Selective Eurasian watermilfoil control – Effects of granular 2,4-D BEE herbicide on native and exotic plants in Wisconsin lakes.

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Abstract:

A total of 29 pre- and post treatment plant frequency data sets were analyzed from 14 Wisconsin lakes treated with granular 2,4-D BEE (2,4-dichlorophenoxyacetic acid - butoxyethyl ester) herbicide for the control of Eurasian watermilfoil (Myriophyllum spicatum L.*). The application rates of granular 2,4-D BBE were 112 and 168 kg/ha. Analysis of pre-and post-treatment changes in percent frequency for 41 species of aquatic plants indicated Eurasian watermilfoil was the only species to show significant declines in all the surveys (averaging -65.0% at 112 kg/ha, and -84.2% at 168 kg/ha.). Northern watermilfoil underwent declines in frequency at the highest 2,4-D application rate (-82.6%) but showed an increase (8.8%) at the lowest rate. This high degree of selectivity of 2,4-D BEE against Eurasian watermilfoil suggests that this herbicide is an important tool for restoring plant communities that have been degraded by Eurasian watermilfoil.*

Key Words:

Eurasian watermilfoil, butoxyethyl ester, species, exotic aquatic plants

Introduction

Eurasian Watermilfoil

Eurasian watermilfoil (*Myriophyllum spicatum* L.) often grows to the water's surface and has three to five leaves arranged in whorls around the stem with each leaf having 12-21 pairs of leaflets. At mid-summer, small reddish flower spikes may emerge above the water's surface. The most distinguishing characteristic of Eurasian watermilfoil is its ability to form dense, impenetrable beds that inhibit boating, swimming and fishing. Eurasian watermilfoil is native to Europe, Asia and Northern Africa. Of the eight milfoil (*Myriophyllum* L.) 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 (Brakken 2000).

Eurasian watermilfoil begins growth earlier in the season than native plants which gives it a competitive advantage. The dense surface mats formed by the plant block sunlight and 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 (Pullman 1993) and reduces foraging opportunities for waterfowl and aquatic mammals. Eurasian watermilfoil has 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).

The continued spread of Eurasian watermilfoil has significant economic consequences. In the Truckee River Watershed below Lake Tahoe, located in western Nevada and northeastern California, 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 real estate values, stop recreational activities, clog municipal and industrial water intakes and increase mosquito production (Smith 1971).

Dense mats of Eurasian watermilfoil alter temperature and oxygen profiles and cause anoxic conditions in bottom water layers (Unmuth et al. 2000) which can cause localized mortality in 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). Because of these economic and environmental concerns, Eurasian watermilfoil management has become the primary objective of many lake management organizations.

Management Alternatives

A number of different methods are used to manage Eurasian watermilfoil throughout the United States. Physical control methods include hand pulling and raking, mechanical harvesting, lake drawdown, and even rotovation of bottom sediments (www.wapms.org/plants/milfoil).

Chemical methods include EPA labeled aquatic herbicides, such as fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone), triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid), endothall (7-oxabicyclo[2,2,1]heptane-2,3-dicarboxylic acid) and several formulations of 2,4-D (2,4-dichlorophenoxyacetic acid). Biological methods include stocking of milfoil weevils (*Euhrychiopsis lecontei* Dietz) (Newman et al. 2001).

Lake management organizations typically consider several criteria when selecting a milfoil management approach. These include: 1) effectiveness; how well the method will work in a given situation, 2) affordability; the cost of the project versus its expected benefits, 3) safety; the level of risk to humans and the environment, 4) public acceptance; the support of lake users, 5) practicality; the suitability of a given management tool for a specific situation, and 6) selectivity; the ability of a management tool to target only Eurasian watermilfoil.

Each of the commonly used Eurasian watermilfoil management alternatives may excel at one or more of these criteria in a given situation, however granular butoxyethyl ester formulation of 2,4-D (2,4-D BEE) has been a commonly employed management alternative because of the benefits it offers in many situations compared to other methods.

2,4-D herbicides have been widely used in lakes throughout North America (Lembi 1996). In fact, the number of lakes in Michigan having Eurasian watermilfoil problems has actually declined as a result of 2,4-D use (Pullman 1993). The greatest disadvantage of 2,4-D BEE treatments is that they rarely produce 100% control in a single application. In most cases, the herbicide tends to work only where applied which allows unnoticed and untreated plants to eventually expand and form dense mon-typic stand. Factors such as pH, plant maturity, water flow, etc. may also reduce herbicide efficacy. Several follow-up treatments, whether in-season or in subsequent years, are often needed to reduce exotic species to target levels.

