymphaea odorata* Aiton), spatterdock (*Nuphar variegata* Durand), watershield (*Brasenia schreberi* J.F.Gmel.), and coontail (*Ceratophyllum demersum* L.) as plants that may be controlled. According to the product label, water stargrass and watermilfoils are susceptible to 2,4-D applied at 112 to 224 kg ha^{-1} (100 to 200 lb $acre^{-1}$). Coontail, watershield, and water lilies are slightly to moderately resistant to 2,4-D and may be controlled at higher rates of 168 to 224 kg ha^{-1}. However, anecdotal reports from lake managers suggest a higher degree of selectivity to Eurasian watermilfoil than the label indicates.

Despite many years of operational field use, there is relatively little information in the scientific literature pertaining to the species selectivity of granular 2,4-D BEE. Parsons et al. (2001) found no significant declines in any native aquatic plants following treatment of Eurasian watermilfoil with 2,4-D BEE in a Washington lake. In a Wisconsin lake, Helsel et al. (1996), reported that coontail,

Table 1. Time frame of Eurasian watermilfoil management activities on the 15 Wisconsin study lakes. Statistical analysis was performed on the paired pre- and posttreatment data sets.

Lake name	Pretreatment survey date	Treatment date	Application rate (kg ha^{-1})	Posttreatment survey date
Data set 1				
Bughs	June 2001	May 2002	112	July 2002
Gilbert	September 2000	May 2001	168	September 2001
Gilbert	September 2000	May 2001	168	July 2002
Gilbert	September 2000	May 2001	168	June 2003
Hancock	September 2003	May 2004	168	July 2004
Kettle Moraine	May 2003	May 2003	112	August 2003
Kettle Moraine	August 2003	May 2004	112	July 2004
Loon	June 2002	June 2003	112	September 2003
Loon	September 2003	September 2003	168	July 2004
Twin	July 2003	July 2003	112	June 2004
Washington	May 2004	June 2004	112	August 2004
Wilson	May 2000	June 2001	112	September 2001
Wilson	May 2000	June 2001	112	June 2002
Wolf	May 2002	June 2002	112	May 2003
Data set 2				
Big Blacksmith	May 2006	May 2006	112	August 2006
Big Blacksmith	August 2006	May 2007	168	August 2007
Little Blacksmith	May 2006	May 2006	112	August 2006
Little Blacksmith	August 2006	May 2007	168	August 2007
Main Channel	May 2006	May 2006	112	August 2006
Main Channel	August 2006	May 2007	168	August 2007
Peshtigo	May 2006	May 2006	112	August 2006
Peshtigo	August 2006	May 2007	168	August 2007
Pywaosit	May 2006	control	0	August 2006
Pywaosit	August 2006	control	0	August 2007
Sapokesick	May 2006	control	0	August 2006
Sapokesick	August 2006	May 2007	168	August 2007
Spring	May 2006	control	0	August 2006
Spring	August 2006	May 2007	168	August 2007
Wahtohsah/Skice	May 2006	control	0	August 2006
Wahtohsah/Skice	August 2006	control	0	August 2007

elodea (*Elodea canadensis* Michx.), variable-leaf watermilfoil (*Myriophyllum heterophyllum* Michx.) and wild celery (*Vallisneria americana* Michx.) declined initially after treatment with 2,4-D BEE, but recovered to 80 to 120% of their standing crops within 10 to 12 wk after application. In a study of the species selectivity of triclopyr and 2,4-D herbicide treatments conducted on Hayden Lake in Idaho, Wersal et al. (2010) found that Eurasian watermilfoil was reduced in all treated areas by 88% with no significant declines in 21 native aquatic plant species. However most other references on species selectivity in the literature pertain to mesocosm studies or are anecdotal in nature. In an effort to gather more information on 2,4-D BEE efficacy and selectivity, we assessed numerous applications to large-scale infestations of Eurasian watermilfoil in natural lakes having diverse communities of aquatic plants.

