ERDC/TN APCRP-CC-14
April 2010

Combinations of Endothall with 2,4-D and Triclopyr for Eurasian Watermilfoil Control

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PURPOSE: This study evaluates combinations of a contact herbicide (endothall) and two systemic auxin-type herbicides (2,4-D and triclopyr) to exploit the strengths of each herbicide class, with an objective of providing both rapid and complete control of the invasive, submersed plant, Eurasian watermilfoil.

BACKGROUND: Eurasian watermilfoil (*Myriophyllum spicatum* L.) is a widespread submersed aquatic plant that causes nuisance problems across the continental United States. Eurasian watermilfoil is an herbaceous perennial submersed aquatic plant that typically grows in water depths of 1 to 3 m (Aiken et al. 1979). Vegetative propagation is either by direct stem fragments or by autofragmentation through the development of an abscission layer in stem segments (Madsen et al. 1988). The production of these stem fragments, either by external forces or by autofragmentation, allows for widespread plant dispersal. Eurasian watermilfoil can form a dense surface canopy, which is the cause of both ecological harm and nuisance impacts. Eurasian watermilfoil is often responsible for reductions in oxygen exchange, depletions in dissolved oxygen, increases in water temperatures, and internal nutrient loading (Madsen 1998). Eurasian watermilfoil has been directly associated with declines in native plant species richness and diversity (Madsen et al. 1991a, 1991b); and indirectly with reductions in habitat complexity resulting in declines in macroinvertebrate abundance (Krull 1970, Keast 1984), reductions in fish growth (Lillie and Budd 1992), and an overall reduction in habitat value for invertebrates and fish (Dibble and Harrel 1997). Eurasian watermilfoil also poses nuisance problems to humans in the form of increasing flood frequency and intensity, restricting water intakes, impeding navigation, and limiting recreation opportunities (Madsen et al. 1991a, 1991b). Once Eurasian watermilfoil has become established in a waterbody, it is generally persistent and difficult to control. Management techniques such as biological, mechanical, and physical methods are available and have been instituted alone, or in integrated management regimes; but adequate control with these techniques has been highly variable. To date, chemical techniques have offered the most consistent and effective control of Eurasian watermilfoil at both small and large spatial scales.

Aquatic herbicides available for control of Eurasian watermilfoil include both contact and systemic chemistries; however, each class of herbicide has its strengths and drawbacks. Contact herbicides are fast acting, typically causing extensive cellular damage at the point of uptake but not affecting areas untouched by the herbicide, resulting in maximum plant damage by one week after treatment

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Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE **APR 2010**	2. REPORT TYPE	3. DATES COVERED **00-00-2010 to 00-00-2010**

4. TITLE AND SUBTITLE **Combinations of Endothall With 2,4-D and Triclopyr for Eurasian Watermilfoil Control**	5a. CONTRACT NUMBER 5b. GRANT NUMBER 5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER 5e. TASK NUMBER 5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **U.S. Army Engineer Research and Development Center,Vicksburg,MS,39180**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S) 11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**10**	

(Madsen 2000). Contact herbicides generally relieve nuisance problems quickly, but may allow regrowth of nuisance plants from root crowns and/or shoot tissue that survived treatments. Alternatively, systemic herbicides often will kill the entire plant through translocation of the active ingredient to plant tissues including shoots, root crowns, rhizomes, and roots. However, systemic herbicides are generally slower acting and limited by situations in which water exchange processes reduce chemical exposure. Endothall, 2,4-D, and triclopyr are three herbicides that are frequently used to control Eurasian watermilfoil infestations. Endothall is considered a broad-spectrum, membrane active, contact herbicide that is effective on a wide range of aquatic plants including both monocotyledons and dicotolyledons (Madsen 1997a, Senseman 2007). Endothall rapidly penetrates the leaf cuticle of Eurasian watermilfoil and causes cellular breakdown within two to five days (MacDonald et al. 1993). Symptoms associated with endothall exposure include plant defoliation and/or brown desiccated tissue (Senseman 2007). The mechanism of action is not well understood, but endothall appears to inhibit lipid and protein synthesis in some plants, it has an inhibitory effect on mRNA synthesis, and in some plants endothall has caused ion leakage and increased levels of polyphenols (Senseman 2007). MacDonald et al. (1993) suggested that endothall acts as a respiratory inhibitor in treated plants, but due to the rapid loss of cellular activity, endothall is not phloem mobile and does not translocate through plant tissue.