With the exception of hand-pulling small beds, few people would consider any Eurasian watermilfoil management alternative to be inexpensive. Herbicide treatments however are usually less costly than most other methods due to the relatively low labor costs in comparison to options such as mechanical harvesting. Perhaps the greatest consideration is that 2,4-D, as a systemic herbicide, can provide long-term control of Eurasian watermilfoil. Annual maintenance costs are usually reduced once the target species are brought under control by the initial herbicide treatments.

The Environmental Protection Agency (EPA) product label for Navigate®, the only registered granular 2,4-D BEE product currently on the market, lists no water use restrictions for swimming or fish consumption following treatment. The EPA lists 2,4-D as a Class D herbicide which means there is insufficient data to classify the compound as a carcinogen or harmful to humans

(U.S. E.P.A. 2005). The University of Michigan School of Public Health conducted a review of more than 160 toxicological and epidemiological studies on 2,4-D and concluded that there was insufficient evidence to link 2,4-D exposure to any forms of cancer (Garabrant and Philbert 2002). In addition, 2,4-D from treated lakes has not been reported to contaminate well water adjacent to treated areas. A Michigan Department of Environmental Quality 4-year study found no traces of 2,4-D in drinking water wells adjacent to twelve lakes heavily treated with the herbicide (Bondra 2002).

2,4-D biodegrades quickly in aquatic environments and does not bioaccumulate. For example, even if fish consume 2,4-D pellets, the chemical is quickly excreted without entering muscle tissues. For these reasons, there are no label restrictions on fish consumption (Walters 1998; Carpenter and Eaton 1983).

While it is not possible to guarantee that any herbicide is 100% safe, the overwhelming body of evidence suggests that 2,4-D herbicides, when properly used, pose minimal risks to humans and the environment.

The biggest disadvantage of using aquatic herbicides is the challenge of gaining public acceptance. The methods by which herbicides work are not readily observed or understood, thus they are an unknown entity to many people. The abstract nature of herbicide chemistry may elicit a negative response from the public; therefore extensive educational efforts are often required before herbicides are accepted as a Eurasian watermilfoil management tool.

Among the herbicides used for controlling Eurasian watermilfoil however, 2,4-D may be the most readily accepted. 2,4-D herbicides are among the most widely used in the world. They are labeled for use on 65 agricultural crops (Burnside 1996) and have been registered by the EPA since the 1950’s. 2,4-D herbicides are arguably the most widely studied products on the market.

Liquid formulations of aquatic herbicides tend to drift in aquatic environments, making them most suitable for treatments of contained bays or entire lakes. The potential for drift makes many liquid formulations, particularly those requiring long contact times, such as fluridone, ill-suited for treatment in flowing water and treatment of small beds (Netherland et al. 1993). Conversely, granular products, such as Navigate®, are designed to release the herbicide in the target area and usually provide much more localized control. This feature allows granular 2,4-D BEE to be used on pioneering Eurasian watermilfoil infestations comprised of only a few plants as well as much large areas.

Several of the herbicides commonly used in Eurasian watermilfoil management are considered non-selective, meaning that they will affect numerous plant species. Selectivity to Eurasian watermilfoil may be dependant on treatment timing (endothall), or on applications at very low rates (fluridone). 2,4-D is considered a selective herbicide and typically only affects dicotyledonous plants. Monocotyledons, such as the pondweed (*Potamogeton* L.) species, are unaffected. The Navigate® label lists watermilfoils, water stargrass (*Heteranthera dubia* (Jacq.) MacMill.), bladderwort (*Utricularia* spp.L.), 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 – 224 kg/ha (100-200 lbs/acre). Coontail, watershield and water lilies are slightly to moderately resistant to 2,4-D and may be controlled at higher rates of 168 – 224 kg/ha (150-200 lbs/acre). However, many lake managers have observed a much higher degree of selectivity to Eurasian watermilfoil than the label indicates.