Materials and Methods

Study Area. Two separate data groups were analyzed in this study. The first data group included 14 pre- and posttreatment aquatic plant survey data sets collected from nine Wisconsin lakes between 2000 and 2004 (Table 1; Figure 2). Data from these nine lakes were analyzed because large-scale milfoil treatments were conducted on each lake, and the management strategies were similar. The management objective for these lakes was to provide long-term control of Eurasian watermilfoil. The treatment strategy involved treating all known beds of Eurasian watermilfoil with granular 2,4-D BEE herbicide. Treatments were conducted in spring when Eurasian watermilfoil was actively growing and water temperatures were in the 15 to 20 C range. These nine Wisconsin lakes (data

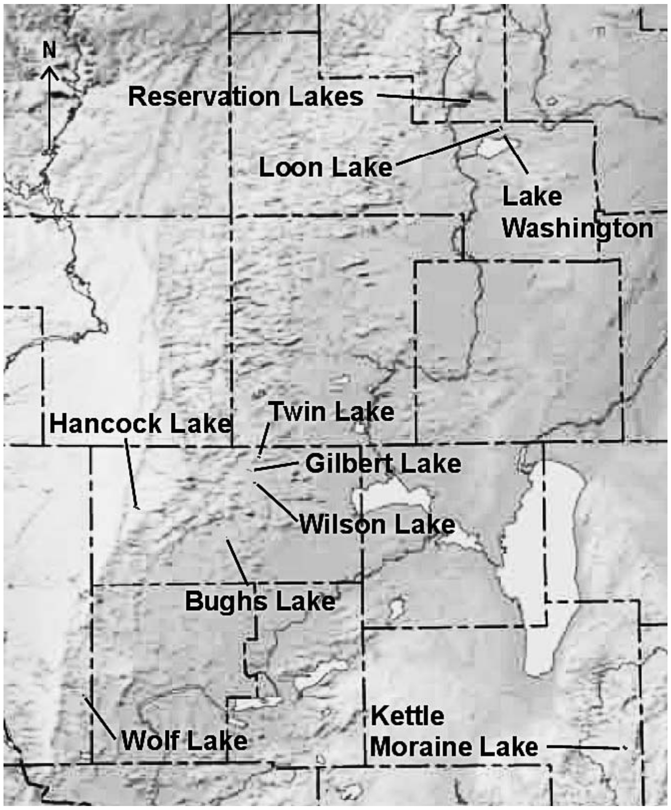


Figure 2. Location of the nine study lakes surveyed from 2000 to 2004, and the location of the Menominee Indian Reservation lakes that were surveyed in 2006 and 2007.

Table 2. The physical characteristics of the study lakes and their preliminary Eurasian watermilfoil distribution.

Lake name	Surface area (ha)	Maximum depth (m)	Average depth (m)	Eurasian watermilfoil area (ha)
Data set 1				
Bughs	12	5.5	2.8	7.2
Gilbert	56	20.0	9.8	3.2
Hancock	37	4.9	1.8	21.2
Kettle Moraine	91	9.2	1.8	44.8
Loon	122	6.8	2.8	33.6
Twin	44	14.1	3.1	5.2
Washington	30	5.5	3.1	11.2
Wilson	32	4.9	1.5	7.2
Wolf	20	14.5	6.1	8.0
Data set 2				
Big Blacksmith	94	23.1	2.9	35.2
Little Blacksmith	34	4.9	1.6	24.0
Main Channel	7	3.1	1.9	2.8
Peshtigo	38	12.9	2.0	12.0
Pywaosit	49	21.5	8.0	11.2
Sapokesick	101	10.8	2.4	70.0
Spring	46	12.9	2.8	35.2
Wahtohsah/ Skice	115	12.3	3.0	72.1

set 1) ranged in size from 12 to 122 ha and pretreatment Eurasian watermilfoil distributions ranged from 3.2 to 44.8 ha (Table 2). Each lake also contained a diverse native plant community.