Herbicides such as 2,4-D and triclopyr translocate throughout the plant and typically require two to four weeks for plant mortality to occur. The chemical 2,4-D is a systemic, auxin-type herbicide that is transported through plants via the symplastic pathway and accumulates in the growing points of shoots and roots (Senseman 2007). The herbicide mimics the auxin hormone in plants; which in Eurasian watermilfoil affects respiration and food reserves, and causes excessive growth, cell division, and mortality of affected plants (Christopher and Bird 1992). Symptomology includes epinastic bending and twisting, stem swelling and elongation, and leaf curling, followed by chlorosis, growth inhibition, wilting, and plant mortality over a 7- to 14-day period. (Senseman 2007). Similar to 2,4-D, plants exposed to triclopyr exhibit the auxin-type herbicide symptomology with a similar mechanism of action. Triclopyr is absorbed through roots, shoots, and leaf tissues. It is translocated through treated plants via the symplastic pathway and accumulates in the meristematic regions of the plant (Getsinger et al. 2000). Plant mortality usually occurs over a 7- to 14-day period.

The effects of endothall, 2,4-D, and triclopyr on Eurasian watermilfoil have been well documented in microcosm- to operational-scale evaluations. Studies conducted in microcosms have determined concentration and exposure times for endothall, 2,4-D, and triclopyr (Westerdahl and Hall 1983; Green and Westerdahl 1990; Netherland et al. 1991; Netherland and Getsinger 1992). Studies conducted in small plots and whole lake scenarios have documented the efficacy range for 2,4-D and triclopyr rates, as well as selectivity removing Eurasian watermilfoil populations and leaving native plant communities (Getsinger et al. 1982; Sprecher and Stewart 1995; Getsinger et al. 1997, 2000; Parsons et al. 2001; Poovey et al. 2004). Similarly, empirical evidence suggests that some species selectivity may be achieved when applying endothall (Skogerboe and Getsinger 2001, 2002; Parsons et al. 2004). More importantly, the addition of endothall may also allow for the control of curlyleaf pondweed (*Potamogeton crispus* L.), a species that frequently co-exists with populations of Eurasian watermilfoil and is typically not affected by either 2,4-D or triclopyr (Netherland et al. 2000; Poovey et al. 2002). Hence, this combination may offer control of two invasive species in the same water body using reduced herbicide concentrations and/or exposure times.

MATERIALS AND METHODS: The study was conducted in an outdoor mesocosm tank system at the R. R. Foil Plant Science Research Center, Mississippi State University, for 8 weeks beginning in April 2006 and ending June 2006. The experiment consisted of a randomized complete block design with two aqueous concentrations of endothall as Aquathol® K[1], one concentration of 2,4-D as DMA 4 IVM®[2], one concentration of triclopyr as Renovate®3[3], two exposure times, and an untreated reference. Each treatment was replicated four times in 378-L, above-ground tanks. A shade cloth (30 percent light reduction) was used to cover the tanks to keep water temperatures < 30 °C to mitigate heat effects on the plants. Water was pumped into each tank from an adjacent irrigation reservoir, and filtered via a basket strainer and sand filter prior to filling the tanks. Temperature loggers were deployed in each block of tanks, for a total of four loggers, to record water temperature in 1-hr intervals throughout the study. Each tank was aerated to simulate water circulation and mixing. Eurasian watermilfoil used in this study was cultured from greenhouse stock. Two shoots of Eurasian watermilfoil, each approximately 20 cm in length, were planted into 3.78-L pots. Pots were filled with a soil medium (top soil, loam, and masonry sand mixture) and amended with 2 g L^{-1} of 19–6–12 Osmocote®[4] fertilizer and placed into the tanks. Seven pots were placed into each of 36 tanks for a total of 252 pots. Eurasian watermilfoil was allowed to grow in the tanks for approximately 4 weeks until plants reached the water surface.