Despite many years of operational field use, there is 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, 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 weeks after application. However most other references on species selectivity in the literature pertain to mesocosm studies or are anecdotal in nature. This study therefore assesses the species selectivity of granular 2,4-D BEE when applied 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 post-treatment aquatic plant survey data sets collected from nine Wisconsin Lakes between 2000 and 2005 (**Table 1**). Data from these nine lakes were analyzed because large-scale Eurasian watermilfoil treatments were conducted on each lake, and the management strategies were similar. The objective of the management plans 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. These nine Wisconsin lakes (Data Set 1) ranged in size from 12 to 122 hectares and pre-treatment Eurasian watermilfoil distributions ranged from 3.2 to 44.8 hectares (**Table 2**). Each lake also contained a diverse native plant community.

The second data group (Data Set 2) included pre-and post-treatment aquatic plant survey data collected between 2006 through 2007 from eight interconnected lakes located on the Menominee Indian Reservation (**Table 1**). During 2006, four of the eight lakes were treated with granular 2,4-D BEE at a rate of 112 kg/ha. The remaining four untreated lakes served as control lakes. During 2007 the same four lakes plus two additional lakes were treated with granular 2,4-D BEE at a rate of 168 kg/ha. The remaining untreated lakes (3) were once again surveyed to serve as controls. Management strategies for both years for each of these lakes were similarly directed toward providing long-term control of Eurasian watermilfoil by treating all known Eurasian watermilfoil in the lakes.

The Reservation lakes ranged in size from 7.1 to 114.6 ha and their pre-treatment Eurasian watermilfoil distributions ranged from 2.8 to 72.1 ha (**Table 2**).

Data Collection

Each survey conducted in each of the 18 lakes utilized standardized aquatic plant survey protocols developed by the Wisconsin Department of Natural Resources, however this survey protocol changed markedly in 2005. Prior to 2005, plant communities of the study lakes were surveyed using line-transect methods. Beginning in 2005, 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 non-vegetated central basin. This littoral zone typically extends to a depth of four to seven meters. The line-transect methods used in these surveys involved plotting a series of evenly spaced transects around the lakeshore typically at 100 meter intervals. These transects extend from the shoreline usually out to the maximum extent of rooted vegetation. Four sampling plots were typically established along each transect by estimating a 10-foot diameter circle around the anchored boat. The circular plot was then divided into four quarters, with each quarter representing a quadrant. Each species in the quadrant would be 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 and hauling it into the boat. 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 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 Wisconsin Department of Natural Resources provided individual maps and downloadable GPS coordinates for individual sample points. The method used in the study lakes involved plotting a series of grid points typically at 60 meter 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 meters to collect plants. 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.

Statistical analysis was performed between the pre- and post-treatment 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 and provided for 95% confidence intervals. Because several of the closely related *Potamogeton* species can be difficult to distinguish, and because 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 – 2007 however, is presented as recorded. Sapokesick Lake, which was an untreated control during 2006, had a partial Eurasian watermilfoil treatment during 2007. Since this differed from the other treated lakes where all know Eurasian watermilfoil infestations were treated, data from the 2007 survey of Sapokesick Lake was eliminated from analyses.

Results and Discussion

The pre- and post treatment percent frequencies of aquatic plants, along with the results of statistical analyses, from nine surveys conducted on seven lakes from 2000 to 2005 is presented in **Table 3**. These surveys reflect the changes after treatment with granular 2,4-D BEE applied at 112 kg/ha. Eurasian watermilfoil showed declines in eight of the nine post-treatment surveys in the treated lakes. Among the other potentially susceptible species, northern watermilfoil (*Myriophyllum sibiricum* Kom.), coontail and water stargrass were the only species to exhibit any significant declines. However each of these species only declined in one of the nine surveys while northern watermilfoil and coontail increased in one of the nine surveys. White watercrowfoot (*Ranunculus longirostris* Godr.) and common bladderwort (*Utricularia vulgaris* L.) also increased. *Chara* spp.(L.) and *Potamogeton* spp. were the only other plants to have any declines, with each declining in one of nine surveys.

The survey results of the 168 kg/ha treatments consisted of five surveys conducted on three lakes (**Table 4**). *M. spicatum* declined in all five surveys and *M. sibiricum* declined in one of five surveys. *U. vulgaris* increased in one of five surveys and most other plants tended to show no change or increase. *Chara* spp. and *Najas flexilis* (Willd.) were the only native plant species to have any declines with each species declining in one of the five surveys.