The second data group (data set 2) included pre- and posttreatment aquatic plant survey data collected between 2006 through 2007 from eight interconnected lakes located on the Menominee Indian Reservation of Wisconsin (Table 1; Figure 2). During 2006, four of the eight lakes were treated with granular 2,4-D BEE at a rate of 112 kg ha^{-1}. The remaining four untreated lakes served as control lakes. During 2007 the same four treated lakes plus two additional lakes were treated with granular 2,4-D BEE at a rate of 168 kg ha^{-1}. The two remaining untreated lakes were once again surveyed to serve as controls. Management strategies for each of these lakes were similarly directed toward providing long-term control of Eurasian watermilfoil by treating all known locations of the plant in the lakes. Treatments were conducted in May when Eurasian watermilfoil was actively growing and water temperatures were in the 15 to 20 C range. The Reservation lakes ranged in size from 7 to 115 ha and their pretreatment Eurasian watermilfoil distributions ranged from 2.8 to 72.1 ha (Table 2).

Data Collection. These pre- and posttreatment aquatic plant surveys were done to fulfill treatment permit conditions established by the Wisconsin Department of Natural Resources (WDNR). The surveys were conducted by private lake management consultants or by the WDNR. Each survey utilized standardized aquatic plant survey protocols developed by the WDNR; however, this survey protocol changed markedly in 2006. Prior to 2006, plant communities of the study lakes were surveyed using line-transect methods. Beginning in 2006, the plant communities of the study lakes were surveyed with a point-intercept method.

Line-Transect Survey Methods. The majority of lakes in this study could be considered glacial potholes, which tend to be ringed with heavily vegetated aquatic plant beds that surround a deep nonvegetated central basin. This littoral zone typically extends to a depth of 4 to 7 m. The line-transect methods used in these surveys involved plotting a series of evenly spaced transects around the lakeshore, typically at 100-m intervals. These transects extended from the shoreline out to the maximum extent of rooted vegetation. Four sampling plots were typically established along each transect by estimating a 3-m-diam circle around

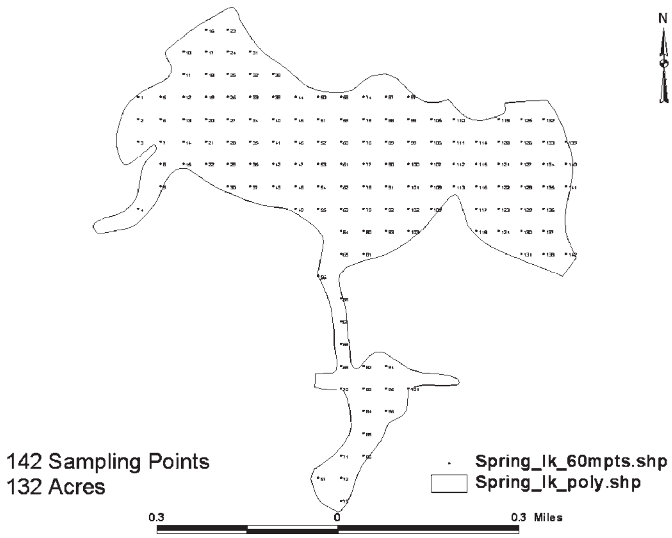


Figure 3. Sample of the point-intercept maps used to perform aquatic plant surveys on the Menominee Indian Reservation lakes.

an anchored boat. The circular plot was then divided into four quarters, with each quarter representing a quadrant. Each species in the quadrant was given an abundance ranking of 1 to 4, depending on how many times it was encountered in each plot. Plants were collected in each quadrant by short-toothed rake. From each rake haul, all plants collected were identified to species whenever possible. Data were recorded separately for each rake haul. Latitude and longitude data were collected with global positioning system (GPS) units at each sample plot in order to make the surveys readily reproducible.