After 4 weeks and just prior to herbicide treatment, one pot of Eurasian watermilfoil was harvested from each tank by cutting shoot mass at the sediment surface. Plants were dried to a constant weight to assess pre-treatment biomass. The liquid formulation of endothall was applied at 1.0 mg ae L^{-1} and 1.5 mg ae L^{-1} alone or in combination with either 2,4-D (0.5 mg ae L^{-1}) or triclopyr (0.5 mg ae L^{-1}) with a 12- or 24-hr exposure time (Table 1). Herbicides were combined using only the 1.0-mg ae L^{-1} concentration of endothall. All herbicides were applied to the water column. Following the exposure times, corresponding tanks were drained and refilled with water three times to ensure aqueous herbicide residues had been removed from the tanks. Visual ratings of percent control were recorded weekly after treatment (WAT) for 4 weeks. Eurasian watermilfoil control was assessed on a scale of 0 to 100 percent, where 0 = no control and 100 = complete plant mortality. At 4 WAT, viable Eurasian watermilfoil was harvested by cutting shoots at the sediment surface, drying at 70 °C for 72 hr, weighing, and comparing to untreated reference plants to assess herbicide efficacy. The 4-week post-treatment period has been noted to be sufficient time for herbicide injury of plant mass and for plants to recover from the initial herbicide injury in small-scale studies (Green and Westerdahl 1990). A one-way Analysis of Variance (ANOVA) with a Fisher's Protected LSD analysis was used to assess differences in biomass between herbicide treatments and within 1 week for the visual ratings. All analyses were conducted at $p = 0.05$ using Statistix 8.0 (Analytical Software 2003). Water temperature data were averaged and the mean ± 1 standard error (SE) values are reported.

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Table 1. Herbicides, treatments, rates, and exposure times for the current study. All combinations of endothall and 2,4-D or triclopyr used the 1.0-mg ae L^{-1} rate of endothall.

Treatment	Exposure Time (hr)	Endothall mg ae L^{-1}	2,4-D mg ae L^{-1}	Triclopyr mg ae L^{-1}
1	24	1.5	0	0
2	24	1	0	0
3	24	1	0.5	0
4	24	1	0	0.5
5	24	0	0.5	0
6	24	0	0	0.5
7	12	1	0.5	0
8	12	1	0	0.5
Untreated Reference	0	0	0	0

RESULTS AND DISCUSSION: At 1 WAT, all tanks treated with endothall alone at 1.0 and 1.5 mg ae L^{-1} exhibited greater than 60 percent control of Eurasian watermilfoil when exposed for 24 hr (Table 2). Eurasian watermilfoil exposed to endothall turned black and began to break apart at 1 WAT. There was some green tissue in all tanks treated with endothall 1 WAT; however, these observations were not consistent in all tanks and green tissue was confined to the very tips of Eurasian watermilfoil shoots, or a single shoot in one pot in a given tank. The presence of green tissue in these treatments prohibited a higher control rating. Control of plants treated at a 24-hr exposure time with 2,4-D or triclopyr was only 20 percent and 5 percent, respectively, at 1 WAT, which was less than the endothall treatments. Eurasian watermilfoil treated with 2,4-D or triclopyr exhibited some epinastic bending and twisting at 1 WAT, but plant tissue was still generally green. When endothall was combined with 2,4-D or triclopyr with a 24-hr exposure, control increased to 60 percent and 45 percent, respectively. Similarly, when the same combinations of herbicides were used with a 12-hr exposure, control increased to 50 percent for endothall + 2,4-D and 60 percent for endothall + triclopyr. Plants treated with the combinations of herbicides generally showed the rapid symptomology typical of endothall exposure; however, epinastic bending typical of auxin herbicides was observed in most tanks.