Table 5 shows the average change in percent frequency of aquatic plants from all of the pre- and post treatment data sets collected from 2000 to 2005. At 112 kg/ha, Eurasian watermilfoil declined by an average of 68% and water stargrass declined by 29%. All other plants, including native milfoils, responded positively to these treatments. When rates were increased to 168 kg/ha, Eurasian watermilfoil reduction improved to 91%, however northern watermilfoil and watershield were reduced by 87% and 14% respectively. All other plants responded positively. Clearly, the species having the greatest negative response to treatment with granular 2,4-D BEE 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. At this rate, the only potentially susceptible species was water stargrass which declined in only one of the 14 surveys. Similarly, northern watermilfoil was the only species, other than Eurasian watermilfoil, that showed a decline in two of the 14 surveys at the application rate of 168 kg/ha. There was no decline recorded in any survey for watershield.

Undoubtedly, survey timing and natural seasonal variations in plant abundance are responsible for some of the significant changes in frequency of occurence found among theses surveys. However, field observations clearly suggested that non-target native plants were actively recolonizing areas where Eurasian watermilfoil *had* been controlled. Thus the many significant increases recorded for non-target plants are likely to be positive responses to the treatments.

Aquatic Plant Survey Results 2006 - 2007

112 kg/ha Treatments

Table 6 shows the decline in Eurasian watermilfoil frequency found in the four Reservation lakes treated at 112 kg/ha during 2006. Main Channel and Big Blacksmith Lakes had declines of 79% and 78% reduction in frequency, respectively. Little Blacksmith had a 42% reduction, while Peshtigo Lake experienced only a 35% reduction. The overall average decline among the four treated lakes was 59%.

Untreated Lakes Wahtosah, Skice, Spring and Sapokesick experienced no significant changes in Eurasian watermilfoil frequency, but Pywaosit Lake experienced a 409% increase in Eurasian watermilfoil percent frequency (**Table 7**). Therefore it is concluded that the declines in Eurasian watermilfoil in the treated lakes were in fact due to the application of 2,4-D BEE.

The only other potentially susceptible species that showed any significant decline was northern watermilfoil which declined in two treated lakes but had no change in frequency in the other two treated lakes. However, northern watermilfoil declined in two of the untreated lakes so the decline in this plant may be due to natural seasonal variation. Among the species considered tolerant to 2,4-D, a total of eight significant declines in frequency were found among the four treated lakes. In the four untreated lakes, a total of 12 significant declines were found. Again, these changes are likely due to natural seasonal variation. In all, the 112 kg/ha treatments appeared to have little, if any, negative impacts to native plants.

168 kg/ha Treatments

The results of the 168 kg/ha treatments on the Reservation lakes is presented in **Table 8**. Eurasian watermilfoil was once again the only plant that consistently showed decreased frequency in all four treated lakes. Northern watermilfoil showed declines in two of the four surveys, coontail in one of the four surveys and small bladderwort (*Utricularia minor* L.) declined in two of the four surveys. Among tolerant species, white-stem pondweed

(*Potamogeton gramineus* L.) and small pondweed (*Potamogeton pusillus* L.) both increased and decreased, respectively. The frequency of occurrence of all other susceptible and tolerant species did not change as a result of the herbicide treatments.

Similar patterns were observed for northern watermilfoil, white-stem pondweed and small pondweed among the untreated control lakes (**Table 9**), *which* suggests that natural seasonal variation may account for some of the changes observed in the treated lakes.

Eurasian watermilfoil declined by an average of 58% at 112 kg/ha and 76% at 168 kg/ha (**Table 10**), while northern watermilfoil declined by 61% and 71%, respectively. The only other potentially susceptible plant to decline after treatment at 112 kg/ha was white water lily which decreased by 24%. However, this same plant increased by 21% after treatment at 168 kg/ha. The only plant to decrease in treated lakes but not in untreated lakes other than northern watermilfoil, was spatterdock*,* which declined by 13%. There were no patterns of decline among the tolerant plants that would suggest a response to herbicide treatments.

Among the 29 pre- and post-treatment data sets analyzed in this study, the only species having consistent decline following treatment with granular 2,4-D BEE at all rates among all of the surveys was Eurasian watermilfoil with an average 65% decline at 112 kg/ha, and an 84% decline at 168 kg/ha. Northern watermilfoil, a closely related native species, was the only other species to commonly exhibit significant declines in frequency; averaging an 8.8% increase at 112 kg/ha, and an 82.6% decrease at 168 kg/ha. While these declines were not consistent, and some declines occurred in control lakes as well, the percentage of decline was greater in the treated lakes and the decline in northern watermilfoil frequency increased 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 species may be negatively affected by 2,4-D BEE treatment in certain situations, but the data suggest that they will not be affected more often than they will exhibit declines. There were no patterns in the data to suggest that any of the 36 other aquatic plant species studied were negatively affected by2,4-D BEE treatments. The high degree of species selectivity documented for 2,4-D BEE makes this herbicide an extremely valuable tool for combating Eurasian watermilfoil while protecting native plants. Species selectivity, along with the other favorable attributes of 2,4-D BEE, makes it a tool for true restoration of aquatic habitats that have been degraded by Eurasian watermilfoil.