Point-Intercept Methods. The point-intercept aquatic plant survey method was developed in order to provide one standardized method that could be used for all different lake morphologies. In order to ensure the reproducibility of the methods, the WDNR provided individual maps and downloadable GPS coordinates for sample points. The method used in the study lakes involved plotting a series of grid points at 60-m intervals across the lake. Aquatic plant samples were collected where grid lines intercepted, and a single rake tow was made at each point intercept. At each sample point, the rake was dragged along the bottom for approximately 0.75 m to collect plants. All navigable points were sampled. All plant samples collected were identified to species whenever possible and each species was given an abundance rating of 1 to 3, based on the density of plants collected on the rake. A sample of a point-intercept map is shown in Figure 3. A more detailed description of this method is found in Mikulyuk et al. (2010).

Statistical Analysis. Statistical analysis was performed between the pre- and posttreatment frequency percentage data for each plant in each lake to determine if differences were statistically significant. These analyses consisted of paired *t*-tests for two sample means with 95% confidence intervals. Each pre- and posttreatment data set was analyzed individually. Data were not pooled among surveys or between years. Because several of the closely related *Potamogeton* species can be difficult to distinguish, and because several different collectors were involved in the 2000 to 2005 surveys, *Potamogeton* species were considered together to avoid potential statistical error due to misidentification. Data from 2006 to 2007, however, are presented as recorded. Sapokesick Lake, which was an untreated control during 2006, had a partial milfoil treatment during 2007. Because this differed from the other treated lakes where all known milfoil infestations were treated, data from the 2007 survey of Sapokesick Lake were eliminated from analyses.

Because of the different survey methods and potential issues with comparability, data from the line-transect surveys were analyzed and presented separately from the data collected in the point-intercept surveys.

Results and Discussion

Line-Transect Aquatic Plant Survey Results 2000 to 2004. The average percentage of change in frequency of aquatic plants from nine surveys conducted on seven lakes from 2000 to 2004 is presented in Figure 4. These surveys reflect the changes after treatment with granular 2,4-D BEE applied at 112 kg ha^{-1}. Eurasian watermilfoil, the target plant, declined by an average of 65.9%. Among the other potentially susceptible species, water stargrass was the only plant to exhibit a significant decline (−11.1%). However, water stargrass exhibited no significant change in frequency at the higher application rate (Figure 5), so this decline may be unrelated to treatment. Northern watermilfoil (*Myriophyllum sibiricum* Kom.), a plant that is closely related to Eurasian watermilfoil, increased by an average of 88.9%. All other plant taxa exhibited increases in frequency or no change.

Figure 5 presents the average percentage of change in frequency of aquatic plants found in five surveys conducted on three lakes that were treated with granular 2,4-D BEE at a rate of 168 kg ha^{-1}. The level of Eurasian watermilfoil control increased dramatically at the higher rate. Eurasian watermilfoil frequency declined an average of 94.4% among these surveys. Northern watermilfoil declined by an average of 20%. As a potentially susceptible species, this decline is also likely due to treatment. Bushy pondweed (*Najas flexilis* Willd.) and *Potamogeton* species also exhibited declines. However the declines were small, and the taxa are considered tolerant to 2,4-D herbicides. It is likely that these declines were due to other factors. All other plant taxa exhibited increases in frequency or no change.