Eurasian watermilfoil control was 50 percent for both endothall rates at 2 WAT (Table 2) and regrowth was evident in all tanks as new shoots were emerging from old shoots and root crowns. Conversely, control in the 2,4-D tanks increased to 95 percent at 2 WAT, with plants necrotic and beginning to break down. At 2 WAT, Eurasian watermilfoil controlled was 60 percent when treated with triclopyr alone, with plants showing increased epinastic bending and chlorosis of the majority of tissue. Control for the combinations of endothall and 2,4-D or triclopyr increased to 100 percent for all combinations and exposure times with the exception of endothall + triclopyr at 24-hr exposure, although the 97 percent rating for this treatment was not significantly different from the others. Eurasian watermilfoil plants in these treatments were breaking apart with little shoot tissue intact.

Control for the endothall alone treatments remained at 50 percent at 4 WAT, and plant regrowth was evident in all tanks. Eurasian watermilfoil was regrowing from root crowns and shoots that were not killed by the endothall treatments, and by 4 WAT, some shoots had reached the water surface again. The concentration and exposure time for endothall in this study produced results that were less than those established in previous studies (Netherland et al. 1991). Under an endothall rate of 1.0 mg ae L^{-1} and an exposure time of 24 hr, Netherland et al. (1991) observed approximately 70 percent control of Eurasian watermilfoil.

Table 2. Percent control (± 1 SE) of Eurasian watermilfoil following herbicide treatment with endothall, 2,4-D, triclopyr, or combinations of endothall with 2,4-D or triclopyr.

Herbicide (mg ae L^{-1})		Exposure Time (h)	Weeks After Treatment[1]			
			1	**2**	**3**	**4**
Untreated Reference			0e	0c	0d	0c
Endothall	(1.0)	24	60ab	50b	50c	50b
	(1.5)	24	65a	50b	50c	50b
2,4-D (0.5)		24	20d	95a	100a	100a
Triclopyr (0.5)		24	5de	60b	90b	97a
Endothall (1.0) + 2,4-D (0.5)		24	60ab	100a	100a	100a
Endothall (1.0) + 2,4-D (0.5)		12	50bc	100a	100a	100a
Endothall (1.0) + Triclopyr (0.5)		24	45c	97a	100a	100a
Endothall (1.0) + Triclopyr (0.5)		12	60ab	100a	100a	100a
		LSD	13	14	9	11

[1] Means in a column followed by the same letter do not differ significantly at $p = 0.05$ according to Fisher's Protected LSD. Mean comparisons were conducted within weeks and not between weeks.

Conversely, control was greater for 2,4-D and triclopyr based on concentration and exposure than in previous laboratory studies (Green and Westerdahl 1990; Netherland and Getsinger 1992). Eurasian watermilfoil control was 100 percent at 3 and 4 WAT for the 2,4-D treatment, which coincide with control observed by Gray et al. (2007). Control was 97 percent at 4 WAT for the triclopyr treatment and took roughly a week longer to achieve a similar level of control compared to 2,4-D (Table 2). Control was 100 percent for all combination treatments regardless of exposure time from 3 WAT until the conclusion of the study. Eurasian watermilfoil control was similar after 4 weeks for 2,4-D, triclopyr, and combination treatments. Additionally, these same treatments were significantly greater than the endothall alone treatments; however, the endothall combination treatments achieved 100 percent control more rapidly than either 2,4-D or triclopyr alone.