Acknowledgements

I am indebted to the numerous lake organizations that supported this study, to Robyn McAllister for assistance with literature review and data entry, and to Nichole Weir and Brad Roost for their assistance with fieldwork, data analysis, and review of this paper.

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Footnotes

1. Wisconsin Lake & Pond Resource, LLC PO Box 109 Berlin, WI 54923, chad@wisconsinlpr.com, Received for publication ____ and in revised form ____

Tables

Table 1. Timeframe of Eurasian watermilfoil management activities on selected Wisconsin lakes treated between 2000 and 2007.

	Pre-Treatment Survey	First Treatment	Application Rate (kg/ha)	Post-Treatment Survey	Second Treatment	Application Rate (kg.ha)	Post-Treatment Survey	Post-Treatment Survey
Data Set 1								
Bughs Lake	6/01	5/02	112	7/02	--	--	--	--
Gilbert	9/00	5/01	168	9/01	--	--	7/02	6/03
Hancock	9/03	5/04	168	7/04	--	--	--	--
Kettle Moraine Lake	5/03	5/03	112	8/03	5/04	112	7/04	--
Loon Lake	6/02	6/03	112	9/03	9/03	168	7/04	--
Twin Lake	7/03	7/03	112	--	6/04	112	6/04	--
Washington Lake	5/04	5/04	112	8/04	--	--	--	--
Wilson	5/00	6/01	112	9/01	--	--	6/02	--
Wolf	5/02	6/02	112	--	5/03	112	5/04	--
Data Set 2								
Big Blacksmith	5/06	5/06	112	8/06	5/07	168	8/07	--
Little Blacksmith	5/06	5/06	112	8/06	5/07	168	8/07	--
Main Channel	5/06	5/06	112	8/06	5/07	168	8/07	--
Peshtigo	5/06	5/06	112	8/06	5/07	168	8/07	--
Pywaosit	5/06	--		8/06	--	--	8/07	--
Sapokesick	5/06	--		8/06	5/07	168	8/07	--
Spring	5/06	--		8/06	5/07	168	8/07	--
Wahtohsah/Skice	5/06	--		8/06	--	--	8/07	--

Table 2. The physical characteristics of selected Wisconsin lakes and their preliminary Eurasian watermilfoil distribution.

Lake Name	*Surface Area (ha)*	*Maximum Depth (m)*	*Average Depth (m)*	*EWM Area (ha)*
Data Set 1				
Bughs	12	5.5	2.8	7.2
Gilbert	56	20	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

Table 3. Changes in frequency of aquatic plants in Wisconsin lakes following treatment with 2,4-D BEE at 112 kg/ha based on students paired t-tests and 95% C.I.

	Bughs Lake			Kettle Moraine					Loon Lake		
Susceptible and Slightly to Moderately Resistant Species	PRE	POST	CHANGE	PRE	POST 12 WAT1	CHANGE	POST 36 WAT2	CHANGE	PRE	POST	CHANGE
Myriophyllum spicatum	29	6	D	63	24	D	20	D	64	28	D
Brasenia schreberi				6	12	I	18	I	7	5	N
Ceratophyllum demersum	8	9	N	45	60	I	76	N	22	27	N
Heteranthera dubia	2	0	N	1	0	N	1	N	3	2	N
Megalodonta beckii									2	0	N
Myriophyllum sibiricum	0	1	N	1	1	N	3	N	0	1	N
Myriophyllum tenellum									5	9	N
Nuphar variegata				3	1	N	7	N	8	6	N
Nymphaea odorata				5	19	I	27	I	5	6	N
Ranunculus longirostris				1	0	N	0	N	0	5	I
Utricularia vulgaris				0	1	N	2	N	9	20	I
Insusceptible Species											
Chara spp.	31	24	D	32	31	N	27	N	16	17	N
Elodea canadensis				26	42	I	70	I	15	22	N
Najas flexilis	13	16	I	20	27	N	34	N	9	22	I
Potamogeton spp.	12	6	D	5	19	I	7	N	6	8	I
Vallisneria americana				0	6	I	5	N	12	35	I

Table 3 (cont.). Changes in frequency of aquatic plants in Wisconsin lakes following treatment with 2,4-D BEE at 112 kg/ha based on students paired t-tests and 95% C.I.