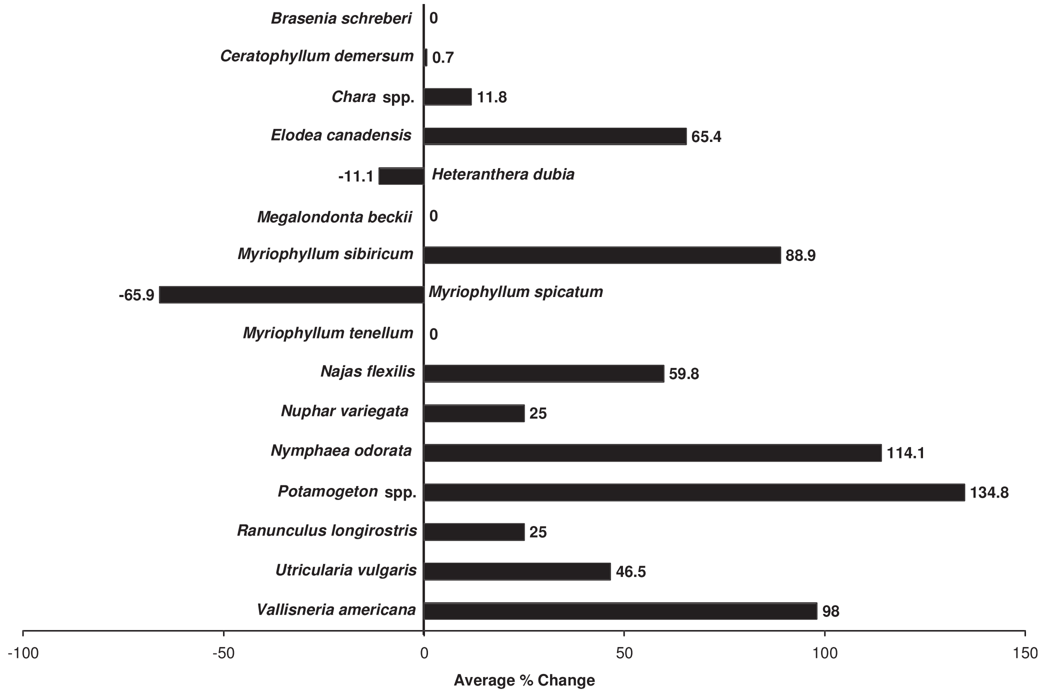
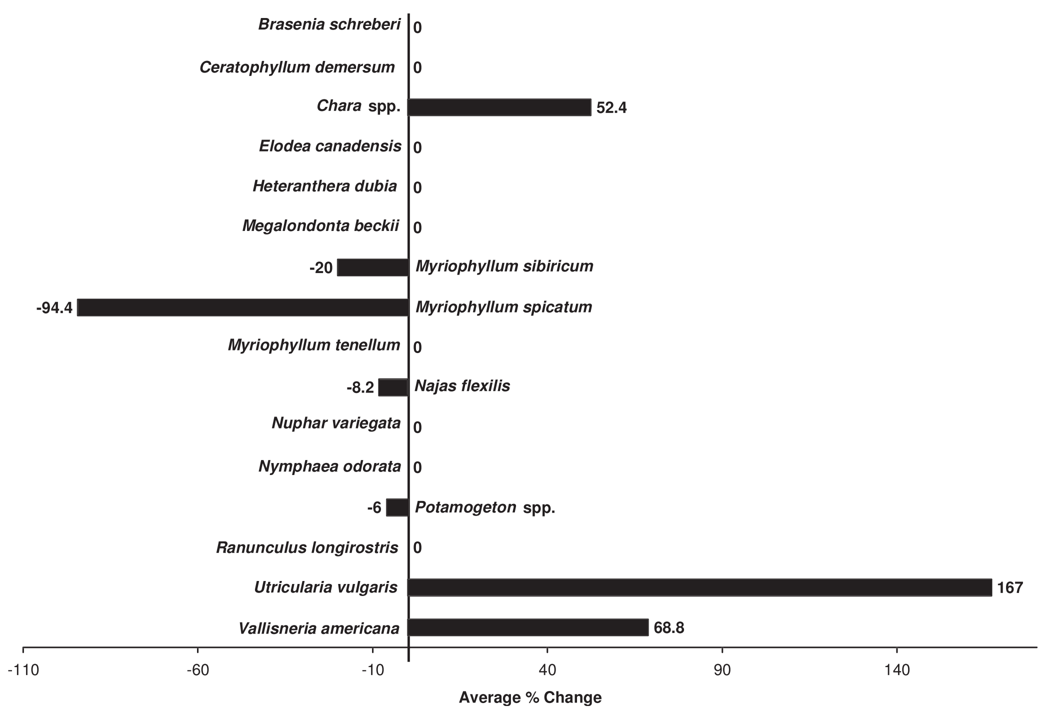


Figure 4. Average percentage of change in frequency of aquatic plants in study lakes (nine total) after treatments with 2,4-D BEE at a rate of 112kg ha^{-1}, 2000 to 2004. Statistically insignificant results ($P > 0.05$) were given a value of 0% when averages were calculated.