More importantly, excellent control of Eurasian watermilfoil was achieved with a 12-hr exposure time when low concentrations of endothall, 2,4-D, or triclopyr were combined. Most native pondweeds are less sensitive to auxin herbicides than is Eurasian watermilfoil and, therefore, should survive concentrations of 0.5 mg L^{-1} of these compounds (Sprecher and Stewart 1995; Sprecher et al. 1998). Skogerboe and Getsinger (2002) reported minimal impact of endothall concentrations of 0.5 to 2.0 mg ae L^{-1} on some native plant species exposed for 24 hr, and this non-target plant injury should be further reduced when shorter exposure times occur, such as the12-hr period in the current study. In addition, this combination of herbicides may allow for applications to be made earlier in the growing season when water temperatures are cooler and some native plant species are not growing, resulting in further species selectivity. Early season, low-temperature trials have indicated that endothall is effective on curlyleaf pondweed below the label minimum temperature of 18 °C (Netherland et al. 2000; Poovey et al. 2002). The application of endothall in water temperatures ranging from 10 to 25 °C resulted in excellent biomass reduction of curlyleaf pondweed and inhibition of turion formation (Netherland et al. 2000). Similarly, 90 percent control of curlyleaf pondweed and suppression of turion formation were obtained with endothall when the water temperature was 16 °C (Poovey et al. 2002). Moreover, a combination of endothall plus an auxin product may offer the desired control of both Eurasian watermilfoil and curlyleaf pondweed, where they grow in mixed stands, with little impact to non-target plant species.

Eurasian watermilfoil plant mass harvested at the conclusion of 4 weeks corroborated results from control ratings (Figure 1). The untreated reference plants had significantly ($p < 0.01$) greater plant mass than plants harvested prior to treatment, indicating that the Eurasian watermilfoil was healthy and growing prior to herbicide applications. Plant mass of Eurasian watermilfoil was lower in tanks treated with endothall compared to the untreated reference after 4 weeks. Similarly, the combinations of endothall + 2,4-D or triclopyr, and 2,4-D or triclopyr alone, resulted in less plant mass than the untreated reference regardless of exposure time. There was also a difference in plant mass between endothall applied alone and the other herbicide treatments. The regrowth of Eurasian watermilfoil in the endothall treatments resulted in greater plant mass than other treatments; however, this mass was still less than that of pre-treatment plants and much less than the untreated reference plants at 4 WAT. In a mesocosm study, Skogerboe and Getsinger (2002) obtained a 99 percent reduction in Eurasian watermilfoil biomass using a rate of only 0.5 mg ae L^{-1} of endothall, and at 8 WAT there was no difference in biomass reduction between any of their endothall rates. Also, Parsons et al. (2004) achieved a 99 percent reduction in Eurasian watermilfoil biomass during the year of application in Kress Lake, WA, using a rate of 1.5 mg ae L^{-1}; and at 2 and 3 years post application, Eurasian watermill biomass was 97 percent less than pre-treatment levels.

Eurasian watermilfoil control at 0.5 mg L^{-1} requires a minimum exposure time of 72 and 48 hr for 2,4-D and triclopyr, respectively, to achieve the level of control found in this study, based on previous laboratory studies (Green and Westerdahl 1990, Netherland and Getsinger 1992). These previous studies were conducted over a 6-week period, whereas a 4-week time period was used in the current study; so the shorter time period likely contributed to the differences in control reported. Additionally, triclopyr efficacy was greater when applied in the field as opposed to laboratory conditions (Getsinger et al. 1997). This enhanced field efficacy has also been observed with other aquatic herbicides (Langeland 1993, Netherland et al. 1993) and is attributed to levels of post-treatment environmental stress that potentially slow plant recovery (Getsinger et al. 1997).

Mean (± 1 SE) daily water temperature for the current study is depicted in Figure 2. The shade cloth was effective in keeping water temperature below 30 °C; however, water temperature did rise above 25 °C several times during the study. In Texas, Eurasian watermilfoil showed significant leaf senescence during mid-summer, when water temperatures approached 35 °C (Madsen 1997b). Also, vegetative spreading of Eurasian watermilfoil was shown to decrease when water temperatures were above 25 °C (Madsen and Smith 1997). This study found no evidence of environmental stress (e.g. temperature stress) in any treatment or reference tank. In a study conducted with curly leaf pondweed, it was reported that warmer water temperatures resulted in increased efficacy of endothall and diquat (Netherland et al. 2000).