Susceptible and Slightly to Moderately Resistant Species	Twin Lake			Washington Lake			Wilson Lake					Wolf Lake		
	PRE	POST	CHANGE	PRE	POST	CHANGE	PRE	POST 12 WAT	CHANGE	POST 52 WAT	CHANGE	PRE	POST	CHANGE
Myriophyllum spicatum	23	9	D	35	40	N	67	4	D	10	D	62	0	D
Brasenia schreberi				12	16	N	4	1	N	4	N	1	0	N
Ceratophyllum demersum	55	40	D	39	44	N	7	11	N	7	N	9	14	N
Heteranthera dubia	1	0	N	3	0	D	3	2	N	2	N	3	7	N
Megalodonta beckii														
Myriophyllum sibiricum	2	20	I	0	1	N	6	2	N	0	D	16	8	N
Myriophyllum tenellum														
Nuphar variegata	2	0	N	4	11	I	1	1	N	1	N			
Nymphaea odorata				14	25	I	5	7	N	7	N	3	7	N
Ranunculus longirostris	1	1	N											
Utricularia vulgaris				7	18	I	1	2	N	1	N			
Insusceptible Species														
Chara spp.	7	6	N	8	15	I	39	46	I	48	I	34	47	N
Elodea canadensis	0	3	N	3	7	N	11	10	N	20	I	19	59	I
Najas flexilis	1	0	N	4	15	I	40	59	I	59	I	3	10	N
Potamogeton spp.	25	21	N	4	4	N	4	7	I	7	I	1	9	I
Vallisneria americana				0	23	I								

N = no change
D = decrease in frequency
I = increase in frequency

Table 4. Changes in frequency of aquatic plants in Wisconsin lakes following treatment with 2,4-D BEE at 168 kg/ha based on students paired t-tests and 95% C.I.

	Gilbert Lake							Hancock Lake			Loon Lake		
Susceptible Species	PRE	POST 14 WAT	CHANGE	POST 58 WAT	CHANGE	POST 102 WAT	CHANGE	PRE	POST	CHANGE	PRE	POST	CHANGE
Myriophyllum spicatum	21	0	D	0	D	1	D	84	0	D	64	15	D
Brasenia schreberi											7	6	N
Ceratophyllum demersum	0	1	N	0	N	0	N	0	3	N	22	18	N
Heteranthera dubia	1	0	N	1	N	0	N				3	4	N
Megalodonta beckii											2	5	N
Myriophyllum sibiricum	1	1	N	0	N	0	N	16	0	D	0	1	N
Myriophyllum tenellum											5	6	N
Nuphar variegata	1	1	N	0	N	1	N	0	2	N	8	10	N
Nymphaea odorata	1	1	N	2	N	2	N	2	7	N	5	10	N
Ranunculus longirostris											0	0	N
Utricularia vulgaris											9	24	I
Insusceptible Species													
Chara spp.	81	91	I	88	N	84	N	22	75	I	16	27	N
Elodea canadensis	0	1	N	1	N	2	N	0	1	N	15	27	I
Najas flexilis	32	36	N	19	D	21	N	58	47	N	9	14	N
Potamogeton spp.	10	7	N	11	N	7	D	13	12	N	6	6	N
Vallisneria americana	0	0	N	0	N	3	I				12	33	I

N = no change
D = decrease in frequency
I = increase in frequency

Table 5. Average percent change in percent frequency of aquatic plants in selected Wisconsin Lakes after treatments with 2,4-D BEE from 200-2005 (Data Set 1).