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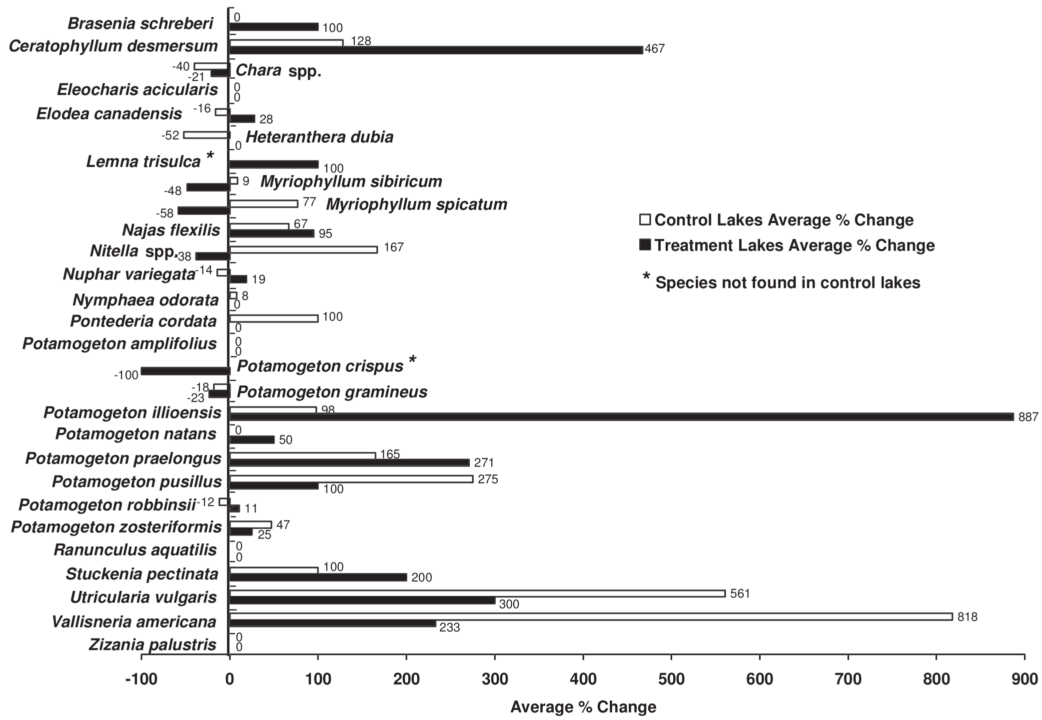

Figure 6. Average percentage of change in frequency of aquatic plants in Menominee Indian Reservation lakes in 2006. Treatment lakes (four) were treated with granular 2,4-D at a rate of 112 kg ha^{-1}. Control lakes (four) were untreated. Statistically insignificant results ($P > 0.05$) were given a value of 0% when averages were calculated.

Clearly, the species having the greatest negative response to treatment with granular 2,4-D BEE among these surveys was Eurasian watermilfoil. The selectivity to Eurasian watermilfoil observed during these surveys was very high, particularly at the lower labeled rate of 112 kg ha^{-1}. Plant abundance naturally varies among different species throughout the season. These natural variations are no doubt responsible for some of the significant increases in frequency of occurrence found among these surveys. However, field observations suggested that nontarget native plants were actively recolonizing areas where Eurasian watermilfoil was previously dominant. The increases in native plant frequency are most likely due to reduced competition from Eurasian watermilfoil.