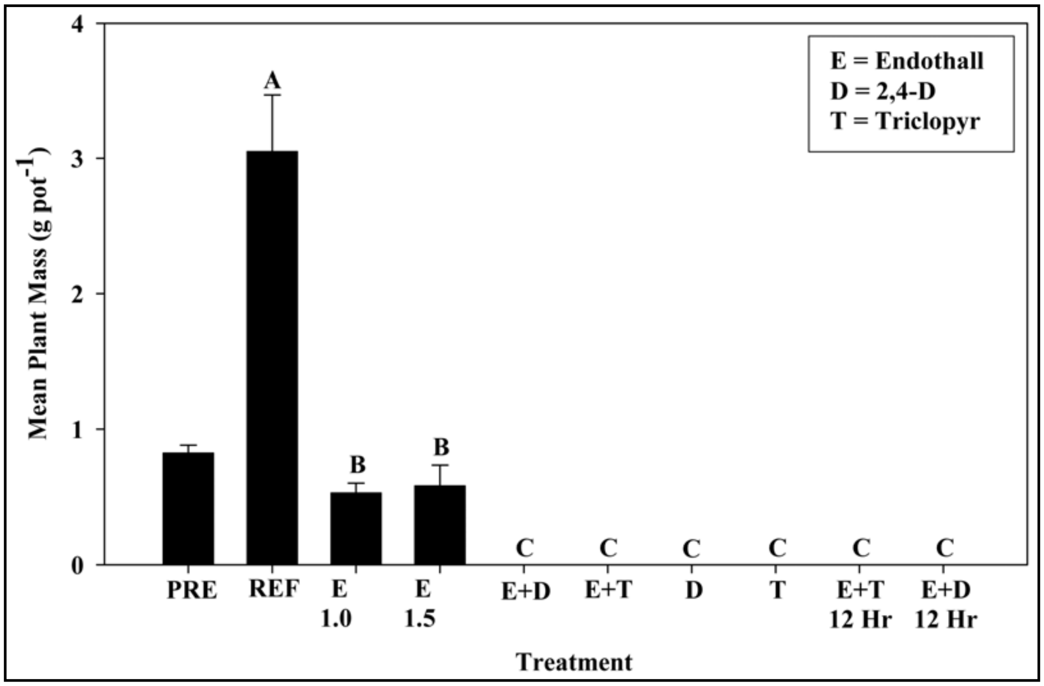

Figure 1. Mean (± 1 SE) plant mass of Eurasian watermilfoil 4 weeks after treatment with endothall 1.0 or 1.5 mg ae L^{-1}, 2,4-D 0.5 mg ae L^{-1}, triclopyr 0.5 mg ae L^{-1}, or combinations of endothall 1.0 mg ae L^{-1} with either 2,4-D or triclopyr. All herbicide treatments were 24 hr exposure unless noted in the figure. Bars sharing the same letter do not differ significantly at $p = 0.05$ according to Fisher's Protected LSD.