	Average % change in frequency after:			
Species	112 kg/ha treatment	No. of lakes	168 kg/ha treatment	No. of lakes
Myriophyllum spicatum	-68	7	-91	3
Utricularia vulgaris	153	3	167	2
Ceratophyllum demersum	22	7	0	3
Myriophyllum tenellum	80	1	20	1
Myriophyllum sibiricum	40	7	-87	3
Nuphar variegata	23	6	43	2
Megalodonta beckii	--	--	150	1
Ranunculus longirostris	100	2	--	--
Heteranthera dubia	-29	6	0	3
Brasenia schreberi	44	4	-14	2
Nymphaea odorata	145	6	122	2
Chara spp.	12	7	62	3
Potamogeton spp.	30	7	-14	3
Elodea canadensis	113	7	93	2
Vallisneria americana	475	4	193	2
Najas flexilis	102	7	-2	3

Table 6. Changes in percent frequency of aquatic plants in the Reservation lakes of Menominee County Wisconsin, following treatment with 2,4-D BEE at 112 kg/ha in 2006 (based on students paired t-tests and 95% C.I).

	Big Blacksmith			Little Blacksmith			Main Channel			Peshtigo Lake		
Susceptible Species	PRE	POST	CHANGE	PRE	POST	CHANGE	PRE	POST	CHANGE	PRE	POST	CHANGE
Myriophyllum spicatum	50	11	D	80	47	D	79	17	D	54	36	D
Ceratophyllum demersum	15	22	I	19	25	N	2	36	I	15	33	I
Myriophyllum sibiricum	34	3	D	14	0	D	12	2	N	27	34	N
Nuphar variegata				2	6	N				7	11	I
Nymphaea odorata	6	4	N				7	7	N	4	5	N
Utricularia vulgaris	1	10	I	0	7	I	0	5	I	0	40	I
Insusceptible Species												
Chara sp.	51	39	D	19	19	N	50	21	D	26	20	N
Eleocharis acicularis							12	12	N			
Elodea canadensis	19	28	I	16	26	I					49	N
Najas flexilis	34	57	I	26	59	I	14	31	I	28	46	I
Nitella sp.	15	7	D				5	2	N	6	0	D
Potamogeton gramineus	15	1	D				7	2	N			
Potamogeton illinoensis	3	36	I	15	37	I	17	26	N	1	24	I
Potamogeton praelongus	9	24	I	4	27	I	0	10	I	5	17	I
Potamogeton robbinsii	25	11	D	25	25	N	29	14	N	0	24	I
Potamogeton zosteriformis	13	17	N	10	10	N	12	0	D	16	13	N
Stuckenia pectinata	0	6	I	1	6	I				3	8	N
Vallisneria americana	0	33	I	0	37	I	2	12	I	1	24	I

N = no statistically significant change
D = statistically significant decrease in frequency
I = statistically significant increase in frequency

Table 7. Changes in percent frequency of aquatic plants in the Reservation lakes of Menominee County Wisconsin, that served as untreated controls in 2006 (based on students paired t-tests and 95% C.I).

	Pywaosit Lake			Sapokesick Lake			Spring Lake			Wahtosah/Skice Lakes		
Susceptible Species	PRE	POST	CHANGE	PRE	POST	CHANGE	PRE	POST	CHANGE	PRE	POST	CHANGE
Myriophyllum spicatum	17	69	I	58	58	N	61	60	N	35	30	N
Ceratophyllum demersum	28	23	N	32	33	N	10	21	I	1	5	I
Heteranthera dubia	25	4	D	7	2	D						
Myriophyllum sibiricum	20	44	I	19	11	D	32	18	D	21	24	N
Nuphar variegata							14	8	D			
Nymphaea odorata				5	5	N	14	5	D			
Utricularia vulgaris							1	23	I			
Chara spp.	31	17	D	15	4	D	44	32	D	52	45	D
Elodea canadensis	43	33	D	38	29	D	39	37	N			
Najas flexilis	60	58	D	38	41	N	24	44	I	12	34	I
Nitella spp.	0	6	I									
Potamogeton gramineus							9	11	N			
Potamogeton illinoensis	25	46	I	10	27	I	10	18	I	24	38	I
Potamogeton praelongus	8	39	I	9	28	I	22	38	I	17	15	D
Potamogeton pusillus	0	27	I	1	9	I				0	10	I
Potamogeton richardsonii										0	15	I
Potamogeton robbinsii	26	17	D	29	13	D	8	16	N	9	13	I
Potamogeton zosteriformis	12	15	N	12	20	I	16	21	N	5	11	I
Stuckenia pectinata							0	8	I	0	15	I
Vallisneria americana	5	46	I	0	22	I	1	21	I	2	9	I

N = no statistically significant change
D = statistically significant decrease in frequency
I = statistically significant increase in frequency

Table 8. Changes in percent frequency of aquatic plants in the Reservation lakes of Menominee County Wisconsin, following treatment with 2,4-D BEE at 168 kg/ha in 2007 (based on students paired t-tests and 95% C.I).