Point-Intercept Aquatic Plant Survey Results 2006 to 2007. Figure 6 shows the average percentage of change in frequency of aquatic plants found in the four Menominee Indian Reservation lakes treated at 112 kg ha^{-1} during 2006, as well as the four untreated control lakes that were surveyed during the same time period. Eurasian watermilfoil declined by an average of 58% in treated lakes, while increasing by an average of 77% in the untreated lakes. Declines were also found for *Chara*, northern watermilfoil, *Nitella*, curly-leaf pondweed (*Potamogeton crispus* L.), and variable pondweed (*Potamogeton gramineus* L.). *Chara* and variable pondweed also showed declines in control lakes, thus these changes are likely due to natural seasonal variation and not treatment. This is also likely the case for *Nitella*, which showed no significant change following treatment at 168 kg ha^{-1} (Figure 7). Curly-leaf pondweed was not found in posttreatment surveys. However, this plant dies off naturally by midsummer, so the decline cannot be attributed to treatment. Northern watermilfoil declined by 48% in treated lakes while increasing by 9% in the control lakes, and is the only nontarget decline readily attributable to treatment among these surveys.

Figure 7 shows the average percentage of change in frequency of aquatic plants found in the four Reservation lakes treated at 168 kg ha^{-1} during 2007, as well as the two untreated control lakes that were surveyed during the same time period. An increase in Eurasian watermilfoil control efficacy was also observed among these surveys following the higher treatment rate. Eurasian watermilfoil declined by an average of 76.5% in treated lakes, while increasing by 24% in untreated lakes. Northern watermilfoil declined by 50% in treated lakes and by 15% in untreated lakes. The only other significant declines were found in coontail, small pondweed (*Potamogeton pusillis* L.), and small bladderwort (*Utricularia minor* L.). However these declines are an average and they were not found in all of the treated lakes.

Among the 24 pre- and posttreatment data sets analyzed in this study, the only species having consistent decline