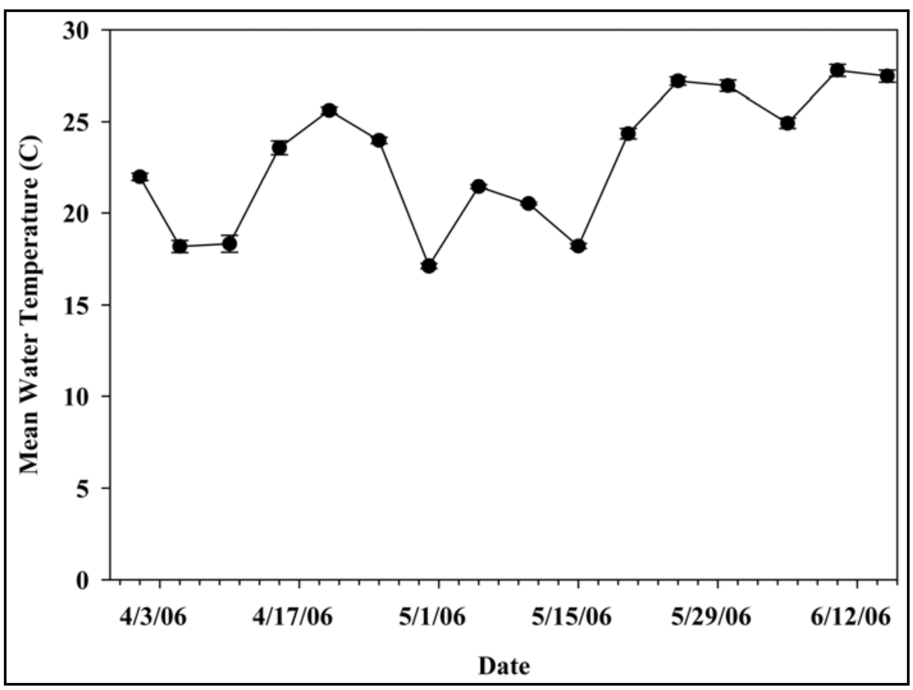

Figure 2. Mean (± 1 SE) water temperature recorded throughout the study. Water temperature was logged in four tanks at 1-hr intervals from April 1, 2006 through June 16, 2006. Water temperature data were averaged for all tanks.

Control of Eurasian watermilfoil was achieved in this study using endothall alone or combinations of endothall with 2,4-D or triclopyr at both 12- and 24-hr exposure periods. All treatments with endothall and a systemic herbicide provided at least 45 percent control in the first week of treatment, and > 90 percent control by 2 weeks regardless of exposure time. Combinations of endothall with either 2,4-D or triclopyr provided the benefits of immediate action and complete control within 3 weeks. Triclopyr and 2,4-D alone provided > 90 percent control by 3 weeks, but initial control was slow and less than 20 percent. In addition, use of these herbicide combinations could allow for simultaneous control of two invasive species, Eurasian watermilfoil and curlyleaf pondweed (*Potamogeton crispus* L), which frequently co-exist in northern tier water bodies (Skogerboe and Getsinger, in preparation). Curlyleaf pondweed is not controlled by the auxins (2,4-D and triclopyr), but is susceptible to low rates of endothall. Combinations of these herbicides had no antagonistic effects and may lead to increased efficacy in large-scale treatments or reduce the amount of herbicide needed to achieve similar control using only one of these products alone. This combination may be beneficial in controlling mixed populations of Eurasian watermilfoil and curlyleaf pondweed with lower herbicide concentrations. Future research should be conducted to determine combination concentrations of these products that are efficacious in controlling both plant species. An evaluation of lower rates and exposure times may also determine the additive or synergistic effects of these herbicides.

ACKNOWLEDGEMENTS: This research was conducted under the U.S. Army Corps of Engineers' Aquatic Plant Control Research Program (APCRP). This research was also supported by the Aquatic Ecosystem Restoration Foundation via a Cooperative Research and Development Agreement with the U.S. Army Engineer Research and Development Center, and with additional support from United Phosphorus, Inc. The authors thank Gary Ervin, Eric Dibble, and Victor Maddox for technical reviews of this manuscript. Thanks are also extended to Jimmy Peeples, Dan White, and Steven Gruchy for their assistance in the study. Permission was granted by the Chief of Engineers to publish this information. Citation of trade names does not constitute endorsement or approval of the use of such commercial products.

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This technical note should be cited as follows:

> Madsen, J. D., R. M. Wersal, K. D. Getsinger, and J. G. Skogerboe. 2010. *Combinations of Endothall with 2,4-D and Triclopyr for Eurasian Watermilfoil Control*. APCRP Technical Notes Collection (ERDC/TN APCRP-CC-14). U.S. Army Engineer Research and Development Center, Vicksburg, MS. *http://el.erdc.usace.army.mil/aqua/*

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