	Big Blacksmith			Little Blacksmith			Peshtigo			Spring		
Susceptible Species	PRE	POST	CHANGE	PRE	POST	CHANGE	PRE	POST	CHANGE	PRE	POST	CHANGE
Myriophyllum spicatum	9	3	D	39	5	D	49	18	D	55	6	D
Ceratophyllum demersum	26	16	D	19	14	N	33	26	N	16	12	N
Heteranthera dubia	35	36	N									
Myriophyllum sibiricum							13	12	N	22	0	D
Nuphar variegata							11	5	N			
Utricularia gibba	13	15	N	14	15	N	35	28	N	41	41	N
Utricularia minor							39	4	D	12	0	D
Utricularia vulgaris							47	31	N	32	41	N
Chara sp.	56	62	N	26	44	N	25	30	N	45	46	N
Elodea canadensis	35	36	N	38	53	N	58	65	N	36	49	N
Najas flexilis	50	49	N	39	41	N	56	53	N	47	33	N
Nitella sp.	20	20	N				21	17	N	3	15	N
Potamogeton gramineus				11	3	N						
Potamogeton illinoensis	33	26	N	20	35	N	15	19	N	36	35	N
Potamogeton praelongus							11	0	N			
Potamogeton pusillus	16	5	D				6	12	I			
Potamogeton richardsonii	27	27	N	16	21	N	13	30	N	18	11	N
Potamogeton robbinsii	17	23	N	40	34	N	29	24	N	29	36	N
Potamogeton zosteriformis	13	15	N	10	15	N	17	21	N	17	12	N
Stuckenia pectinata							3	23	N	10	5	N
Vallisneria americana	49	45	N	33	42	N	24	35	N	36	36	N
Species Richness	29	27		26	26		31	32		26	26	

Table 9. Changes in percent frequency of aquatic plants in the Reservation lakes of Menominee County Wisconsin, that served as untreated controls in 2007 (based on students paired t-tests and 95% C.I).

	Wahtohsah/Skice			Pywaosit		
Susceptible Species	PRE	POST	CHANGE	PRE	POST	CHANGE
Myriophyllum spicatum	29	43	I	28	30	N
Ceratophyllum demersum				43	33	N
Myriophyllum sibiricum	24	17	D	22	18	N
Chara sp.	59	52	N	17	30	N
Elodea canadensis				45	0	N
Najas flexilis	48	48	N	54	50	N
Potamogeton illinoensis	37	45	N	17	35	N
Potamogeton pusillus				5	12	I
Potamogeton richardsonii	31	28	N	22	32	N
Potamogeton robbinsii	19	19	N	23	29	N
Potamogeton strictifolius	14	0	N	12	0	N
Potamogeton zosteriformis	11	14	N	17	20	N
Stuckenia pectinata	18	16	N			
Vallisneria americana	14	19	N	48	44	N

N = no change
D = significant decrease in frequency
I = significant increase in frequency

Table 10. Average changes in percent frequency of aquatic plants in the Reservation lakes of Menominee County Wisconsin in 2007 (based on students paired t-tests and 95% C.I).

Susceptible Species	2006 Treated Lakes Average Change	2006 Control Lakes Average Change	2007 Treated Lakes Average Change	2007 Control Lakes Average Change
Myriophyllum spicatum	-58	73	-76	28
Ceratophyllum demersum	397	191	-29	-15
Heteranthera dubia	52		6	-50
Myriophyllum sibiricum	-61	13	-77	-22
Nuphar variegata	159	10	-13	71
Utricularia gibba			-2	
Utricularia minor			-64	-100
Utricularia vulgaris	404	591	11	-100
Chara sp.	-27	-40	26	33
Elodea canadensis	10		22	-45
Najas flexilis	94	70	-8	-4
Nitella sp.	-26		111	-84
Potamogeton gramineus	-33	39	37	103
Potamogeton illinoensis	839	101	18	64
Potamogeton praelongus	264	164	-28	188
Potamogeton pusillus	100	344	-25	32
Potamogeton richardsonii		33	32	18
Potamogeton robbinsii	-1	18	7	12
Potamogeton strictifolius			-23	-100
Potamogeton zosteriformis	-22	62	13	20
Stuckenia pectinata	257	100	283	68
Vallisneria americana	618	856	16	13