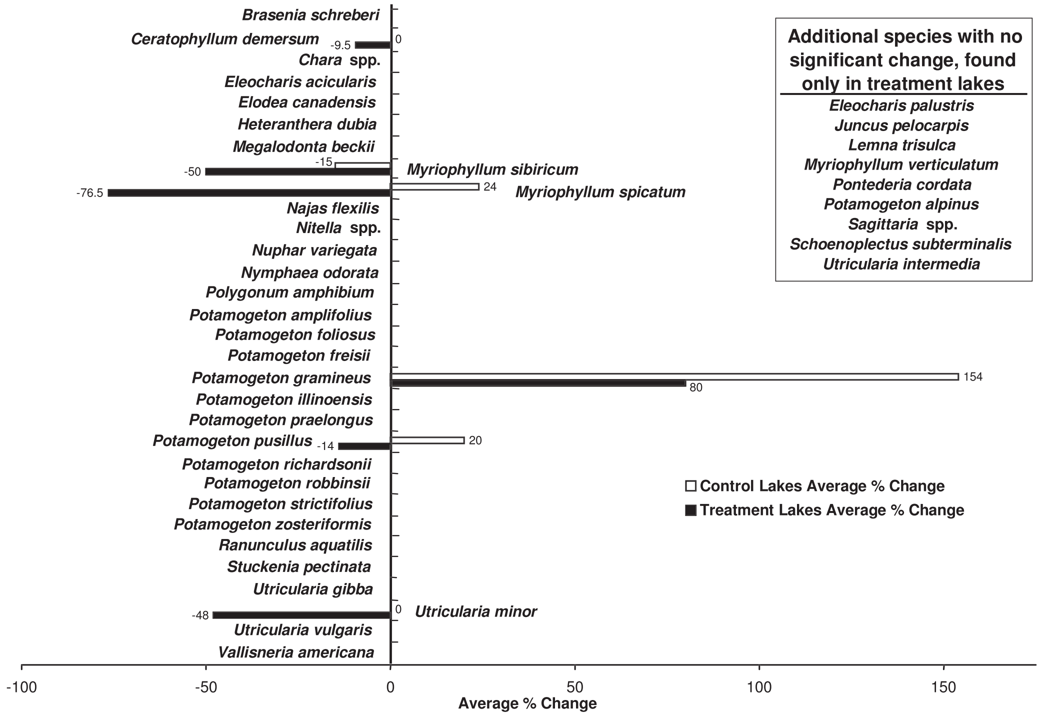

Figure 7. Average percentage of change in frequency of aquatic plants in Menominee Indian Reservation lakes in 2007. Treatment lakes (four) were treated with granular 2,4-D at a rate of 168 kg ha^{-1}. Control lakes (two) were untreated. Statistically insignificant results ($P > 0.05$) were given a value of 0% when averages were calculated.

following treatment with granular 2,4-D BEE at all rates among all of the surveys was Eurasian watermilfoil. Northern watermilfoil was the only other species to commonly exhibit significant declines in frequency. Although these declines were not consistent, they did tend to increase with higher application rates of 2,4-D BEE. Therefore the declines in northern watermilfoil frequency were directly related to treatment. Significant declines occurred infrequently for coontail, water stargrass, and small bladderwort. These dicotyledons may be negatively affected by 2,4-D BEE treatment in certain situations, but the data suggest that they will be unaffected more often than they will exhibit declines. There were no patterns in the data to suggest that any of the 41 other aquatic plant species studied were negatively affected by 2,4-D BEE treatments.

It is not surprising to see few negative responses among monocots, which are considered tolerant to 2,4-D herbicides. It is, however, surprising that so few negative responses were observed among the dicots that are considered susceptible to 2,4-D. The responses of other dicots may be a function of 2,4-D concentration and exposure time. Given the rapid early season growth of Eurasian watermilfoil, it can be assumed that the plant's metabolism is accelerated as well. Accelerated metabolism may result in increased uptake and translocation of herbicide. The accelerated metabolism of Eurasian watermilfoil may account for its susceptibility to 2,4-D BEE at concentrations that do not affect other species.

Herbicide formulation could also be a factor in the high degree of species selectivity for granular 2,4-D BEE that was observed in this study. Liquid herbicides, when applied to aquatic systems, achieve their highest concentration shortly after application. Herbicide concentration then steadily decreases as the herbicide dissipates and degrades. Granular aquatic herbicide formulations are designed to slowly release herbicide over time in the target area. Thus granular formulations may never provide as high a concentration to the treatment area as liquid formulations, even when the exact same quantities of active ingredient are applied.

The relationships between 2,4-D BEE concentration, exposure time, and species selectivity warrants further investigation.

Management Implications. Species selectivity, the ability of a management technique to affect only the target organism, is a very important criterion in Eurasian watermilfoil management. Nontarget native plants not only provide important habitat in lake ecosystems, but they protect water quality and clarity as well. Therefore protecting native plant communities from adverse effects of herbicide treatments has been a priority for lake managers and regulatory agencies alike. The species selectivity of different herbicides and how selectivity relates to factors such as application method and treatment timing has been a source of much confusion. The results of this study indicate not only that granular 2,4-D BEE can be used selectively against Eurasian watermilfoil, but that the herbicide formulation is inherently selective. Species selectivity was achieved when applications were made amongst diverse, actively growing native plant communities; thus selectivity was not due to outside factors such as treatment timing.

The high degree of species selectivity documented for granular 2,4-D BEE in this study suggests that this herbicide can be an extremely valuable tool for combating Eurasian watermilfoil while protecting native plants. Treatment efficacy along with species selectivity makes 2,4-D BEE a tool for true restoration of aquatic habitats that have been degraded by Eurasian watermilfoil.

Sources of Materials

[1] Navigate®, granular butoxyethyl ester formulation of 2,4-D (2,4-D BEE), Applied Biochemists, Germantown, WI 53022.

Acknowledgments

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